Original article

Circulating anti-Müllerian hormone levels and markers of subclinical cardiovascular disease in middle-aged and older men

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ABSTRACT

Context: Recent research suggests that higher circulating anti-Müllerian hormone (AMH) levels are associated with less frequent occurrence of (subclinical) cardiovascular disease (CVD) in women, but evidence in men is limited.
Objective: We investigated whether circulating AMH levels are associated with measures of subclinical CVD in middle-aged and older men.
Design: Prospective cohort study with a median follow-up time of 8.7 years. Serum AMH was measured at baseline. We assessed both cross-sectional and longitudinal associations using linear regression models adjusted for confounders.
Setting: Dutch middle-aged and older men from the community.
Participants: 394 men (aged 40–80 years) with an available AMH measurement at baseline.
Main outcome measures: At baseline (2001−2002): carotid intima-media thickness (CIMT), pulse wave velocity (PWV), abdominal aortic diameter, and Framingham risk score (FRS) predictions. At follow-up (2010−2011): CIMT, mean carotid aortic plaque score, PWV, and FRS predictions. All outcomes were transformed using rank-based inverse normal transformation to meet the normality assumption.
Results: Higher AMH levels were associated with lower CIMT at baseline (β = −0.04; 95%CI = 0.07, −0.01), but not with the other measures of subclinical CVD at baseline. Longitudinal analyses suggested that higher baseline AMH levels were associated with lower mean plaque scores at follow-up (β = −0.03, 95%CI = −0.07, 0.00), but not with the other follow-up outcomes.
Conclusions: Our results suggest that AMH is associated with current CIMT and future carotid aortic plaque burden in men, implying that circulating AMH levels are potentially associated with local atherosclerosis rather than with total aortic stiffness.

1. Introduction

Anti-Müllerian hormone (AMH) is a gonadal hormone that is primarily known for its crucial role in sexual differentiation during embryogenesis [1]. In adult men and women, AMH is produced by immature Sertoli cells and antral stage ovarian follicles [2], respectively. Based on few fundamental studies [3,4], it has been proposed that AMH may also have a role in the pathogenesis of cardiovascular diseases (CVD).

Recent epidemiological research indeed suggests that higher circulating AMH levels may be associated with a lower risk of both subclinical CVD [5,6] and clinical CVD [7,8] in women, but studies in men are scarce [9,10]. In addition, most previous studies had a cross-sectional design and did not adjust for potential confounding by circulating sex hormones, which hampers establishing whether AMH could play a causal role in CVD pathology in both sexes.

To gain more insight into the mechanisms through which AMH levels may affect CVD risk, we aimed to investigate whether circulating AMH levels are associated with different subclinical CVD measures in middle-aged and older men. More specifically, we examined associations of AMH with markers of atherosclerosis, arterial stiffness, abdominal aortic dilation, and 10-year risk of coronary heart disease (CHD), using cross-

sectional and longitudinal data.

2. Materials and methods

2.1. Study population

We used data from a Dutch population-based cohort study, which included 400 middle-aged and older men aged 40 to 80 years. Details about the scope of this cohort and about recruitment of study participants have been described into detail previously [11]. Briefly, participants were recruited through either convenience sampling or a random selection of the municipal register in 2001–2002. After a median follow-up period of 8.7 years, all study participants who were still alive and not living abroad (n = 346) were invited for a follow-up visit in 2010–2011. Of these 346 study participants, 270 were re-examined at follow-up (participation rate 68%). Reasons for non-attendance at follow-up examinations were being physically or mentally unable to visit the study center (n = 40), not interested (n = 22), and non-response (n = 14). During baseline and follow-up visits, questionnaires were completed, fasting blood samples were collected, and physical examinations were conducted. This study received approval from the Institutional Review Board of the University Medical Center Utrecht, and all study participants signed an informed consent prior to study inclusion. For the current study we excluded study participants without an available serum sample (n = 6). As a result 394 men were included in subsequent analyses.

2.2. AMH measurements

AMH levels were measured in serum samples collected at baseline, but not in samples collected at follow-up of the study. These baseline serum samples were stored at −80 °C. In September 2019, AMH was measured in serum samples using the cobas e 411 analyzer (Roche Diagnostics) by the Clinical Chemistry Laboratory at the University Medical Center Utrecht. The cobas e 411 is a fully automated analyzer that uses a ElectroChemiLuminescence technology for immunoassay analysis. This method has a variation coefficient of ≤6%, and for AMH its lower limit of quantification is 0.03 µg/l. Of the 394 samples, one measurement exceeded the upper limit of quantification (23 µg/l). For this sample AMH was remeasured in a diluted aliquot. None of the AMH measurements were below the lower limit of quantification.

2.3. Subclinical cardiovascular disease measures

2.3.1. Atherosclerosis

We included carotid intima-media thickness (CIMT) and carotid artery plaque burden as markers of atherosclerosis. CIMT (mm) was measured at baseline and follow-up through ultrasonography of both the left and right carotid arteries using a 7.5-MHz linear array transducer (at baseline: Acuson Aspen, Siemens; at follow-up: Acuson Sequoia, Siemens). For each study participant, the average CIMT of eight predefined angles (90°, 120°, 180°, and 150° for the right carotid artery and 180°, 210°, 240°, and 270° for the left carotid artery) was included as measure of CIMT. Carotid artery plaque burden was only assessed at follow-up, using ultrasound images of 12 arterial sites (near and far walls of right and left common carotid artery, the bifurcation and internal carotid artery). A 4-level rating scale was used to quantify plaque burden at each of these sites; 0 = no plaque, 1 = minimal plaque, 2 = moderate plaque and 3 = severe plaque. We used these 12 scores to calculate mean plaque scores for each participant, which we included as outcome in subsequent analyses.

2.3.2. Arterial stiffness

We included pulse wave velocity (PWV) as marker of arterial stiffness. PWV (m/s) was measured at baseline and follow-up using a SphygmoCor device (PWV Medical, Sydney, Australia), as described previously [12]. To cover a complete respiratory cycle, an average of ten successive waveforms was measured. The whole procedure was repeated three times per participant and average PWV values were included as measure of PWV. We set two biologically implausible baseline PWV measurements (2.75 and 30.51 m/s) to missing and subsequently imputed these missing values.

2.3.3. Abdominal aortic dilation

We included abdominal aortic diameter as indicator of abdominal aortic dilation, which can be used as indicator for abdominal aortic aneurysms. Abdominal aortic diameter (cm) was measured at both baseline and follow-up visits, but due to the high proportion of missing aortic diameter data at follow-up (91%) we only included aortic diameter at baseline in our analyses. Aortic diameter was measured through ultrasonography (Acuson Aspen, Siemens).

2.3.4. 10-year risk of CHD

For each participant, 10-year risk of CHD was predicted using the Framingham risk score formula published by Wilson et al. (1998) [13]. This formula requires information on the following predictors: age, total cholesterol, HDL-cholesterol, blood pressure, diabetes and smoking. Details about the quantification of these factors are described in the next paragraphs. Framingham risk score probabilities were calculated using both baseline data and follow-up data.

2.4. Covariates

Information on age (years), smoking status (current/former/never) and educational attainment (low/middle/high/university) were collected through questionnaires. Body mass index (BMI) (kg/m^2) was calculated using height and weight measurements obtained during physical examination. Systolic blood pressure (mm Hg) was measured in supine position using a semi-automated oscillometric method. We included the average of the two systolic blood pressure measurements in subsequent analyses.

Total cholesterol was measured in serum samples using an automatic enzymatic procedure (Synchron LX Systems, Beckman Coulter, Mydrecht, The Netherlands). Details on serum sex hormone measurements have been described previously [11]. Briefly, total testosterone was measured using an in-house competitive radioimmunoassay employing a polyclonal anti-testosterone (Dr JH Pratt, Indianapolis, IN, USA). This radioimmunoassay involved a diethyl ether extraction procedure, and purity of the used tracer ([1α-2α —^{3}H]Testosterone) was chromographically verified. The lower limit of detection of the assay was 0.24 nmol/l, and inter-assay variation was 6.0, 5.4 and 8.6% at 2.1, 5.6 and 23 nmol/l, respectively (n = 85). Sex hormone binding globuline (SHBG) was measured using an immunometric technique on an Immulite Analyzer (Diagnostic Products Corporation, Los Angeles, CA, USA). The lower limit of detection of this method was 5 nmol/l. Dihydroepiandrosterone sulphate (DHEAS) was measured on an Advantage Chemiluminescence System (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA), which has a lower limit of detection of 0.1 mmol/l. Total estradiol was measured using an in-house competitive radio-immunoassay employing a polyclonal anti-E2 antibody (Dr F de Jong, Erasmus MC, Rotterdam, The Netherlands). Prior to the used assay diethyl ether extraction and Sephadex chromatography were performed. The purity of the tracer used for this assay ([2,4,6,7-^{3}H]E$_2$) was chromographically verified. The lower limit of detection was 20 pmol/l, and interassay variation was 10.0 and 3.1% at 81 (n = 24) and 660 pmol/l (n = 17) respectively. Free testosterone, and free estradiol were calculated using algorithms previously described by Vermeulen et al. [14] and Södergard et al. [15], respectively. All specified hormones were only measured in baseline samples.

2.5. Statistical analyses

Baseline characteristics of study participants were described using mean median [interquartile range (IQR)], or percentage (n). We summarized baseline characteristics by AMH tertiles. In addition, we calculated Spearman's rank correlation coefficients to assess correlations between age, AMH, free testosterone, free estradiol and DHEAS. We also calculated correlations between AMH levels and levels of the three sex hormones.

2.5.1. Imputation procedure

Missing information on baseline variables was below 6%, but missing follow-up data ranged from 34% for CIMT to 52% for mean aortic plaque scores. Previous research showed that multiple imputation of missing follow-up data yields unbiased effect estimates if the follow-up data is missing at random [16]. The missing at random assumption is met if the occurrence of missing data can be contributed to other observed characteristics of the study population. To assess if our missing data met this assumption, we compared baseline characteristics of men without missing values on relevant variables (n = 164) to characteristics of men with missing values on at least one of these variables (n = 230) prior to imputation. We imputed missing values on both baseline and follow-up variables using multiple imputation (100 iterations, 50 imputed datasets), for which we used the R-package "mice" (version 3.6.0) [17]. Subsequent regression analyses were performed in each imputed dataset; regression coefficients and standard errors were pooled according to Rubin's Rule of combination [18], using the pool function implemented in "mice".

2.5.2. Cross-sectional analyses

Because of their non-normal distributions, we transformed CIMT, PWV, aorta diameter and Framingham risk score predictions using rank-based inverse normal transformation (INT) implemented in R-package "RNOmni" (version 0.7.1) [19]. This approach first transforms observations onto the probability scale using the empirical cumulative distribution function. As a second step, the observations are transformed into *Z*-scores using the probit function [19]. Consequently, effect estimates corresponding to the association of baseline AMH with the specified parameters for subclinical CVD are not straightforward to interpret. Presented results should therefore rather be interpreted as the presence or absence, and the direction, of an association than as a quantification of an effect.

We investigated associations of continuous AMH and AMH tertiles with baseline INT CIMT, INT PWV, INT aorta diameter and INT Framingham risk score predictions using linear regression models. First, we performed unadjusted analyses (Model 1). Second, we adjusted regression models for age (Model 2) and, third, for other known CVD risk factors (Model 3); BMI, smoking status, educational attainment, systolic blood pressure and total cholesterol. Finally, we conducted analyses additionally adjusted for levels of free testosterone, free estradiol and DHEAS (Model 4). Analyses including INT Framingham risk score predictions as outcome were not adjusted for age and Model 3 only included BMI and educational level, as age, smoking status, systolic blood pressure and total cholesterol, were already used to calculate Framingham risk score predictions.

2.5.3. Longitudinal analyses

Follow-up CIMT, mean plaque burden scores, PWV and Framingham risk score predictions were also transformed using rank-based INT. We assessed the association between AMH and each INT outcome using linear regression models. Models were adjusted for baseline measurements of the corresponding outcome and for the same baseline covariates as described for the cross-sectional analyses. Because we did not have baseline measurements for carotid artery plaque burden, we fitted an additional model for mean plaque scores, in which we additionally included CIMT at baseline (Model 5). All analyses were performed using

R, version 3.5.1 [20].

2.5.4. Sensitivity analyses

Since higher circulating AMH levels have been associated with a lower risk of diabetes and CVD, we performed sensitivity analyses excluding men with prevalent diabetes or CVD, to assess how this affected our results. Sensitivity analyses excluding men with prevalent diabetes were not performed for analyses including Framingham risk score predictions as outcome, as diabetes is included as predictor in the Framingham risk score itself. Prevalent diabetes was determined based on the combination of questionnaire data about diagnosis and treatment with insulin or oral hypoglycemic agents and fasting glucose measurements (>6.9 mmol/l). Fasting blood glucose levels were measured using a reagent-strip glucose oxidase method using a GlucoTouch reflectometer (LifeScan, Inc., Benelux). Participants were considered to have clinically prevalent CVD if they reported a physician diagnosis of coronary artery disease (i.e. myocardial infarction, coronary artery bypass graft, percutaneous transluminal coronary angioplasty), peripheral artery disease or stroke. We also performed sensitivity analyses excluding two outlying AMH measurements (>15 μg/l) to assess their effect on our results. In addition, we performed a sensitivity analysis in which we truncated follow-up outcome measures prior to imputation for participants who died during follow-up (n = 51). Excluding these participants completely from the longitudinal analyses potentially could have introduced selection bias. If imputations in participants who died were done on the basis of the distribution of CIMT, PWV, mean plaque score and FRS predictions in participants who survived, this could also have biased subsequent regression analyses. Besides, such an approach would create an immortal study population, which is not realistic. Therefore, we performed worst-case scenario analyses for which we assigned arbitrary values representing death due to CVD to each outcome at follow-up to assess how this influenced our results. We truncated CIMT, mean plaque score, PWV and FRS proportions at follow-up to 2.0 mm, 3 (representing severe plaque at each measured site), 25.0 m/s and 1.0, respectively. Subsequently, we repeated multiple imputation and association analyses using the same approach as described for the main analysis.

3. Results

Baseline characteristics of the study population are presented by AMH tertiles in Table 1. Men with AMH levels in the middle and highest tertiles were younger and less likely to have prevalent CVD than men with AMH levels in the lowest tertile. In addition, men in the highest AMH tertile were less likely to be current smokers or prevalent diabetes cases compared to men in the middle and lowest AMH tertiles.

We observed a weak negative correlation between age and AMH levels (Spearman's rho = −0.21). Higher age was also correlated with lower free testosterone (Spearman's rho = −0.50), free estradiol (Spearman's rho = −0.18) and DHEAS levels (Spearman's rho = 0.54) (Fig. 1). We observed weak positive correlations between AMH and free testosterone (Spearman's rho = 0.23) and DHEAS levels (Spearman's rho = 0.13). AMH levels were not correlated with levels of free estradiol.

Comparison of baseline characteristics among study participants with (n = 230) and without complete data (n = 164) indicated that men with one or more missing values on variables included in association analyses were older, less likely to be never smokers, had a higher systolic blood pressure, and were more likely to have prevalent diabetes or CVD (Supplemental Table 1). In general, participants with missing data were less healthy than participants without missing data. We observed a similar pattern of differences in baseline characteristics between men who attended and who did not attend the follow-up visit.

3.1. Cross-sectional analyses

After adjustment for age and other risk factors for CVD, higher AMH

Table 1

Baseline characteristics of the study population (n = 394) presented by AMH tertiles.

	AMH tertiles		
	1st AMH tertile (n = 129)	2nd AMH tertile (n = 134)	3rd AMH tertile (n = 131)
AMH (μg/l)[a]	1.9 [1.4, 2.2]	3.2 [2.8, 3.6]	5.9 [4.8, 7.2]
Age (years)[a]	65.0 [56.0, 72.0]	59.0 [48.0, 70.0]	59.0 [48.0, 66.5]
BMI (kg/m²)[a]	26.2 [24.0, 28.2]	26.5 [24.2, 28.6]	25.5 [23.8, 27.3]
Educational attainment[b]			
Low	17.1 (22)	16.4 (22)	16.8 (22)
Middle	26.4 (34)	27.6 (37)	32.8 (43)
High	35.7 (46)	40.3 (54)	27.5 (36)
University	20.9 (27)	15.7 (21)	22.9 (30)
Smoking status[b]			
Current	26.4 (34)	26.9 (36)	19.1 (25)
Former	59.7 (77)	49.3 (66)	55.0 (72)
Never	14.0 (18)	23.9 (32)	26.0 (34)
Systolic blood pressure (mm Hg)[a,c]	142.0 [124.8, 157.0]	139.0 [127.0, 156.0]	140.0 [129.0, 157.0]
Total cholesterol (mmol/l)[a,c]	5.6 [4.9, 6.2]	6.0 [5.3, 6.7]	5.8 [5.2, 6.4]
Prevalent diabetes[b]	7.0 (9)	7.5 (10)	5.3 (7)
Prevalent CVD[b]	25.6 (33)	13.4 (18)	13.0 (17)
Free testosterone (pmol/l)[a]	316.5 [266.7, 374.6]	343.1 [293.6, 407.3]	360.2 [309.3, 443.8]
Free estradiol (pmol/l)[a]	1.5 [1.3, 1.8]	1.5 [1.3, 1.8]	1.5 [1.3, 1.7]
DHEAS (pmol/l)[a]	5.5 [3.6, 8.2]	6.4 [4.5, 9.1]	6.9 [4.3, 9.1]

Abbreviations: AMH, anti-Müllerian hormone; BMI, body mass index; CVD, cardiovascular disease; DHEAS, Dihydroepiandrosterone sulphate.

[a] Median [IQR].

[b] Percentage (n).

[c] Missing values (n): systolic blood pressure [21], total cholesterol [1].

levels were associated with lower baseline INT CIMT ($\beta_{\text{continuous AMH}}$ = −0.04; 95% CI = −0.07, −0.01), but not with INT PWV, or INT aorta diameter measured at baseline (Table 2). Analyses including AMH tertiles supported a linear association between circulating AMH levels and INT CIMT (p_{trend} = 0.01) Adjustment for free testosterone, free estradiol and DHEAS levels did not change these results. Post-hoc analyses adjusted for total testosterone, total estradiol, SHBG and DHEAS instead, did not change estimates either (Supplemental Table 2). In addition, we observed that higher AMH levels were associated with lower INT Framingham risk score predictions ($\beta_{\text{continuousAMH}}$ = −0.05; 95% CI = −0.09, −0.02; Table 2), but effect estimates attenuated after adjustment for free testosterone, free estradiol and DHEAS ($\beta_{\text{continuousAMH}}$ = −0.03; 95% CI = −0.06, 0.00; Table 2). Stepwise adjustment for these sex hormones showed that free testosterone affected effect estimates most.

Sensitivity analyses excluding men with prevalent diabetes attenuated the association between the middle AMH tertile and baseline INT CIMT, but did not affect other estimates (Supplemental Table 3). Exclusion of men with prevalent CVD and exclusion of outlying AMH measurements did not change results from fully adjusted models either.

3.2. Longitudinal analyses

Over a median follow-up period of 8.7 years, CIMT, PWV and FRS predictions increased compared to baseline (Table 3). We observed no associations between AMH and INT CIMT at follow-up (Table 4). However, our results suggested that higher continuous AMH levels were associated with lower INT mean plaque scores, independent of CIMT at baseline ($\beta_{\text{continuousAMH}}$ = −0.03, 95% CI = −0.07, 0.00). Adjustment for total testosterone, total estradiol, SHBG and DHEAS instead of adjustment for free hormone levels and DHEAS produced practically identical results (Supplemental Table 2). Effect estimates for the AMH tertiles were in accordance with findings for continuous AMH levels, but

associations were no longer statistically significant after adjustment for baseline CIMT (Table 4). AMH levels were not associated with INT PWV or INT FRS at follow-up (Table 4). Sensitivity analyses excluding prevalent diabetes, CVD and the two outlying AMH measurements did not change our conclusions regarding CIMT, PWV and FRS predictions, but the association between AMH and mean plaque score was no longer statistically significant. Analyses including truncated values for participants that died during follow-up did also not change any of our conclusions (Supplemental Table 4).

4. Discussion

In the current study, cross-sectional analyses suggested that higher AMH levels are associated with lower CIMT, independent of risk factors for CVD and circulating sex hormones. Higher AMH levels were also associated with lower Framingham risk score predictions, but adjustment for free testosterone, DHEAS and free estradiol attenuated this association. Our results did not provide evidence for associations between AMH and aorta diameter or PWV. Longitudinal analyses did not provide evidence for associations between circulating AMH levels and CIMT at follow-up, but our results indicated that higher AMH is potentially associated with a lower plaque score after a median follow-up period of 8.7 years. Circulating AMH levels were not associated with PWV and FRS predictions at follow-up.

The main strength of this study is its prospective design, which enabled us to investigate both cross-sectional and longitudinal associations between AMH and different measures of subclinical CVD. Most previous studies only studied these associations cross-sectionally, and mostly in women [5–10]. Furthermore, previous research on the relation of AMH with CVD related outcomes did not examine potential confounding by testosterone, estradiol and DHEAS. A third strength is the relatively large sample size of this study compared to previous studies that investigated the association between circulating AMH and subclinical CVD. Nevertheless, the size of the current cohort may still have been limited to detect modest true associations between AMH and PWV, aorta diameter and FRS predictions.

A limitation of the current study is its selective loss to follow-up. Due to the age distribution of the study population at baseline (25% >70 years) a considerable proportion of the participants was not able to attend the follow-up examination after ~9 years. Extra effort was taken to maximize the participation rate at follow-up, among others through house calls, although follow-up measurements for CIMT, PWV and plaque scores could not be performed outside of the outpatients' clinic. To limit bias due to this selective loss to follow-up, we imputed missing data using a multiple imputation approach, which is preferable over not addressing missing follow-up data at all, even if the missing at random assumption is only partially met [16]. We observed that study participants with missing CIMT and PWV data at follow-up were older at baseline, more likely to have prevalent diabetes or CVD and in general had less favorable levels of CVD risk factors (e.g. higher blood pressure and more likely to be ever smoker) compared to study participants without missing follow-up data. These results suggest that missingness was, at least partly, related to other observed data and, accordingly, that the missing at random assumption was at least partially met. We also included an indicator for reasons for non-attendance at follow-up as predictor in our imputation model to increase plausibility of the missing at random assumption being met. In addition, we performed a worst-case scenario sensitivity analysis in which we assigned participants who died during follow-up values reflecting death due to CVD. Results from this analysis did not indicate that the results of our main analyses were biased.

Our findings regarding AMH and CIMT at baseline and mean carotid artery plaque score at follow-up are in concordance with a previous cross-sectional study that reported that higher circulating AMH levels were associated with lower CIMT in 70 healthy women [5]. In addition, higher baseline circulating AMH levels correlated with smaller
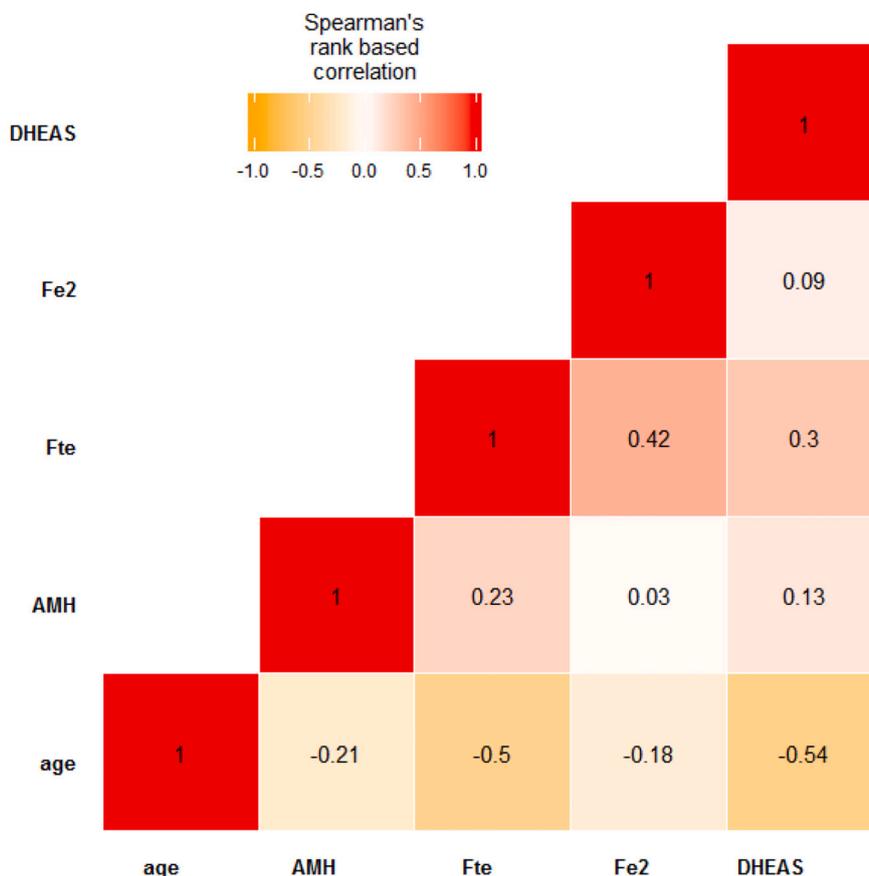
Fig. 1. Spearman's rank-based correlation coefficients for age, AMH and sex hormones.
Abbreviations: AMH, anti-Müllerian hormone; Fte, free testosterone; Fe2, free estradiol; DHEAS, Dihydroepiandrosterone sulphate.

atherosclerotic plaques after ~2 years in female monkeys [4]. Our findings add to these previous findings that AMH is also associated with CIMT in men, and that this association appears to be independent of both calculated free levels and total levels of circulating sex hormones. Other previous studies examining the relation between AMH and CIMT included a small number of women with type 1 diabetes [6,21], which limits direct comparison to findings in healthy individuals.

Similar to two previous studies [5,21] in women, our results do not support that AMH is associated with PWV. In other words, our results suggest that circulating AMH is associated with local atherosclerosis but not with total aortic stiffness. We did also not observe an association between AMH and abdominal aortic diameter, whereas Dennis et al. reported that lower AMH levels were correlated with a larger infrarenal aortic diameter [10]. An explanation for this discrepancy may be the difference in locations at which the aortic diameter was measured, since the study by Dennis et al. did not find a difference in AMH levels between men with an abdominal aortic aneurysm and healthy men [10]. Another explanation may be that the number of participants with an aortic diameter larger than 3.0 cm, which is indicative of a dilated abdominal aorta, in the current study was too small to detect an association (n = 12).

Finally, our results did not provide evidence for an association of circulating AMH levels with 10-year risk predictions for CHD after adjustment for circulating sex hormones. To date, no other studies have directly investigated this association, but our finding is in agreement with a previous longitudinal study that did not find an association between AMH and silent CHD in women [8], which was quantified as possible or probable CHD on an electrocardiogram. On the other hand, higher AMH levels have been found to be associated with lower incidence of CHD and total CVD in women [7]. Research investigating the association between circulating AMH levels and clinical cardiovascular

disease in men is currently lacking.

Future research is required to confirm whether the observed associations between AMH and cardiovascular health reflect a direct effect of AMH or merely correlation. Since higher circulating AMH levels have also been independently associated with lower ~10-year all-cause mortality in men (HR = 0.94; 95%CI = 0.90–0.98) [22], future studies should be conducted to disentangle whether AMH may be a proxy for biological aging instead of a cardiovascular hormone. Ideally, such studies would examine if the relation between circulating AMH levels and cardiovascular outcomes is confounded by biological aging through inclusion of, for example, epigenetic data. Like biological aging, infertility has been linked to long-term health, including but not limited to cardiovascular health, in both men and women. Although current scientific evidence on AMH being a suitable proxy for male infertility is still limited, future studies that collected data on fertility related traits (e.g. history of infertility or number of biological children fathered) could explore the hypothesis that AMH is rather a proxy for male infertility. Finally, we recommend that future studies set out to investigate associations between AMH and cardiovascular health compare these between participants who take cardiometabolic medication and participants who do not.

In conclusion, our results indicate that AMH is associated with current CIMT and potentially with future mean carotid aortic plaque score. This novel observation is in line with a previous study that reported a similar association in women. However, future studies are required to confirm if our findings are clinically relevant and if AMH is causally associated with, or merely a potential biomarker for, atherosclerosis.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.maturitas.2022.05.009.

Table 2

Cross-sectional associations between circulating AMH levels and rank-based inverse normal transformed measures for subclinical CVD measured at baseline.

| | Continuous AMH (µg/l) | AMH tertiles | | | |
| | | T1 (<2.5 µg/l) | T2 (2.5–4.0 µg/l) | T3 (>4.0 µg/l) | |
	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)	p-value for trend
Subclinical CVD measures at baseline					
CIMT					
Model 1: unadjusted	**−0.07 (−0.10, −0.03)**	Ref	**−0.41 (−0.65, −0.17)**	**−0.49 (−0.72, −0.25)**	
Model 2: adjusted for age	**−0.04 (−0.07, −0.01)**	Ref	−0.17 (−0.35, 0.02)	**−0.20 (−0.38, −0.01)**	
Model 3: model 2 + adjustment for CVD risk factors[a]	**−0.04 (−0.07, −0.01)**	Ref	**−0.21 (−0.39, −0.03)**	**−0.24 (−0.43, −0.06)**	
Model 4: model 3 + adjustment for free testosterone, free estradiol and DHEAS	**−0.04 (−0.07, −0.01)**	Ref	**−0.21 (−0.39, −0.03)**	**−0.24 (−0.43, −0.06)**	**0.01**
PWV					
Model 1: unadjusted	−0.03 (−0.07, 0.00)	Ref	**−0.31 (−0.55, −0.07)**	**−0.29 (−0.53, −0.04)**	
Model 2: adjusted for age	0.00 (−0.03, 0.02)	Ref	−0.06 (−0.25, 0.14)	0.01 (−0.18, 0.20)	
Model 3: model 2 + adjustment for CVD risk factors[a]	−0.01 (−0.04, 0.01)	Ref	−0.12 (−0.28, 0.05)	−0.09 (−0.26, 0.08)	
Model 4: model 3 + adjustment for free testosterone, free estradiol and DHEAS	−0.01 (−0.03, 0.01)	Ref	−0.11 (−0.27, 0.06)	−0.08 (−0.25, 0.09)	0.34
Abdominal aortic diameter					
Model 1: unadjusted	−0.02 (−0.06, 0.01)	Ref	−0.15 (−0.39, 0.09)	−0.16 (−0.39, 0.08)	
Model 2: adjusted for age	−0.01 (−0.04, 0.03)	Ref	−0.05 (−0.28, 0.19)	−0.03 (−0.27, 0.20)	
Model 3: model 2 + adjustment for CVD risk factors[a]	0.00 (−0.04, 0.03)	Ref	−0.04 (−0.27, 0.19)	0.02 (−0.22, 0.26)	
Model 4: model 3 + adjustment for free testosterone, free estradiol and DHEAS	0.00 (−0.04, 0.03)	Ref	−0.03 (−0.26, 0.20)	0.03 (−0.21, 0.27)	0.78
Framingham risk score predictions					
Model 1: unadjusted	**−0.05 (−0.09, −0.02)**	Ref	−0.20 (−0.44, 0.04)	**−0.40 (−0.64, −0.16)**	
Model 2: adjusted for age	N/A	N/A	N/A	N/A	
Model 3: model 2 + adjustment for CVD risk factors[b]	**−0.05 (−0.09, −0.02)**	Ref	−0.21 (−0.44, 0.02)	**−0.39 (−0.63, −0.16)**	
Model 4: model 3 + adjustment for free testosterone, free estradiol and DHEAS	−0.03 (−0.06, 0.00)	ref	−0.05 (−0.26, 0.16)	−0.20 (−0.42, 0.02)	0.07

Abbreviations: AMH, anti-Müllerian hormone; CVD, cardiovascular disease; CIMT, carotid intima-media thickness; PWV, pulse wave velocity; DHEAS, Dihydroepiandrosterone sulphate.

Figures in bold indicate that corresponding p-values were < 0.05.

[a] Model adjusted for: age (years), body mass index (kg/m^2), educational attainment (low/middle/high/university), smoking status (current/former/never), systolic blood pressure (mm Hg), and total cholesterol (mmol/l).

[b] Model adjusted for: body mass index and educational attainment.

Table 3

Median [IQR] measures for crude subclinical CVD measures at baseline and follow-up presented by AMH tertiles.

| | AMH tertiles | | |
	T1 (<2.5 µg/l)	T2 (2.5–4.0 µg/l)	T3 (>4.0 µg/l)
Subclinical CVD measures[a]			
Atherosclerosis			
CIMT at baseline (mm)	0.85 [0.76, 0.97]	0.78 [0.71, 0.91]	0.76 [0.67, 0.92]
CIMT at follow-up (mm)	0.94 [0.85, 1.09]	0.90 [0.80, 1.01]	0.88 [0.77, 1.01]
Average plaque score at follow-up	0.58 [0.33, 0.92]	0.33 [0.17, 0.75]	0.42 [0.08, 0.67]
Arterial stiffness			
PWV at baseline (m/s)	9.47 [8.17, 10.93]	8.71 [7.31, 10.25]	8.77 [7.40, 10.45]
PWV at follow-up (m/s)	9.80 [8.25, 11.90]	9.25 [7.85, 10.80]	9.10 [7.55, 11.15]
Abdominal aortic diameter at baseline (cm)	1.90 [1.70, 2.00]	1.80 [1.60, 2.00]	1.80 [1.60, 2.00]
10-year risk of coronary heart disease			
Framingham risk score prediction at baseline (proportion)	0.18 [0.13, 0.27]	0.15 [0.10, 0.25]	0.14 [0.08, 0.22]
Framingham risk score prediction at follow-up (proportion)	0.22 [0.13, 0.41]	0.18 [0.12, 0.31]	0.17 [0.10, 0.37]

Abbreviations: IQR, interquartile range; CVD, cardiovascular disease; AMH, anti-Müllerian hormone; CIMT carotid intima-media thickness; PWV, pulse wave velocity

[a] Presented median values [IQR] are averages across 50 multiply imputed datasets. These are crude values, i.e. values are unadjusted and untransformed.

Contributors

Renée M.G. Verdiesen was involved in the initial study design, carried out analyses and drafted the article.

N. Charlotte Onland-Moret was involved in the initial study design, gave input to the analyses, was involved in the interpretation of results and critically revised the manuscript.

Carla H. van Gils was involved in the initial study design, gave input to the analyses, was involved in the interpretation of results and

Table 4
Longitudinal associations between circulating AMH levels and rank-based inverse normal transformed CIMT and PWV.

	Continuous AMH (µg/l)	AMH tertiles			
		T1 (<2.5 µg/l)	T2 (2.5–4.0 µg/l)	T3 (>4.0 µg/l)	
	β (95%CI)	β (95% CI)	β (95%CI)	β (95%CI)	p-value for trend
Subclinical CVD measures at follow-up					
CIMT					
Model 1: adjusted for baseline CIMT[a]	−0.01 (−0.03, 0.02)	Ref	0.07 (−0.08, 0.22)	0.01 (−0.15, 0.18)	
Model 2: model 1 + adjustment for age	−0.01 (−0.03, 0.02)	Ref	0.08 (−0.08, 0.23)	0.02 (−0.15, 0.18)	
Model 3: model 2 + adjustment for CVD risk factors[b]	−0.01 (−0.03, 0.02)	Ref	0.05 (−0.10, 0.20)	0.01 (−0.15, 0.17)	
Model 4: model 3 + adjustment for free testosterone, free estradiol and DHEAS	−0.01 (−0.03, 0.02)	Ref	0.04 (−0.11, 0.20)	0.00 (−0.17, 0.16)	0.96
Mean plaque score					
Model 1: unadjusted	**−0.06 (−0.10, −0.03)**	Ref	**−0.32 (−0.58, −0.06)**	**−0.44 (−0.69, −0.18)**	
Model 2: adjusted for age	**−0.04 (−0.08, −0.01)**	Ref	−0.13 (−0.37, 0.11)	−0.22 (−0.45, 0.02)	
Model 3: model 2 + adjustment for CVD risk factors[b]	**−0.04 (−0.08, −0.01)**	Ref	−0.17 (−0.4, 0.06)	**−0.26 (−0.49, −0.03)**	
Model 4: model 3 + adjustment for free testosterone, free estradiol and DHEAS	**−0.04 (−0.08, −0.01)**	Ref	−0.17 (−0.4, 0.07)	**−0.25 (−0.48, −0.02)**	
Model 5: model 4 + adjustment for CIMT at baseline[a]	**−0.03 (−0.07, 0.00)**	Ref	−0.11 (−0.33, 0.12)	−0.19 (−0.41, 0.04)	0.10
PWV					
Model 1: adjusted for baseline PWV[a]	−0.02 (−0.05, 0.01)	Ref	−0.05 (−0.26, 0.16)	−0.12 (−0.32, 0.09)	
Model 2: model 1 + adjustment for age	−0.01 (−0.04, 0.01)	Ref	−0.01 (−0.22, 0.20)	−0.05 (−0.26, 0.15)	
Model 3: model 2 + adjustment for CVD risk factors[b]	−0.01 (−0.04, 0.02)	Ref	0.00 (−0.21, 0.21)	−0.02 (−0.23, 0.18)	
Model 4: model 3 + adjustment for free testosterone, free estradiol and DHEAS	−0.01 (−0.04, 0.02)	Ref	0.00 (−0.21, 0.22)	−0.01 (−0.22, 0.20)	0.93
Framingham risk score predictions					
Model 1: adjusted for baseline	0.00 (−0.04, 0.03)	Ref	−0.13 (−0.34, 0.09)	−0.04 (−0.26, 0.18)	

Table 4 (*continued*)

	Continuous AMH (µg/l)	AMH tertiles			
		T1 (<2.5 µg/l)	T2 (2.5–4.0 µg/l)	T3 (>4.0 µg/l)	
	β (95%CI)	β (95% CI)	β (95%CI)	β (95%CI)	p-value for trend
Framingham risk score predictions[a]					
Model 2: model 1 + adjustment for age	N/A	Ref	N/A	N/A	
Model 3: model 2 + adjustment for CVD risk factors[b]	0.00 (−0.03, 0.03)	Ref	−0.14 (−0.36, 0.07)	−0.03 (−0.24, 0.19)	
Model 4: model 3 + adjustment for free testosterone, free estradiol and DHEAS	0.00 (−0.03, 0.03)	Ref	−0.12 (−0.33, 0.10)	−0.01 (−0.22, 0.21)	0.97

Abbreviations: AMH, anti-Müllerian hormone; CIMT, carotid intima-media thickness; PWV, pulse wave velocity; CVD, cardiovascular disease; DHEAS, Dihydroepiandrosterone sulphate.

[a] Rank-based INT baseline measurements were included.

[b] CVD risk factors included were: body mass index (kg/m^2), educational attainment (low/middle/high/university), smoking status (current/former/never), systolic blood pressure (mm Hg), and total cholesterol (mmol/l).

critically revised the manuscript.

Yvonne T. van der Schouw was involved in the initial study design and acquisition of the data, gave input to the analyses, was involved in the interpretation of results and critically revised the manuscript.

All authors saw and approved the final version of the manuscript.

Ethical approval

This study received approval from the Institutional Review Board of the University Medical Center Utrecht in the Netherlands, and all study participants signed an informed consent prior to study inclusion.

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This article was not commissioned and was externally peer reviewed.

Research data (data sharing and collaboration)

Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

Declaration of competing interest

The authors declare that they have no competing interest.

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