Premenopausal Antimullerian Hormone Concentration is Associated with Subsequent Atherosclerosis

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Abstract

Objective—To determine if premenopausal ovarian reserve is associated with susceptibility for atherosclerosis.

Methods—Female cynomologus macaques (n = 66, women's equivalent age = 45 yrs) consumed an atherogenic diet for ~5 months prior to the measurement of a marker of ovarian reserve (antimüllerian hormone, AMH), plasma lipids, follicular phase estradiol (E2) and body weight (BW). Monkeys were then ovariectomized (OVX, n =17) remained premenopausal (PRE, n=20) or induced to have reduce ovarian reserve (ROR, n=29). After 26 additional months on the diet, atherosclerosis measurements and risk variables were reassessed.

Results—No differences in baseline AMH, plasma lipids, BW, E2 or post-diet lipids and BW, were observed among the groups subsequently assigned to OVX, PRE or ROR conditions. Post-diet measurements of atherosclerosis extent did not differ among the groups. However, analysis of plaque size by tertile of baseline AMH revealed that plaques were largest in monkeys that began the experiment with the lowest baseline AMH, followed by those in the middle and high tertiles (plaque extent mm2: Low AMH = 0.76 ± 0.12, Mid AMH = 0.46 ± 0.1, High AMH = 0.34 ± 0.08, p=0.02). Baseline AMH and plaque size were also correlated negatively (r = −0.31, p = 0.01). Plasma lipids were also correlated significantly with plaque extent (all p's <0.01), but not with AMH.

Conclusions—We report for the first time an inverse relationship between a marker of ovarian reserve (AMH) and subsequent atherosclerosis risk.

Keywords

Atherosclerosis; antimüllerian hormone; ovariectomy; ovarian reserve; cynomologus; nonhuman primate

Introduction

Cardiovascular disease remains the leading cause of mortality in postmenopausal women [1]. Coronary heart disease (CHD) due to atherosclerosis accounts for the majority of those deaths, with greater than 50% of women experiencing sudden cardiac death. Furthermore,

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many of these women succumb without previously reported symptoms [2]. Because the underlying atherosclerosis likely develops over a period of decades, these data emphasize the need for improved methods of assessment that would allow earlier identification of women at risk for CHD. However, gaps in knowledge still exist regarding the initiation and progression of atherosclerosis in this population. For example, the Framingham Risk Score has been reported to underestimate risk in peri- and early postmenopausal women. Hence, data from a group of young (~55yrs), non diabetic, postmenopausal women revealed detectable coronary artery calcium, indicative of subclinical atherosclerosis, despite having a low Framingham risk score [3].

Although evidence is accumulating in support of an interventional "window of opportunity" for estrogen treatment in the reduction of CHD in early postmenopausal women [4], the nature of the association between CHD risk and loss of ovarian function (menopause) is still controversial. For example, in a meta-analysis of 18 studies, no relationship between postmenopausal status and cardiovascular disease was found and only a modest effect of early menopause (surgical > natural) was observed[5]. Conflicting data on this subject may be due, in part, to the inability to account for the variability in type of "menopause" being investigated, including natural vs. surgical (hysterectomy with or without oophorectomy) and early (premature ovarian failure) vs. later menopause. Further, it has not been possible in most studies to account for differences among women in menopausal transition experiences, such as long (6–10 yrs) vs. short (~2yrs) menopausal transitions, or to account for differences in premenopausal reproductive dysfunction prior to the menopausal transition and menopause. Data from women and nonhuman primates suggest that premenopausal ovarian function may affect risk for chronic disease postmenopausally [6, 7]. More precise estimations of ovarian reserve using antimüllerian hormone (AMH), along with application of improved clinical staging of reproductive aging using criteria such as those from STRAW criteria [8] for the classification of women in menopausal transition, may increase understanding of the aforementioned relationships.

AMH is a protein hormone in the transforming growth factor-β family and is produced by granulosa cells of small growing (preantral and small antral) follicles in the ovary of rodents, nonhuman and human primates. AMH is positively correlated with primordial follicle numbers (ovarian reserve) in both women [9, 10] and Old World nonhuman primates [11] ($r > 0.7$ for both). Declines in AMH parallel follicle loss as women approach menopause [12] and AMH has been reported to fall to very low levels as early as 5 years prior to the final menstrual period [13]. Although AMH's main role is believed to be the regulation of primordial follicle recruitment [14–17], recent reports in mice indicate that AMH may have neuroprotective effects in the brain [18]. It is not known currently whether AMH has direct effects on other non-ovarian tissues.

The purpose of the study presented here was to determine retrospectively whether premenopausal ovarian reserve, as indicated by plasma AMH concentration, is associated with subsequent atherosclerosis progression. The data thus represent an opportunistic observation from a larger study designed to determine the effect of ovarian reserve on risk for chronic disease [19].

Methods

Study Design and Diet

Sixty six cynomologus females (*Macaca fascicularis*, imported from the Institut Pertanian Bogor, Indonesia) were used for this study. These monkeys are part of an ongoing study designed to investigate the effects of ovarian reserve on cardiovascular and metabolic disease risk. The study design has been described previously [19]. Briefly, adult status was

confirmed by dentition and evidence of epiphyseal closure, and the mean age and body weight of the monkeys was 15 years (range 7–21yrs) and 3.1 kg. All monkeys were housed in social groups of 2 to 4 individuals and were fed a semi-purified diet formulated to be similar to diets consumed by women in North America. The diet was prepared in the Wake Forest Primate Center diet laboratory and contained 0.20–0.28 mg cholesterol per calorie and derived 19% of its energy from protein (casein and lactalbumin), ~35% from fat and ~47% from carbohydrates. The monkeys were fed 120 Cal of diet/kg body weight daily.

For the first 5 months of the study, monkeys were exposed to a moderate amount of cholesterol (0.20mg/Cal). Cholesterol content of the diet was then increased to 0.28mg chol/Cal for the remainder of the study. After consuming diet for ~ 7 months (baseline period), monkeys were randomized to one of three conditions: 1) bilateral ovariectomy – OVX, n = 17, 2) premenopausal, PRE, n = 20 or 3) premenopausal with reduced ovarian reserve, ROR, n= 29. Following ovariectomy, plasma concentrations of estradiol were monitored to document completeness of the ovariectomy (estradiol concentrations below the level of assay detection). Three monkeys were excluded from the original 20 monkeys in the OVX group, due to resumption of intermittent menstrual bleeding and measureable estradiol concentrations, indicative of the presence of ectopic ovarian tissue [20]. Reduced ovarian reserve was achieved by the surgical placement of a biodegradable peri-ovarian implant containing a compound that specifically targets primordial follicles (4-vinlycyclohexenediepoxide, VCD), thereby decreasing ovarian reserve and mimicking the early stages of the menopausal transition [21]. This procedure coincided with the ovariectomy surgeries. Treatment with VCD reduced AMH ~ 56% (14.1 ± 1.7 to 6.7 ± 0.5 ng/ml) within four months [19] and remained above the level of detection for the duration of the experiment.

Following ovariectomy and induction ROR, monkeys consumed the Western diet consumption for 26 months. The iliac artery was then biopsied to allow the measurement of atherosclerosis extent (plaque area mm^2) without sacrificing the animal. The iliac artery was used because it provides an estimate of coronary artery atherosclerosis extent due to its high correlation with coronary artery atherosclerosis, r > 0.71 [22].

All animal manipulations were approved by the Wake Forest University Animal Care and Use Committee and were conducted in accordance with state and federal laws and following the guidelines of the US Department of Health and HumanServices.

Plasma Measures

Antimullerian hormone (AMH) and plasma lipid determinations were done following 5 months consumption of the diet (baseline) and within two months prior to artery collection. Serum AMH was measured using ELISA (DSL, Texas, Intra assay CV: 4.53% at13.62 ng/ml; Inter assay CV: 19.75% at 0.23 ng/ml, 11.42% at 9.61 ng/ml, and 11.27% at1.89 ng/ml). Total plasma cholesterol (TPC) concentrations, high-density lipoprotein cholesterol (HDL-C) concentrations, and plasma triglycerides (TGs) were determined in the Comparative Medicine Clinical Chemistry and Endocrinology Laboratory using reagents (ACE cholesterol, ACE HDL-C, and ACE triglycerides) and instrumentation (ACE ALERA autoanalyzer) from Alfa Wasserman Diagnostic Technologies (West Caldwell, NJ). TPC and HDL-C were standardized to calibrated controls from the Centers for Disease Control and Prevention-National Heart, Lung, and Blood Lipid Standardization Program. Intra- and inter-assay coefficients of variation were less than 5% for all analytes. Non-HDL-C, which approximates the sum of low-density lipoprotein (LDL) cholesterol and very-low-density lipoprotein (VLDL) cholesterol, was calculated by subtracting HDL-C from TPC. Plasma glucose concentrations were determined by the glucose oxidase method using a Glucose Analyzer 2 (Beckman Instruments, Brea CA, USA).Estradiol was measured during the follicular phase across two baseline menstrual cycles, approximately 4 and 5 months after

arrival from Indonesia. E2 was measured from serum by a modification [23] of a commercially available radioimmunoassay (RIA) from Diagnostic Products Corporation (Los Angeles, CA). Intra-assay and inter-assay CVs were <4% and 10%, respectively.

Measurement of Atherosclerosis

The common iliac artery was collected from all monkeys after consuming the diet for a total of ~ 33 months (7 months baseline plus 26 months post-OVX). Once collected, the left common iliac (LCI) was opened longitudinally, laid flat, fixed in 4% paraformaldehyde for 24 hrs and then transferred to 70% ethanol. After fixation, the artery was divided into the 3 equal segments, embedded in paraffin, and sections (4 μm) from each block were stained with Verhoeffvan Gieson's stain. Images of arteries were digitally captured and the extent of atherosclerosis (mm^2) determined using methods published previously [24].

Statistical Analysis

For this investigation, the treatment groups (OVX, ROR and PRE) were balanced prior to intervention at baseline for body weight, AMH and TPC: HDLC ratio. To meet normality and equality of variance assumptions, atherosclerosis plaque area (mm^2) and AMH were square root (SQRT) transformed and the data were then back transformed for presentation. There were no significant differences in the square root of plaque extent among the 3 arterial blocks measured per LCI (block-1 = 0.7 ± 0.04, block-2 = 0.67 ± 0.05, block-3 = 0.68 ± 0.05, p = 0.22) and therefore the mean of plaque area (mm^2) for all three blocks was used as the outcome variable for all further analysis of atherosclerosis extent. AMH for all monkeys measured at baseline was divided into tertiles with the following ranges: AMH-Low = 0.96–7.6, AMH-Mid = 7.71–14.4 and AMH-High =14.6–50.6 ng/ml. Atherosclerosis extent was also analysed according to baseline median AMH within each treatment group. Statistical analysis was done using SAS 9.2 and the SAS-based software -JMP® V-9. General linear models were used to examine the differences in plaque area, plasma lipids, estradiol (E2) and body weight among tertiles of AMH as well as among treatment groups. A multivariate regression model was used to determine correlations among variables. The significance was set at p<.05.

Results

Atherosclerosis extent is not affected by treatment but is predicted by baseline AMH

The common iliac artery was collected from all monkeys after consuming the diet for a total of ~ 33 months (baseline plus post-treatment period). Mean plaque area (mm^2) did not differ among the treatment groups (OVX = 0.75 ± 0.08, ROR-0.68± 0.07PRE = 0.73 ± 0.08, p = 0.8 overall, non-transformed means) (Figure 1A). Because the treatment groups were balanced prior to treatment according to baseline AMH, a procedure not done in previous studies, data were then analyzed to determine whether baseline AMH for all monkeys was associated with subsequent atherosclerosis extent. Atherosclerosis extent according to tertile of baseline AMH is depicted in Figure 1B. Monkeys with the lowest plasma AMH at baseline developed the most atherosclerosis 26 months later. To investigate the relationship between baseline AMH and subsequent atherosclerosis in each individual treatment group, monkeys were divided into two groups using the median of baseline AMH. Figure 1C shows that in all groups, baseline AMH appears to be related to atherosclerosis outcomes, although only the OVX group reached statistical significance. Finally, the negative correlation between baseline AMH and plaque area was also significant ($r = -0.31$, p <0.01) (Figure 1D). Neither AMH measured at the time of artery collection, nor percent change in AMH from baseline to post-treatment, were significantly associated with plaque extent (p = 0.6 for both comparisons).

Plasma Lipids Correlate Significantly with Atherosclerosis

Plasma lipids measured at baseline (seven months after beginning to consume a cholesterol containing diet) correlated significantly with plaque area (TPC, r = 0.42, p <0.001; LDL +VLDLC, r = 0.46, p <0.001; HDLC r = −0.38, p=0.002; and TPC:HDLC, r=0.44, p <0.001). The correlations between lipids and atherosclerosis remained significant after 33 months of consuming the experimental diet (TPC, r = 0.70, p < 0.001; LDL+VLDLC r = 0.72, p < 0.001; HDLC r = − 0.39, p=0.001; and TPC: HDLC r = 0.61, p < 0.001).

Baseline and Post-Ovariectomy AMH, BW, E2 and Plasma Lipids

AMH, body weight, and plasma lipids were measured after consuming the diet for 7 months (baseline) and 26 months after OVX. Follicular phase estradiol was measured at baseline only. Please note: PRE and ROR groups were combined into a single PRE group (n=49) for analysis because no baseline or post-treatment differences were observed between the two with respect to the aforementioned variables. There were no significant differences at baseline between monkeys assigned to OVX (n=17) or PRE (n= 49) conditions: (AMH, 10.6 ± 2 vs. 13 ± 1.3 ng/mL, p = 0.3; estradiol, 21.8 ± 6 vs. 25.5 ± 4 pg/mL, p = 0.6; BW, 2.99 ± 0.1 vs. 3.15 ± 0.1 kg, p = 0.3; TPC, 241.7 ± 13 vs. 238.1 ± 7.9 mg/dL, p = 0.8; HDLC, 45.3 ± 3.5 vs. 43.9 ± 2.1 mg/dL, p = 0.7; LDL+VLDLC, 196.4 ± 14.5 vs. 193.5 ± 9 mg/dL, p = 0.9 or TPC:HDLC, 6.0 ± 0.7 vs. 6.1 ± 0.4, p=0.9). Four months post-ovariectomy AMH was below the level of detection. After 26 months, no significant differences were observed in between OVX and PRE monkeys respectively in BW (3.14 ± 0.2 vs. 3.46 ± 0.12 kg, p = 0.2), TPC (318.2 ± 86 vs. 300.3 ± 71 mg/dL, p = 0.4), HDLC (46.7± 17 vs. 45.9 ± 19 mg/ dL, p= 0.9) or LDL+VLDLC (271.6 ± 19.5 vs. 254.5 ± 12 mg/dL, p = 0.5), TPC:HDLC (7.8 ± 3 vs. 7.6 ± 3, p=0.9). Similarly, baseline plasma lipids, BW, E2, nor post-treatment plasma lipids and BW differed according to baseline AMH tertile (Table 1).

Discussion

In the present study there was no effect of ovariectomy or reduced ovarian reserve on atherosclerosis extent when compared to premenopausal control monkeys. This finding was unexpected given previous reports of increased CHD risk following oophorectomy in both monkeys and women [25, 26]. However, the current study differs from previous monkeys studies because each treatment condition was balanced for plasma AMH (a previously validated marker of ovarian reserve or reproductive age) prior to the intervention. Consequently, we investigated retrospectively, the potential relationship between AMH and subsequent atherosclerosis development. As a result, we found that AMH, measured prior to long-term (26 months) consumption of an atherogenic diet, was negatively correlated with atherosclerosis extent and there was a significant, monotonic increase in atherosclerosis extent across tertiles of baseline AMH. Furthermore, within each individual treatment group, monkeys that fell below the median AMH at baseline developed more atherosclerosis than those above the median. Interestingly, it was only in the OVX group that this finding reached statistical significance. There were no differences in plasma lipids or lipoproteins among the tertiles of AMH, either at baseline or at the time of atherosclerosis measurement, indicating that the observed relationships between AMH and subsequent atherosclerosis were independent of plasma lipids. Taken together, these data suggest that AMH may be a marker of arterial susceptibility to atherosclerosis, a finding that may have been worsened by ovariectomy. Consistent with some studies of women however, the data also indicate that the trajectory of atherosclerosis progression was not influenced by estrogen depletion (ovariectomy) compared to premenopausal subjects.

The observed relationship between low AMH and subsequent atherosclerosis development in premenopausal monkeys may relate to arterial estrogen exposure prior to initiation of the

atherogenic diet. That is, AMH may be a marker of arterial susceptibility to atherosclerosis rather than having a direct relationship with atherosclerosis progression. It is well accepted that estrogen is atheroprotective through increased vascular responsiveness, attenuation of inflammatory mediators and cytokines, and decreased oxidative stress [27]. In addition, estrogen inhibits smooth muscle cell proliferation and reduces cholesterol and macrophage influx into the artery wall [28]. AMH is produced by the granulosa cells of small growing ovarian follicles (primary, secondary and small pre-antral) and is highly correlated with larger antral follicle number [29, 30]. Waves of growing and antral follicles are responsible for the majority of estradiol production by the ovary during the menstrual cycle. Consequently, lower AMH may indicate lower overall estradiol-production. In support of this hypothesis are the results of a study of premenopausal women > 40 years of age in which peak estradiol was observed to be lowest in women who were also in the lowest tertile of AMH [31]. In addition, it has been reported that premenopausal women with diminished ovarian reserve (and likely low AMH) had impaired urinary excretions of estrone conjugate [32]. Taken together this data indicate that it is possible that the monkeys in our study that were in the lowest tertile of AMH prior to dietary challenge may have had a history of chronically lower estrogen levels, resulting in increased susceptibility to initiation and progression of atherosclerosis. In addition, if AMH is a marker of estradiol exposure and subsequent artery susceptibility to the initiation of atherosclerosis, then it is not surprising that experimental reduction of AMH via chemical or surgical means was not associated with atherosclerosis extent.

We were not able to confirm a relationship between baseline E2 and atherosclerosis in this study. This finding might be explained in relation to the E2 sampling time period. Monkeys were sampled within ~ 5 months of arrival at our center from Indonesia and follicular phase samples were collected from two menstrual cycles only (1 sample each cycle). Cynomologus monkeys are known to experience hypothalamic suppression of menstrual cyclicity and cyclic hormones (estradiol, progesterone) following extreme stressors, such as travel and introduction to new social groups [6–33]. Therefore, our sampling method may not adequately represent premenopausal E2 exposure, and thus degree of pre-existing atheroprotection. More frequent measures of E2 across numerous menstrual cycles, over a longer period (12–18 months) may be required for accurate determinations of pre-existing E2 status.

Another potential explanation for our finding may relate to arterial aging. AMH is a marker of reproductive aging and is becoming accepted as a tool for predicting time to menopause. A recent long-term follow up study of normo-ovulatory women reported that women with low AMH for their age became menopausal earlier than those in the higher percentiles of AMH [34]. It is possible that ovarian aging and somatic aging occur in parallel, and that AMH is also a marker for generalized aging. Studies in rodents indicate that estrogen's vasoprotective and anti-inflammatory effects are diminished in aging arteries of ovariectomized older rats compared to young OVX rats [35]. It is also possible that circulating pro-inflammatory mediators increase with age, irrespective of estradiol production, resulting in increased arterial susceptibility to atherosclerosis [36–38].

It is notable that atherosclerosis extent was not increased in ovariectomized monkeys compared to premenopausal control subjects. It is unclear how this observation may or may not be in contrast to some previous studies dealing with the effect of ovariectomy on atherosclerosis progression. Monkeys in the study presented here consumed an atherogenic diet for 7 months prior to ovariectomy and from historical studies we expect them to have developed small fibro-fatty plaques by this time [39]. The amount of cholesterol in the atherogenic diet and the length of time the monkeys consume the diet prior to ovariectomy may modulate the effect of estrogen depletion on atherosclerosis progression. That is, it is

possible that the trajectory for atherosclerosis progression was set and was not further exacerbated by estrogen depletion, except in monkeys that had low AMH prior to OVX. Never the less, this finding would appear to be in contrast to a previous cynomologus monkey study that observed increased coronary artery atherosclerosis in ovariectomized monkeys compared to premenopausal monkeys [25]. Interestingly, OVX monkeys in that study did not differ in atherosclerosis extent from premenopausal females classified as socially stressed (subordinate) and anovulatory (progesterone values below expected for ovulatory cycles). Thus, the main driver of atherosclerosis protection in that study was dominant social status; a finding reported previously to be associated with normal ovarian function [6]. There was no effect of social status on atherosclerosis in the present study. In contrast to the aforementioned study, findings from the present study are supported by a nonhuman primate experiment that included both premenopausal and OVX groups fed an amount of cholesterol in the diet (0. 28mg chol/Cal) similar to our study, for a similar amount of time (30 months)[40]. In that study, no significant differences in coronary artery atherosclerosis were observed between OVX and premenopausal monkeys (0.20 ± 0.04 vs. 0.11 ± 0.04, p>0.05).

There are limitations to this study that deserve mention. First, our hypothesis was not formulated *apriori.* As such, these findings should be viewed as retrospective and therefore preliminary until tested prospectively. Second, we know from historical studies [41] that cynomologus macaques do not develop significant coronary artery atherosclerosis while consuming low cholesterol diets (i.e. in the wild or at the Indonesian Primate Center) and therefore it is assumed that the monkeys in this study did not have significant iliac artery atherosclerosis at the time of diet initiation. However, arterial function can be compromised in the absence of atherosclerosis (i.e. arterial stiffness), and thus other baseline measures of arterial function (i.e. pulse wave velocity, in vitro vascular reactivity of arteries taken via biopsy, arterial gene expression) might have been informative and should be studied prospectively. Finally, AMH is not perfectly correlated with follicle numbers and the coefficient of variation can be large. However, AMH was measured 3 times at baseline and approximately every 6 months post-treatment (data not shown) and the inter-individual variation across time in premenopausal monkeys was less than 10% overall when the samples were assayed together in a single batch, thus giving us confidence that baseline AMH was representative of ovarian reserve in our monkeys.

Conclusion

In conclusion, our primary findings indicate that a relationship exists between a marker of ovarian reserve (AMH) and atherosclerosis progression in monkeys. This relationship is independent of the strong association between plasma lipid concentrations and atherosclerosis. This finding may have implications for CHD risk determination and early prevention in women who are late reproductive age or transitioning to menopause.

Acknowledgments

Abbreviations

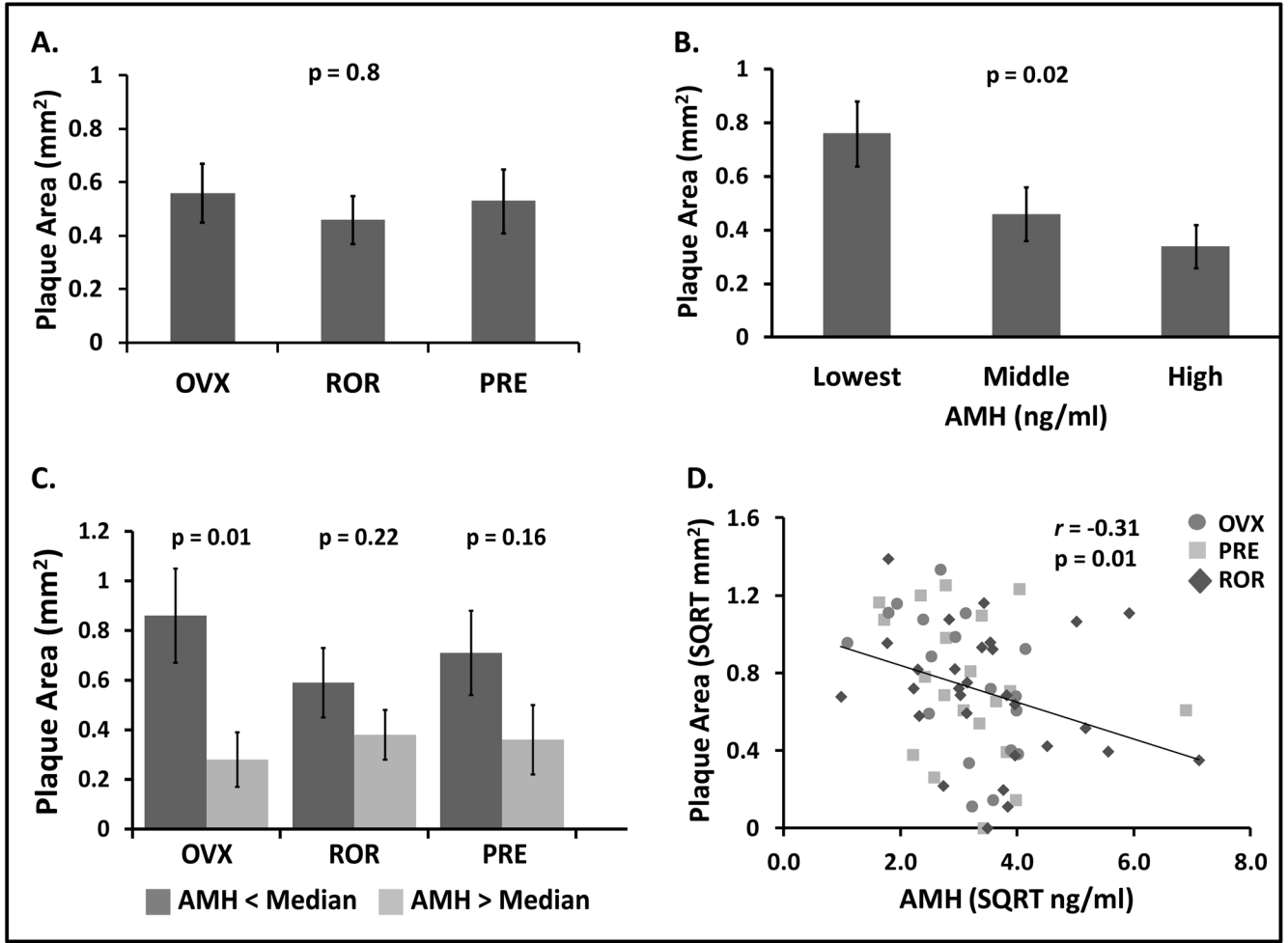**AMH**   antimüllerian hormone

E2   estradiol

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FIG. 1.
A: Iliac artery atherosclerosis plaque area (mm^2) among OVX (n = 17), ROR (n = 29), and PRE (n = 20) cynomolgus monkeys after ~2 years of diet consumption. **B:** Plaque area depicted according to tertiles of baseline plasma antimullerian hormone (AMH), all treatment groups combined. **C:** Plaque area depicted according to median baseline AMH for each individual treatment group. **D:** Correlation between baseline AMH and atherosclerosis, all treatment groups combined: OVX (○), PREMENOP (□), and ROR subset (diamond shape). Data are presented as mean ± SE plaque area (back transformed from SQRT) (A–C) and as the association between the SQRT of plaque size ± SE and SQRT of AMH ($r = -0.31$, $P < 0.01$) (D). OVX, ovariectomized; ROR, reduced ovarian reserve; PRE, premenopausal control; AMH, antimüllerian hormone; PREMENOP, premenopausal; SQRT, square root.

Table 1

Atherosclerosis and CHD Risk Variables According to Tertile of Baseline Antimullerian Hormone

	AMH Low (n = 22)	AMH Mid (n = 22)	AMH High (n = 22)	p
Iliac Artery Plaque Area (mm2)				
Post-Treatment	0.76 ± 0.12	0.46 ± 0.1	0.34 ± 0.08	0.02
AMH (ng/ml)				
Baseline Tertile Mean	4.94 ± 1.4	10.59 ± 1.3	21.3 ± 1.3	0.001
Tertile Range	0.96–7.6	7.71 – 14.4	14.6 – 50.6	NA
Estradiol (pg/ml)				
Baseline	20.1 ± 5.3	21.6 ± 5.5	31.6 ± 5.3	0.3
Body weight (kg)				
Baseline	3.2 ± 0.1	3.1 ± 0.1	3.1 ± 0.1	0.86
Post-Treatment	3.36 ± 0.2	3.31 ± 0.2	3.36 ± 0.2	0.97
TPC (mg/dl)				
Baseline	245.1 ± 11	237.2 ± 13	235.2 ± 11	0.82
Post-Treatment	327.9 ± 16	290.9 ± 15	297.5 ± 17	0.23
HDLC (mg/dl)				
Baseline	44.2 ± 3	45.8 ± 3	42.8 ± 3	0.8
Post-Treatment	44.7 ± 4	46.9 ± 4	43.3 ± 4	0.86
LDLC + VLDLC (mg/dl)				
Baseline	199.5 ± 14	191.4 ± 13	192.5 ± 13	0.9
Post-Treatment	283.2 ± 18	244 ± 17	254.2 ± 19	0.3
TPC: HDLC				
Baseline	6.2 ± 0.6	6.1 ± 0.6	6.0 ± 0.6	0.5
Post-Treatment	8.4 ± 0.7	7.4 ± 0.8	7.6 ± 0.8	0.56
LDLC + VLDLC: HDCL				
Baseline	5.2 ± 0.6	5.1 ± 0.6	5.0 ± 0.6	1
Post-Treatment	7.4 ± 0.7	6.4 ± 0.7	6.6 ± 0.8	0.6

Baseline and 26 months post-diet measures of atherosclerosis, hormone and risk variable data are presented according to baseline (pre-treatment group assignment) tertiles of plasma AMH for all monkeys. Data are Mean ±SE.