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The Level of Serum Anti-Müllerian Hormone Correlates with Vitamin D Status in Men and Women But Not in Boys

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Context: Anti-Müllerian hormone (AMH) is a gonad-specific hormone, which is extensively used as a marker of gonadal status. The level of serum AMH has a high variance in similar individuals for reasons that are unknown. The AMH gene promoter contains a vitamin D response element that may cause vitamin D status to influence serum AMH levels.

Aim: The objective of the study was to determine whether serum levels of AMH are related to 25-hydroxyvitamin D [25(OH)D] status.

Setting: This was a correlative and intervention study.

Participants: Three cohorts of participants were analyzed; mature men ($n = 113$), premenopausal women ($n = 33$), and 5- to 6-yr-old boys ($n = 74$). Women were given a daily supplement of ergocalciferol, cholecalciferol, or a placebo for 6 months and provided baseline and posttreatment blood samples.

Main Outcome Measures: Serum AMH and 25(OH)D were measured and analyzed for covariation.

Results: Serum AMH positively correlated with 25(OH)D in men ($r = 0.22$, $P = 0.02$) but not boys. Both 25(OH)D and AMH levels exhibited seasonal variation in women, with an 18% decrease in AMH levels in winter compared with summer ($P = 0.01$). Change in AMH level correlated with the initial AMH level and the magnitude of change in vitamin D levels ($r = 0.36$, $P = 0.004$). Cholecalciferol supplementation prevented seasonal AMH change.

Conclusion: Vitamin D may be a positive regulator of AMH production in adults, and vitamin D deficiency may confound clinical decisions based on AMH. Vitamin D deficiency should be considered when serum AMH levels are obtained for diagnosis. (*J Clin Endocrinol Metab* 97: 0000–0000, 2012)

Anti-Müllerian hormone [AMH; or Müllerian inhibiting substance] is a protein hormone produced in the Sertoli cells of the testes and the granulosa cells of the ovaries (1, 2). In males, AMH is expressed from early gestation and is a paracrine regulator of the regression of the precursor to the uterus and of the development of the testes in the fetus (2). In females, AMH is expressed from

the onset of puberty until menopause and regulates follicular recruitment and development within the ovary (1, 3).

AMH is also secreted from the gonads into the blood (4, 5) in which it may have endocrine effects on other tissues. The hormonal functions of AMH are not fully elucidated. Recent studies suggest that AMH contributes to sexual

dimorphism in the brains of mice (6–8) and to the control of the rate of maturation in boys (9). Clinically, the level of AMH in blood is used to assess gonadal status because serum AMH is entirely derived from the gonads in both sexes (10–12). Notably, serum AMH levels estimate ovarian reserve (13) and the proximity of menopause (14). Low maternal AMH is a predictor of poor success rate in assisted fertility procedures such as *in vitro* fertilization (15).

Serum AMH has high interperson variability in both children and adults (9, 16, 17), but the determinants of this variability are unknown. To date, no environmental factors have been shown to influence serum AMH, and factors such as the stage of the ovarian cycle (18, 19), weight loss (20), cigarette smoking (21), pregnancy (22), and hormonal contraceptive use (23) do not correlate with serum AMH levels.

The promoter for the *AMH* gene contains a vitamin D response element and the active form of vitamin D up-regulates AMH production in cultured prostate cells (24, 25). Vitamin D is therefore a potential regulator of AMH concentration in blood. The aim of the present study was to determine whether vitamin D status [25-hydroxyvitamin D [25(OH)D] level] correlates with AMH levels in men, women, and boys. In addition, we report that AMH levels in women are seasonal and that vitamin D supplementation during winter prevents this seasonality.

Subjects and Methods

Study populations

The men, women, and boys were recruited through different channels, as noted below. The participants in each group were of mixed descent but predominantly of European origin.

Men

Serum from 113 men was obtained from the Vascular Research Group Biobank (Dunedin School of Medicine, University of Otago, Dunedin, New Zealand). All men were community dwelling and healthy with no history of cardiovascular disease. They were aged from 54 to 93 yr old and donated their blood at varying times of the year. This resulted in a nonuniform distribution with 58 donating in winter, 28 in autumn, 22 in spring, and five in summer. This study group is therefore suitable for studying the relationship between vitamin D and AMH but is not informative about seasonality due to the small number of summer cases. All study participants gave written informed consent, and approval for the study was obtained from the Multi-Region Ethics Committee of New Zealand.

Vitamin D intervention study: women

This study examined whether a person's AMH levels were seasonal and whether daily supplements of either 1000 IU of ergocalciferol (D2) or 1000 IU of cholecalciferol (D3) in autumn and winter prevented the seasonality. The sera of the participants were obtained from a preexisting study of vitamin D status in the

local community. The participants in the original study were contacted via E-mail to seek permission to use their stored serum, with the approval of the University of Otago Human Ethics Committee. The exclusion criteria were the following: 1) women near menopause (>40 yr or an AMH value below 0.5 ng/ml/3.6 pmol/liter) and 2) a seasonal change in weight of greater than 4 kg or a body mass index of greater than 25 kg/m² as body mass index correlates with AMH in some cohorts but not all studies (20, 26, 27). Vitamin D levels are also influenced by body fat mass (28). More women (n = 34) than men (n = 8) responded, and only the female data are therefore illustrated. The women were aged 19–39 yr, with one being excluded because of her low AMH level. AMH does not have a detectable variation during the menstrual cycle (18, 19), and blood samples were acquired randomly throughout the menstrual cycle.

Boys

Seventy-four Dunedin boys aged either 5 or 6 yr were recruited from the community as previously described (9). Briefly, blood samples were collected at the Southern Community Laboratories Ltd. (Dunedin, New Zealand), at which a registered phlebotomist collected up to 2 ml of blood into BD vacutainers. Serum was collected within 1 h and stored in aliquots at –80 °C. The University of Otago Human Ethics Committee approved the project.

Serum analysis

AMH was measured in duplicate with MIS/AMH ELISA (Diagnostic System Laboratories, Webster, TX; DSL-10-14400, analytical sensitivity 0.04 pM, 0.006 ng/ml), with the enzyme immunoassay AMH/MIS kit (Beckman Coulter, Fullerton, CA; A16507, analytical sensitivity 1 pM, 0.14 ng/ml) being used when Beckman Coulter purchased Diagnostic System Laboratories. The two kits have been reported to give similar results (29), with this being confirmed in our laboratory by analyzing over 100 samples from various sources with both kits. All included participants had AMH levels above the sensitivity of the ELISA kits. Inhibin B levels were measured in duplicate by ELISA (Beckman Coulter; A81303). All participants had levels above the detection of the kit (2.6 pg/ml).

The 25(OH)D levels in males were measured in duplicate by ELISA (ALPCO Immunoassays, Windham, NH; K2109, analytical sensitivity 3.2 nM, 1.28 ng/ml). The data obtained with this kit is linear with respect to both liquid chromatography-mass spectrometry (ELISA estimate $1.2082 \times$ liquid chromatography-mass spectrometry estimate + 1.2965, $R = 0.92$) and HPLC (ELISA estimate $0.9466 \times$ HPLC estimate 7.0509, $R = 0.888$) estimates. The specificity of the kit is 100% for 25(OH)D₃ and 24,25-OH-vitamin D₂ and 68% for 25(OH)D₂, with no significant cross-reactivity to D2 (0.3%). All of the experimental samples had 25(OH)D levels above the detection limit of the kit (2.0 nmol/liter).

The serum used in the vitamin D intervention study was obtained from a prior study, as noted above, which included measurements of 25(OH)D. In this study, 25-dihydroxyvitamin D2 and 25-dihydroxyvitamin D3 were measured by isotope-dilution liquid chromatography-tandem mass spectrometry according to the method of Maunsell *et al.* (30), with the sum of 25-dihydroxyvitamin D2 and 25-dihydroxyvitamin D3 reported. The results from the intervention study are not directly compared

with the study of men, and the 25(OH)D were therefore not remeasured by ELISA.

Statistical analysis

The association between AMH and 25(OH)D in the cross-sectional studies of men and boys was examined by linear regression, with potential confounders analyzed using partial correlation. In the seasonal study of women, the mean and SEM were calculated for the summer and winter levels of AMH and 25(OH)D, with the statistical significance determined using paired Student *t* tests. Additionally, the change in AMH (Δ AMH) and 25(OH)D [Δ 25(OH)D] were calculated for each woman and these data used to build a linear regression model. Potential confounders were analyzed by partial correlation. All calculations were performed in Stata/IC 11.2 (StataCorp LP, College Station, TX).

Results

25(OH)D and AMH correlate in mature men

The median 25(OH)D concentration from 113 mature men in the cohort was 99 nmol/liter (range 2–317 nmol/liter). Nine of the men had 25(OH)D levels of less than 25 nmol/liter, indicating vitamin D deficiency. Serum AMH concentration ranged from 1 to 87 pmol/liter with a median of 21 pmol/liter. The level of AMH in the serum of the men positively correlated with their levels of 25(OH)D ($r = 0.22$, $n = 113$, $P = 0.02$) (Fig. 1). If vitamin D is a causal regulator of AMH levels, then the regression equation indicates that moving from vitamin D deficiency to the upper range of vitamin D levels would increase AMH levels by 13 pmol/liter on average.

The association between 25(OH)D and AMH could reflect specific regulation of AMH or general regulation of the Sertoli cells. If the latter is the case, then other Sertoli cell secretions, such as Inhibin B (InhB), should also covary with 25(OH)D. The levels of AMH and InhB were positively associated as expected ($r = 0.40$, $n = 113$, $P = 0.000$). However, there was no association between InhB

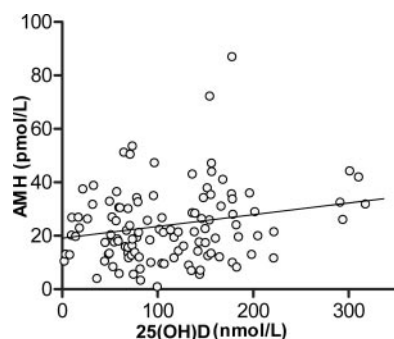


FIG. 1. The levels of AMH correlated with 25(OH)D concentration in men. Serum AMH and 25(OH)D were measured by ELISA in healthy men aged 54–93 yr. AMH positively correlated with 25(OH)D by linear regression [$\text{AMH} = 0.04 (25[\text{OH}]\text{D}) + 18.7$, $r = 0.22$, $n = 113$, $P = 0.021$] (1 ng/ml of AMH = 7.14 pmol/liter).

TABLE 1. 25(OH)D covaries with AMH independent of InhB and age in 113 mature men

Model no.	Model		Predictor of AMH	Partial	
	R	P		R	P
1	0.22	0.021	25(OH)D	0.22	0.021
2	0.50	<0.001	25(OH)D	0.24	0.011
			InhB	0.41	<0.001
3	0.61	<0.001	25(OH)D	0.22	0.018
			InhB	0.38	<0.001
			Age	−0.15	0.106

The relationship between serum AMH, 25(OH)D, InhB, and age was examined using linear regression models, with the partial correlates recorded.

and 25(OH)D ($r = -0.01$, $n = 113$, $P = 0.92$). Furthermore, the inclusion of both InhB and age in a regression model did not affect the relationship between AMH levels and 25(OH)D (Table 1). This suggests that 25(OH)D correlates specifically with the AMH levels rather than with the number or the general function of Sertoli cells.

AMH is seasonally covariant with 25(OH)D in women

The latitude of Dunedin (New Zealand) is 45° 52' south, and 25(OH)D in the local population decreases in winter due to reduced sunlight (31, 32). If vitamin D regulates AMH, then AMH should also be seasonal, with any seasonality being prevented by winter supplementation with vitamin D. This was examined using blood collected from a vitamin D intervention study, which compared summer and winter blood samples in a cohort of Dunedin women. After the baseline summer blood test, the women were given either daily oral supplements of either 1000 IU of D3 or a placebo. The level of 25(OH)D and AMH in the women receiving the placebo both exhibited significant seasonality, with the women's winter levels of AMH being 18% lower on average than their summer levels. In contrast, the women receiving the D3 supplements exhibited no seasonal loss of either 25(OH)D or AMH (Fig. 2). Supplementation with the less potent D2 did not prevent the winter decline in either 25(OH)D or AMH (Fig. 2).

If vitamin D directly regulates AMH, then the magnitude of a women's Δ AMH should correlate with the magnitude of her Δ 25(OH)D. This was examined by combining the three treatment groups (placebo, D2, D3) to generate a single cohort of women with a wide range of Δ 25(OH)D, which was then examined using linear regression. The Δ AMH in the women correlated with the change in her levels of Δ 25(OH)D and with her initial levels of AMH (Fig. 3). The initial level of AMH is related to age because the number of follicular cells producing AMH declines as ovarian reserve decreases (13, 14). The inclusion of age and the initial 25(OH)D levels did not signif-

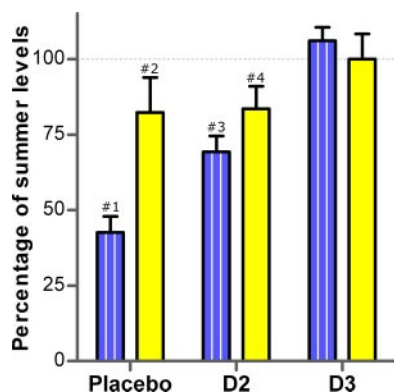


FIG. 2. AMH and 25(OH)D levels are seasonal. Women were given daily doses of vitamin D3 (n = 16), D2 (n = 7), or a placebo (n = 10) over the autumn and winter months. The winter levels of 25(OH)D (blue) and AMH (yellow) are illustrated as the percentage of each woman's summer levels. The bar represent the mean + the SEM. #, Significantly different from the summer values; #1, $P = 0.001$; #2, $P = 0.01$; #3, $P = 0.003$; #4, $P = 0.03$.

icantly alter the partial correlates between $\Delta 25(\text{OH})\text{D}$ and ΔAMH , indicating that these variables do not profoundly affect the vitamin D regulation of AMH. Specific studies are, however, needed to exclude subtle effects of aging or ovarian disease on the regulation of AMH expression.

25(OH)D and AMH do not covary in boys

During development, boys have supraadult levels of AMH, whereas prepubescent girls lack AMH (5, 16). This indicates that AMH levels are subject to boy-specific factors. We therefore examined whether 25(OH)D influences AMH levels in boys. AMH levels are developmentally regulated during male infancy and as boys enter puberty (5, 16) but are stable during middle childhood (9, 16). The study was therefore restricted to 5- and 6-yr-old boys, which also limits environmental variation because New Zealand children begin school on their fifth birthday. There was no correlation between AMH and 25(OH)D in

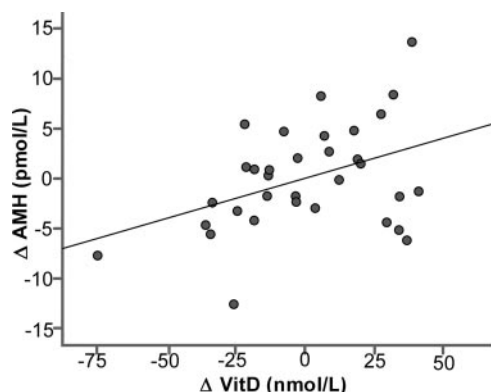


FIG. 3. Partial plot of linear regression presented in Table 2. The association between ΔAMH and $\Delta 25(\text{OH})\text{D}$ is shown adjusted for the initial levels of AMH and 25(OH)D ($R = 0.36$, $P = 0.004$). Regression equation: $\Delta \text{AMH} = 0.08(\Delta 25(\text{OH})\text{D}) + 0.043[\text{initial } 25(\text{OH})\text{D}] - 1.15$. Conversion is: 1 ng/ml of AMH = 7.14 pmol/liter.

TABLE 2. The change in the level of AMH correlates with seasonal change in 25(OH)D levels of 33 women

Model no.	Model		Predictor of ΔAMH	Partial	
	R	P		R	P
1	0.60	0.002	ΔVitD	0.38	0.030
			Initial AMH level	−0.53	0.002
2	0.62	0.007	ΔVitD	0.45	0.014
			Initial AMH level	−0.52	0.003
			Initial vitamin D level	0.21	0.271
			Age	0.20	0.297

The relationship between ΔAMH and the change in her levels of 25(OH)D were analyzed using linear regression models, with the influence of the initial levels of AMH and 25(OH)D being studied. The partial correlates from each model are recorded, and the partial plot of model 2 is illustrated in Fig. 3. ΔVitD , Change in vitamin D level.

the boys ($r = 0.07$, $P = 0.54$, Fig. 4) despite the wide range of 25(OH)D concentrations represented (3–237 nmol/liter). Inclusion of InhB in a linear regression model to control for Sertoli cell number did not change the relationship between 25(OH)D levels and AMH ($r = 0.07$, $P = 0.62$).

Discussion

AMH levels vary considerably between similarly aged individuals (9, 16, 17), which affects its use as a diagnostic marker. Several lines of evidence suggest that 25(OH)D status is one of the factors responsible for this variation. First, the promoter of the *AMH* gene contains a vitamin D response element, which is active in cultured cells (24, 25). Second, the level of AMH in men correlated with their 25(OH)D levels (Fig. 2). This is consistent with the direct regulation of AMH by vitamin D because the levels of the other major Sertoli cell hormone, InhB , did not correlate with 25(OH)D. Third, the extent of seasonal variation in a woman's level of AMH correlated with the extent to which her 25(OH)D levels varied (Fig. 3), with D3 sup-

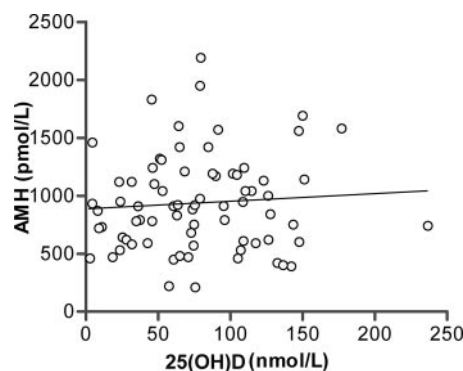


FIG. 4. The levels of AMH did not correlate with 25(OH)D levels in boys. 25(OH)D was variable (3–237 nm) in 74 boys aged 5–6 yr, but there was no correlation between AMH and 25(OH)D in a linear regression [$\text{AMH} = 0.001(25[\text{OH}]\text{D}) + 0.89$, $r = 0.07$, $n = 74$, $P = 0.541$] (1 ng/ml of AMH = 7.14 pmol/liter).

plements being sufficient to block seasonal change in both 25(OH)D and AMH levels (Fig. 2). Collectively, these observations implicate vitamin D status as a determinant of AMH levels in adults.

The average winter decrease in AMH levels of women not taking vitamin D supplements was 18%. AMH also decreases in mature women as their ovarian reserve diminishes, with an 18% decrease in AMH being approximately equivalent to 2 yr of aging (33). The range in seasonal change in AMH levels, however, was wide (1–62%), indicating that seasonal variation will significantly influence the estimation of ovarian reserve for some but not all women. The importance of 25(OH)D to the estimation of ovarian reserve is likely to vary from population to population because 25(OH)D status is influenced by exposure to sunlight, which varies with geographic location. The current study was undertaken in Dunedin (New Zealand), which is nearly equidistant from the equator and South Pole. In Northern Hemisphere terms, this approximates to Harbin (China) in Asia, Milan (Italy) in Europe, and Portland, OR (United States) and Montréal (Canada) in North America. People living closer to the poles typically experience a greater seasonal variation, potentially leading to greater decrease in the winter AMH levels. Similarly, people exposed to lower levels of UV light due to darker skin color, cultural garments, or the use of sunscreen may have chronically lower serum AMH levels. Consistent with this, AMH varies with race in American women, with black women having lower AMH levels than white women of the same age (34).

The serum levels of AMH in women diminish as they age and are influenced by conditions such as polycystic ovarian syndrome. Consequently, most of the variation in AMH levels between women reflects differences in the number of follicles/follicular cells that produce AMH, rather than variation in transcription of the *AMH* gene. Therefore, the age of a woman and/or the status of her ovary would not be expected to influence the effect of 25(OH)D on the production of AMH. Consistent with this, the inclusion of age in the regression analysis did not diminish the relationship between Δ 25(OH)D and Δ AMH and may even have slightly strengthened it. The current study is, however, insufficiently powered to detect whether age or ovarian status subtly influences the effect of 25(OH)D on AMH production, and future studies to specifically address these issues are needed.

AMH did not correlate with 25(OH)D levels in boys, in contrast to both men and women. This is broadly consistent with the known characteristics of the *AMH* promoter. Boys express very high levels of AMH, indicating that powerful boy-specific enhancers drive the *AMH* gene promoter. Consequently, any influence of the vitamin D re-

sponse element would be limited to a small amplification of an already active promoter. It is also possible that the presence of the childhood activators of the *AMH* promoter may prevent the vitamin D receptor binding to the promoter.

In conclusion, serum AMH levels reflect the number of cells producing AMH (13, 35), enabling AMH to be used as a marker of gonadal status in both women and men. This, however, is dependent on levels of serum AMH being independent of environmental factors. The current study suggests that 25(OH)D status affects a person's AMH level. Consequently, vitamin D deficiency may need to be considered when serum AMH levels are obtained for clinical diagnosis, particularly in communities or individuals that are at risk of vitamin D deficiency.

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