

Relationships between Anti-mullerian Hormone, Testosterone, Luteinizing Hormone and Follicle Stimulating Hormone in Men on Testosterone Therapy

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Abstract

Aim: To assess if testosterone therapy, with suppression of LH and FSH altered AMH levels in men.

Study background: AMH is an important male hormone and is increasingly measured by laboratories for men and women's health assessments. This prospective study was conducted in community medical centres to assess the effects of testosterone on AMH levels in men.

Methods: Men (n=15) with androgen deficiency symptoms, who were prescribed a trial of testosterone therapy by their own practitioner for at least 6 months, consented to participate in a study measuring AMH pre-therapy and post-therapy. Testosterone therapy was given to achieve LH and FSH suppression. Measurement of testosterone, LH, FSH and AMH at baseline and post testosterone therapy on at least 2 occasions including at 6 months was completed in all men. Men with abnormal baseline biochemistry (elevated LH or testosterone below age appropriate ranges) were excluded (n=5) from further study.

Results: In the study group baseline LH was normal (<8 U/L) and baseline testosterone was 7-23 (mean 12 nmol/L) and within age specific intervals. Mean baseline AMH was 36 pmol/L (range 19-89) and within age related intervals. A significant rise (p=0.001) of at least 1.5-fold in testosterone occurred post treatment (range 1.5-7.5-fold increase) with suppression of LH to <1 U/L with therapy. AMH showed variable changes after testosterone. There was no significant trend in AMH either rising or falling compared to baseline and levels were not associated with testosterone (p=0.197) nor affected by the suppression of FSH or LH (p=0.683, 0.271 respectively).

Conclusions: No significant pattern of change occurs in AMH in adult men at 6 months undergoing exogenous testosterone therapy. Laboratories do not need to adjust AMH reference intervals for effects of testosterone therapy in men with normal baseline LH and testosterone prior to therapy.

Keywords: AMH; Men; Testosterone therapy

Introduction

Anti-mullerian hormone (AMH) has been widely studied for its role in predicting ovarian reserve and response to fertility treatments in women and in assessment of disorders of sexual differentiation in paediatrics [1,2]. However, despite being a critical male hormone, vital in early male sexual differentiation, its physiologic role and the factors determining levels in adult men remain poorly understood [3,4]. It appears that AMH be a marker of Sertoli cell number and function but new reports suggest a broader role for this "gonadal cytokine" in men [5,6]. Laboratories providing measurement of male AMH should understand factors affecting levels in men so that they can provide appropriate clinical interpretation of results for clinicians.

Intriguing recent data reports reduced AMH levels in males with increased BMI and obesity and lower levels in those with metabolic disease and carbohydrate disturbance [3,7]. Men with enlarged aortic diameter are also reported to have lower AMH levels [8]. In contrast men, free of arterial cardiovascular disease had higher AMH levels which were similar to levels of AMH in younger men [4]. A novel finding of high Growth hormone/IGF-1 activity being associated with reduced AMH levels has also been reported by Andreassen et al. [9]. These studies suggest that AMH levels may have broader health implications than expected and as a consequence understanding the factors affecting AMH levels in men is important.

In males, AMH levels change dramatically at puberty. Prepubertal levels of AMH are very high but as Sertoli cells (SC) mature, levels reduce dramatically to 3-4% of prepubertal levels in adult males [10].

The factors initiating maturation of SC and reduction of AMH are complex but are thought to include expression of the Androgen Receptor (AR), androgen sensitivity of the Sertoli cells, FSH and T3 action and the appearance of meiotic germ cells [11-13]. The action of intra-testicular testosterone (IT-T) on the AR is also thought to be an important inhibitory modulator of AMH levels however this action may be indirect [14-16].

As intra-testicular testosterone action on ARs seems to be associated with reduced AMH at puberty, the potential of testosterone and IT-T to modify AMH in adult men is also of interest. In men, there is little known about the effects of testosterone therapy on AMH. It is known that exogenous testosterone therapy in men results in elevated serum testosterone, suppression of Luteinizing hormone (LH) and Follicle stimulating hormone (FSH) and a profound reduction (~98%) in IT-T [17-20].

The aim of this study was to study the relationship between serum testosterone, LH, FSH and AMH in men undertaking a trial testosterone therapy for symptoms of androgen deficiency and to determine if testosterone therapy affected AMH results in men. Samples were collected pre-therapy and post testosterone therapy to investigate if any significant change occurred in AMH in this setting.

Materials and Methods

Subjects

This study was approved by the Hollywood Hospital Human Research Ethics Committee (Approval number HPH380). Men with symptoms compatible with androgen deficiency (low libido, reduced or absent morning erections, loss of energy) presented to their own medical practitioner and if reviewed and assessed as suitable for a trial of testosterone therapy, were offered participation in this study. A variety of testosterone therapies were offered to men by their doctors including topical testosterone therapy (n=6) and intra-muscular testosterone (n=4). Suppression to <1 U/L of LH and FSH occurred in all men indicating the same pharmacologic effect. Men all had initial baseline pathology testing prior to commencing therapy as requested by their doctor. Baseline bloods were nominated as "Visit 1" and after commencing testosterone therapy, the first follow up blood test was nominated "Visit 2" and a sample collected after at least 6 months of continuous testosterone therapy was nominated as "Visit 3". Men (n=5) who had elevated LH (greater than 1.5-fold the reference range) or testosterone below age appropriate reference intervals, were excluded from further study.

Laboratory analysis

Blood was collected during office hours (9 am to 5 pm) into 5 mL serum separator tubes (BD Vacutainer®; Becton Dickinson), allowed to clot at room temperature and then centrifuged within 30 minutes at 1200 g for 10 minutes. Serum was decanted and frozen at -20°C until analysis.

AMH was analysed in duplicate according to the manufacturer's guidelines using the AMH Gen II enzyme linked immunosorbent assay (ELISA) from Beckman Coulter (Beckman Coulter Ireland Inc. Mervue Business Park, Mervue, Galway, Ireland). For AMH all patient samples were analysed in duplicate and results calculated as the mean of the duplicates. Quality control materials (QC I and II) were used as directed by the manufacturer and long term "in house" patient pools at 7.7 pmol/L (low pool) and 28.9 pmol/L (high pool) were run with

every assay for this study. Coefficient of variation (CV) precision studies from QC and patient pools between runs over 6 months was 4.2% and 3.8% respectively for QC I and II, and 3.8% and 4.6% for low and high patient pools. To convert AMH values in pmol/L to ng/mL or µg/L divide results by 7.14.

Serum Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) were analysed on the Abbott Architect platform (Abbott Laboratories, Abbott Park, Illinois, USA) according to manufacturer's guidelines. For FSH the CV for internal QC material over a 6-month period was 4.0%, 4.2% and 3.9% for FSH levels of 7.8, 23.4 and 38.5 U/L respectively. For LH, the CV for internal QC over 6 months was 3.9%, 3.6%, 3.9% at LH levels of 1.1, 16.5 and 55.4 U/L respectively.

Testosterone was analysed on the Abbott Architect platform (Abbott Laboratories, Abbott Park, Illinois, USA). This assay has very good correlation ($R^2=0.964$) with an in-house liquid chromatography tandem mass spectrometry (LCMS/MS) assay over a <1 to 30 nmol/L range. CV's for the immunoassay for internal QC over 6 months were 7.3%, 4.3% and 4.1% at testosterone levels of 1, 5.6 and 22.4 nmol/L.

Reference ranges

LH values less than 8 U/L were defined as normal consistent with the laboratory reference interval. Testosterone ranges vary with age. Baseline testosterone was within age specific reference intervals in all men when using published data on men aged 21-35 years (range 10.4-30.1 nmol/L), older men aged 70-89 years (range 6.4-25.7 nmol/L) and specific age-related intervals recently reported by Kelsey et al. [21-23]. The AMH reference intervals for men in our laboratory are 20-120 pmol/L for 19-40 year olds and for >40 years 15-72 pmol/L. These intervals were derived from local data, Roche kit literature on male reference intervals and literature [10,24].

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics Version 23. The relationships between each of the study hormones were analysed using bivariate correlations (2-tailed, Spearman) to determine the correlation coefficient (r) and p-value (significance). Correlations were also made between hormones and subject age. General linear models and repeated measures ANOVA were used to measure the relationships between AMH, Testosterone, LH, FSH and visit number. Differences were statistically significant when $p<0.05$. Power calculations indicated that with a sample of 10 or more individuals with data at each phase, there would be 80% power to detect differences equivalent to 1 SD change in transformed AMH. This calculation was made based on a paired difference in log-transformed AMH response, assuming a 5% level of statistical significance.

Results and Discussion

Baseline data prior to testosterone therapy

Ten men with a mean age of 53 years (range 30-75 years) took part in this study. Mean baseline AMH was 36 pmol/L (range 19-89), and baseline LH was normal (<8 U/L) in all men with a mean LH of 4.1 U/L. Baseline testosterone was 7.0-23.4 (mean 12.2 pmol/L) and was within age specific reference ranges for all men. Mean baseline FSH was 6.7 U/L.

Post testosterone therapy

Data prior to and during testosterone therapy is shown in Table 1 and Figure 1. All men had a rise ($r=0.571$, $p=0.001$), in serum testosterone over the 6 months (range 1.5-7.5-fold) and suppression of LH and FSH during testosterone therapy to <1 U/L. Age was negatively correlated with levels of testosterone ($r=-0.439$, $p=0.015$). An increase in testosterone levels was associated with suppression of LH ($r=-0.593$, $p=0.001$) and FSH ($r=-0.656$, $p=0.000$). Testosterone levels were not correlated to AMH ($p=0.197$). LH decreased over the treatment course ($r=-0.649$, $p=0.000$) and was not associated with age ($p=0.730$). FSH levels similarly, significantly decreased over the course of the three visits ($r=-0.665$, $p=0.000$) and were not associated with age ($p=0.880$). LH and FSH were significantly associated with each other ($r=0.896$, $p=0.000$).

Subject	Age	VISIT	AMH (pmol/L)	Testosterone (nmol/L)	LH (U/L)	FSH (U/L)
1	54	1	25	9.4	7.1	9.2
1		2	22	14.7	3.3	3.5
1		3	21	14.6	0.1	0.2
2	59	1	21	10.3	7.8	10.2
2		2	13	31.8	0.1	0.3
2		3	18	15.9	0.5	1.1
3	30	1	59	12.8	6.2	6.6
3		2	55	41.2	0.1	0.1
3		3	46	27.1	0.3	0.6
4	63	1	36	7	2.2	5
4		2	34	18	0	0.3
4		3	40	19.7	0	0.4
5	33	1	41	23.4	1.3	4.2
5		2	35	60	0	0.3

5		3	45	56.5	0	0.2
6	58	1	19	13	2.7	3.9
6		2	25	32.1	0	0.1
6		3	21	35.8	2.1	1.8
7	45	1	89	12.1	1	1.8
7		2	86	23.1	0	0.4
7		3	91	19	0	0.5
8	75	1	20	7.4	6.1	18.3
8		2	20	15.3	0.5	1.8
8		3	26	17.7	0	0.3
9	68	1	21	9.6	2.9	4.4
9		2	20	16.1	0	0.1
9		3	21	20.2	0	0.1
10	45	1	30	17.2	3.2	3
10		2	29	21.1	3.6	3.7
10		3	24	31.6	0.6	0.9

Table 1: Subjects and study hormones prior to and during testosterone therapy. Visit 1 provides baseline (pre-testosterone) hormone levels. Visit 2 is early testosterone therapy and Visit 3 late (>6 months) on testosterone therapy.

AMH showed variable changes in men on testosterone therapy, with some men demonstrating a modest rise or fall in AMH and others showing no consistent change in AMH levels. AMH changes in men are shown in Figure 1. Overall AMH levels did not change significantly over time ($p=0.990$) but were negatively correlated with subject age ($r=-0.660$, $p=0.000$). AMH levels were not affected by the suppression of FSH or LH ($p=0.683$ and $p=0.271$ respectively).

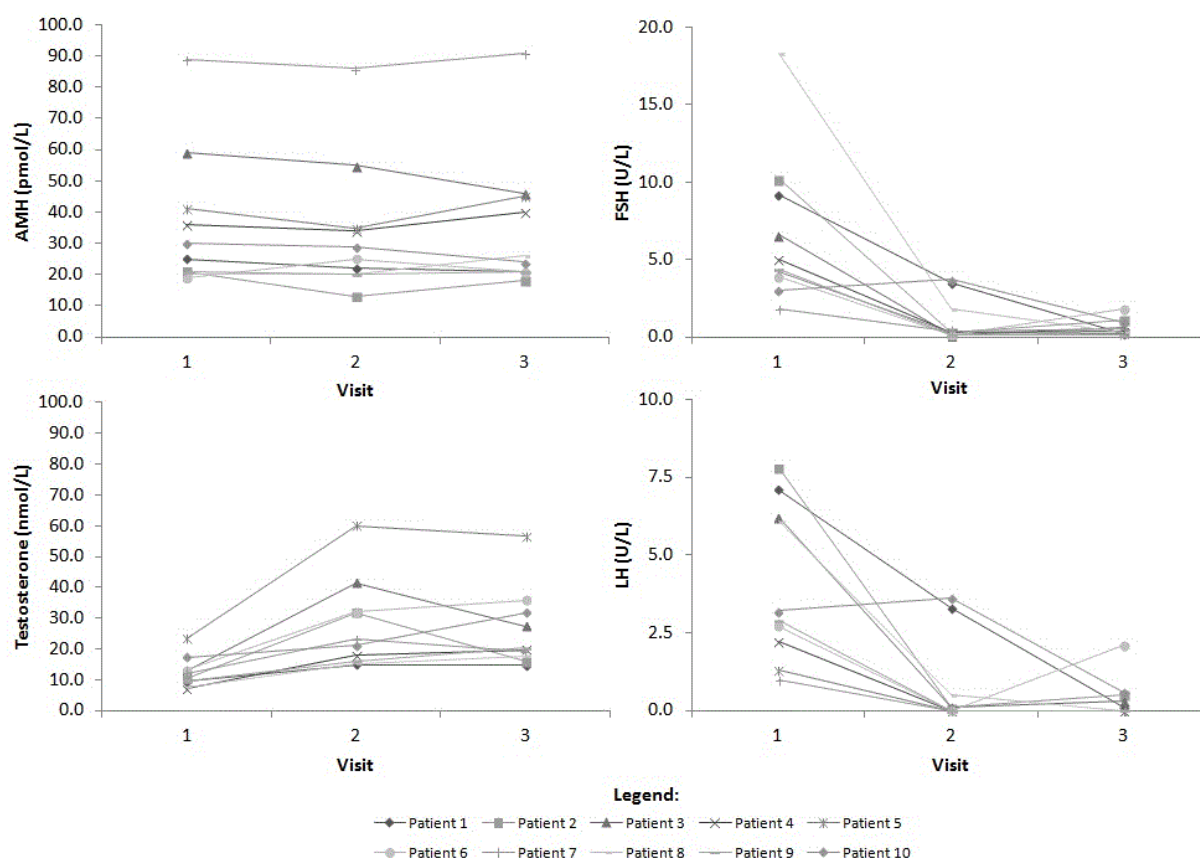


Figure 1: Longitudinal changes in AMH, testosterone, FSH and LH. Visit 1 provides baseline (pre-testosterone) hormone levels. Visit 2 is early testosterone therapy and Visit 3 late (>6 months) on testosterone therapy.

This study did not find any consistent or significant change in AMH levels in a group of men undergoing exogenous androgen therapy for at least 6 months. Compliance with testosterone therapy was confirmed by documenting a significant increase in measured serum testosterone (>1.5-fold rise) and suppression of LH and FSH to <1 U/L in all men. However, despite increased serum testosterone and gonadotrophin suppression in all men, there was no consistent trend of reducing or increasing AMH and no significant change in AMH after therapy. This data is helpful for laboratories reporting AMH values in adult men who are on testosterone therapy.

Factors known to affect AMH levels in adult men

There are strong genetic influences on AMH levels in men after puberty and high interpersonal variation [3,4]. A small diurnal variation of AMH occurs with levels ~5% lower in the early morning (06:00) when testosterone is at its peak, compared to slightly higher AMH at 19:00 [25]. However, AMH is relatively stable during office hours which is when our samples were collected [25]. AMH is negatively correlated with BMI but in 33 men with weight loss 12 months after bariatric surgery, overall there was no significant change in AMH [3,26]. This implies a longer-term effect of obesity of Sertoli cell function and AMH levels. As such, if any short-term changes in BMI did occur in our subjects during the 6-month follow-up these would not be expected to have any impact on AMH levels. Growth

hormone therapy and increased IGF-1 activity has also been associated with reduced AMH levels in the setting of unchanged serum testosterone and FSH levels and increased oestradiol levels [9]. However, the mechanism of the reduced AMH remains unclear. We did not have data on IGF-1 levels in our patients but there is no reason to expect that IGF-1 levels changed in our subjects during this study. It was shown recently that plasma AMH levels are not significantly affected by impaired spermatogenesis per se and are not correlated with FSH [5]. We noted that 3 men in our study with raised FSH levels (9.2, 10.2 and 18.3 U/L) had normal AMH levels of 25, 21 and 20 pmol/L respectively which are within age related reference intervals for AMH [10].

Exogenous testosterone therapy and intra-testicular testosterone levels

Serum LH correlates highly with intra-testicular testosterone and accounts for most of the variation in IT-T [27]. When LH is suppressed, IT-T levels are similarly reduced dramatically [27,28]. Exogenous testosterone therapy has been shown to reduce IT-T to ~2% of control levels by means of LH and FSH suppression [17]. It is expected that our subjects had significant reduction in IT-T given the rise in serum testosterone and suppression of LH and FSH present in each individual.

Relationships between testosterone, IT-T and AMH

During puberty rising IT-T and Sertoli cell AR expression is associated with a decrease in AMH however the precise mechanisms for this decrease are unclear [14]. It might be expected that a falling IT-T would have the opposite effect and result in increased AMH. However, our study demonstrated that this did not occur. A study in 8 adult males by Andreassen et al. [9] supports the concept that circulating concentrations of testosterone may not directly influence AMH levels, as in this study, serum testosterone levels remained the same during a period of Growth hormone therapy which resulted in reduced AMH level.

Does sustained rise in FSH have a role in modulating AMH?

Other studies have similarly found that changing levels of androgens did not affect AMH levels in adult men. An informative study by Eldar-Geva et al. [29] reports treating 10 men with anti-androgens with a subsequent rapid rise in serum testosterone and FSH (as expected) and AMH did not change during this phase of therapy. However, when a GnRH agonist implant was also administered for a year, FSH and testosterone initially fell (again with no change in AMH), but a late and prolonged rise in FSH (from one month onwards with FSH rising back towards baseline levels) with sustained suppression of testosterone, was accompanied by a rise in AMH. The authors concluded that FSH has a role in regulating AMH in the absence of testosterone action [29]. In our study, there was no secondary rise in FSH back towards baseline and this may reflect the different therapies used to achieve FSH suppression, being a GnRH agonist implant in Eldar-Geva's group and exogenous testosterone therapy in our subjects. In another study which included 10 men treated for prostate cancer with GnRH agonist, decreased testosterone and increased AMH levels were reported at 12 months post therapy, however serial FSH and LH levels were not reported [30].

Roth et al. [28] also reported that acute gonadotrophin suppression (over 10 days) resulted in reduced serum testosterone and IT-T and noted that although AMH might be expected to rise in this setting, it did not. They queried whether the AMH assay was reliable and considered whether the lack of FSH stimulation may have also contributed to a lack of AMH rise. They concluded AMH did not significantly change with acute gonadotrophin suppression and was not correlated with IT-T [28]. Our findings confirm those of Roth et al. and provide further evidence that in adult males AMH levels are not affected by changes in serum testosterone or suppression of LH/FSH suppression. However, these findings do not exclude the possibility that longer term rising FSH levels may have an effect on AMH. In support of this concept, Young et al. [31] found that 8 patients with congenital hypogonadotropic hypogonadism who were given recombinant FSH had an increase in their AMH levels.

Terminal differentiation of Sertoli cells.

There are some possible explanations for these findings of a lack of effect of changing testosterone levels on AMH. It is known that Sertoli cell function changes over life span. Immature Sertoli cells are undifferentiated and are able to continue proliferation under the influence of FSH [11]. This may be one of the mechanisms by which FSH levels have an influence on SC numbers and indirectly AMH production. Immature SC's have high expression of AMH but as they mature, SC's become terminally differentiated and largely unable to further proliferate. Alterations in gene transcription and protein

synthesis occur and expression of AMH reduces dramatically [32]. It is possible that terminally differentiated Sertoli cells are unable to further modify AMH production in response to changing IT-T levels.

Mouse androgen receptor knock out model

The mechanism by which rising testosterone may affect AMH levels is unclear as no androgen response element has been found in the regulatory regions of the AMH gene and studies in mice where the AR is knocked out (SCARKO) show that expression of AMH does not change [12,32,33]. It has been suggested that non-genomic or androgen receptor independent pathways for testosterone to suppress AMH may also exist and this seems increasingly likely given the mounting evidence that changing serum testosterone or IT-T testosterone levels in adult men does not affect AMH [29]. It also seems likely that persistently rising FSH levels may have a role in modulating AMH levels in the absence of testosterone, as described by Eldar-Geva [29].

Limitations

The limitations of this study are the small number of subjects studied and further studies of large numbers of individuals on exogenous testosterone therapy would be helpful. It may also be informative to follow men for longer than 6 months to determine if any later changes occur in AMH levels in men on testosterone therapy for 12 months or more. Further, we did not have access to an Inhibin B assay and this would have been informative.

Strengths

This is a community based, un-selected group of men who were presenting to their general practitioners because of androgen deficiency symptoms. As such this group were representative of men being considered for trial of testosterone therapy in the community and provide a useful group in which to assess potential changes to AMH.

Conclusion

The factors controlling AMH levels in men are poorly understood but AMH levels appear to have important implications for men's health. As testosterone therapy is not uncommon in the community it is helpful for laboratories and clinicians to understand if this alters AMH levels. This study showed that rising serum testosterone levels in the setting of exogenous testosterone therapy, together with suppression of LH and FSH, did not result in any significant change in AMH. Factors other than intra-testicular androgens and gonadotrophin suppression, such as FSH, may be implicated in regulation of AMH levels in men and further research to understand the physiological controls of AMH is needed.

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