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Association of Free Testosterone with Hypogonadal Symptoms in Men with Near-Normal Total Testosterone Levels

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Abstract

Objective—To investigate the association between hypogonadal symptoms and free testosterone levels in men with near-normal total testosterone levels (250–350ng/dL) and to determine whether a discriminatory threshold for free testosterone exists below which hypogonadal symptoms become more prevalent.

Methods—We reviewed the charts of 3,167 men who presented to an outpatient men's health clinic. 231 men had symptoms of “low testosterone” and serum testosterone levels between 250 and 350ng/dL. We evaluated hypogonadal symptoms using the ADAM and qADAM questionnaires. Serum levels of total testosterone and SHBG were collected on the same day that men completed their questionnaires. We used linear regression to determine whether or not a threshold of free testosterone exists for hypogonadal symptoms. We performed univariate and multivariable analyses to evaluate factors that predicted a low free testosterone level.

Results—The median age was 43.5 y, and the median testosterone and free testosterone levels were 303ng/dL, and 6.3ng/dL respectively. Prevalence and severity of hypogonadal symptoms (ADAM and qADAM) were similar between men with low (<6.4ng/mL) and normal free testosterone levels. There was an association between age and three of the 10 hypogonadal symptoms (decreased enjoyment in life, sadness, and deterioration of work performance) with a low free testosterone on a univariate analysis. Only younger age was positively associated with free testosterone on multivariable analysis.

Conclusions—We did not observe a relationship between hypogonadal symptoms and free testosterone in men with near-normal testosterone levels.

Symptom-specific free testosterone thresholds could not be defined, as age remains an important confounder.

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Introduction

Free testosterone (FT) is the fraction of the total testosterone (T) that is readily available to the cells and is dependent on the levels of sex hormone binding globulin (SHBG) and albumin.¹ SHBG bound testosterone remains in the circulation with no binding function in this form. Higher total testosterone and lower SHBG levels can increase FT. FT can be diagnostically useful when T does not correspond with the clinical presentation of hypogonadism, especially in aging men with borderline low levels of T and men in whom levels of SHBG are suspected to be altered.² FT can be calculated by the Vermueulen formula using serum T, SHBG, and albumin levels. The use of FT is complicated by the wide range of assays used to determine FT levels and the lack of a standard threshold value.^{1, 3}

Previous studies have analyzed the association of FT and the symptoms of hypogonadism and concluded that FT is associated with hypogonadism at a low level of specificity.⁴⁻⁶ In a multi-center study, more than 3,000 men aged 40 to 79 years answered questionnaires assessing 32 physical, psychological, and sexual symptoms.⁷ Of the 32 symptoms, significant 'syndromic' association with FT was observed for only three sexual symptoms, namely, decreased sexual thoughts, weak morning erections, and erectile dysfunction. Unfortunately, the FT threshold associated with these three symptoms varied widely from 4.5 to 8.0 ng/mL.

The Endocrine Guidelines suggest measurement of free or bioavailable testosterone levels in men in whom total testosterone concentrations are near the lower limit of the normal range.² Therefore, we restricted our analysis to men with near-normal T levels (250 – 350ng/dL) and hypogonadal symptoms. In the present study, we evaluated the association between free T and hypogonadal symptoms and attempted to determine whether there exists a clear-cut discriminatory threshold of free testosterone below which hypogonadal symptoms become more prevalent.

Methods

Following approval by the Institutional Review Board (IRB), we retrospectively reviewed the charts of a total of 3,167 men seen consecutively between May 2013 and July 2014 who complained of symptoms of "low testosterone". Men using testosterone or other androgenic anabolic steroids (AAS) within 6 months of the time of the survey were excluded. We also excluded men who had presented with a primary diagnosis of infertility, Klinefelter syndrome, or secondary hypogonadism after appropriate assessment of history and endocrine evaluation with gonadotropins.

All men answered the Androgen Deficiency in the ADAM and qADAM questionnaires, and their testosterone levels were measured on the same day. The ADAM questionnaire consists of 10 Yes / No questions regarding hypogonadal symptoms. The qADAM questionnaire quantifies the severity of the symptoms on a 1-5 scale (range from 10 – 50).

All venous blood samples were obtained under standardized conditions before 10AM from fasting patients. Serum or plasma was separated at 800 × g. T and SHBG measurements

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were performed using the enzyme immuno-assay assay Beckman Access II platform (Beckman Coulter, Fullerton, CA, USA). FT was calculated on the basis of T and SHBG using the Vermeulen formula³. FT levels were fitted on a scatter plot to determine inflection points and threshold levels. Data was analyzed using Microsoft Excel (Microsoft, Redmond, WA) and SPSS. A Q-Q test was performed to verify that the variables were normally distributed. Univariate and multivariable analyses were performed for age, T, and the 10 symptoms identified on the ADAM questionnaire. Variables that were statistically significant on the univariate analysis were included in the multivariable analysis. All values were reported as median + IQR and t-tests were used to evaluate differences in means between groups. A p-value 0.05 was considered statistically significant.

Results

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Of the 3,167 men, 231 men had a T value between 250 – 350 ng/dL. The median age of the 231 men was 43.5 years. The median T level was 303 ng/dL and the median FT level was 6.3 ng/dL (Table 1). As expected, men with a higher free testosterone (>6.3 ng/dL) were younger (37 vs. 51, $p < 0.001$), had a higher total testosterone (307 vs. 295, $p = 0.02$), and lower SHBG (21 vs. 36, $p < 0.001$). Interestingly, regardless of whether men had low (<6.3) or normal free testosterone levels, they reported similar hypogonadal symptoms (Table 2; as evaluated using ADAM and qADAM scores). Similarly, there was no association between total testosterone levels and hypogonadal symptoms.

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Age and three of the 10 hypogonadal symptoms (the probability of having decreased enjoyment in life, sadness, and deterioration of work performance) were associated with a low FT on a univariate analysis. However, on multivariable analysis, only younger age, and none of the hypogonadal symptoms, was positively associated with higher FT (Table 3). We used locally weighted linear regression to attempt to identify threshold levels of FT below which, the probability of a symptom increased above the background prevalence in the overall study population. Unfortunately, no significant threshold could be established.

Comment

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In evaluating men with suspected hypogonadism, some clinicians start by evaluating total testosterone (T) levels; others evaluate free or bioavailable T levels, which can also identify hypogonadism.⁸ Many factors (including obesity, drugs, comorbidities, assay variability, and time of day) affect total T and free T levels.⁹ Most testosterone circulates tightly bound to sex hormone-binding globulin (SHBG) or weakly bound to albumin. A minor amount circulates as free testosterone, and it is believed that this combined with the fraction bound to albumin is the metabolically active fraction as opposed to the fraction tightly bound to SHBG.

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Free testosterone has been evaluated in two large population-based studies. The Veterans Administration researchers analyzed data from 3700 men (mean age, 60; nearly half obese) in whom evaluation for hypogonadism included measurement of both total T and calculated free T levels.¹⁰ About 15% had low free T levels (<34 pg/mL) and thus were considered to have biochemical hypogonadism. The EMAS study was a multi-center study of more than 3,000 men aged 40 to 79 years who were administered questionnaires assessing 32 physical,

psychological, and sexual symptoms suggested by previous studies to be related to androgen deficiency.⁷ Of the 32 symptoms, significant ‘*syndromic*’ association was observed for only three sexual symptoms, namely, decreased sexual thoughts, weak morning erections, and erectile dysfunction. The authors concluded that a diagnosis of syndromic late-onset hypogonadism can be made in a man with these three sexual symptoms *and* serum total testosterone level <230 ng/dL or serum total testosterone level between 230 to 317 ng/dl *and* free testosterone level <7 ng/mL (220 pmol/L). In fact, free testosterone thresholds for the three sexual symptoms (decreased frequency of morning erection, decreased frequency of sexual thoughts, and erectile dysfunction) were 160, 280, and 280 pmol per liter (46, 81, and 81 pg per milliliter), respectively. No thresholds were identified for either psychological or physical symptoms associated with free testosterone.

Like the EMAS study, we wanted to determine whether there exists a threshold for free testosterone at which symptoms of hypogonadism would become more prevalent. In addition, we wanted to identify a cluster of symptoms that would be more prevalent in men with low free T. However, we identified neither a threshold nor a cluster of symptoms that were more prevalent in men with low free T. This finding is not surprising given that studies with both total¹¹ and free testosterone^{4, 12} have failed to demonstrate an association between serum levels and symptoms. These non-findings suggest that there may be another mechanism influencing sexual symptoms outside of total and bioavailable testosterone levels which may include factors such as androgen receptor binding affinity and strength of downstream response. Despite being validated, the questionnaires currently used in the diagnosis of hypogonadism such as MMAS, ADAM and NERI have poor specificity for the diagnosis of low testosterone.¹³

Our study has both strengths and limitations. We had a relatively small sample size because we restricted our analysis of free testosterone to men who had “near normal” testosterone levels, i.e 250 - 350 ng/dL. We restricted our sample size to follow recommendations suggested by the Endocrine Society guidelines.² The guidelines suggest evaluating FT in men with equivocal total testosterone. We also included only men who had at least two separate serum hormone assessments as suggested by the guidelines, who had not received testosterone therapy in the last six months, and who presented to the men's health clinic with the chief complaint of “low testosterone” or “symptoms of hypogonadism. We did not evaluate factors such as obesity, comorbidities, or medications that could have influenced total and free testosterone levels.

In summary, we found that only total testosterone and SHBG are positively associated with declining free testosterone levels in men with near-normal total testosterone levels. There is neither a clear-cut threshold of free testosterone nor a cluster of symptoms that identifies men with low free testosterone levels. The true value of free testosterone testing and evaluation remains to be identified in larger controlled studies.

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References

1. Moreno SA, Shyam A, Morgentaler A. Comparison of free testosterone results by analog radioimmunoassay and calculated free testosterone in an ambulatory clinical population. *The journal of sexual medicine*. 2010; 7:1948–1953. [PubMed: 19732301]
2. Bhasin S, Cunningham GR, Hayes FJ, et al. Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. *The Journal of clinical endocrinology and metabolism*. 2010; 95:2536–2559. [PubMed: 20525905]
3. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocr Metab*. 1999; 84:3666–3672. [PubMed: 10523012]
4. Kelleher S, Conway AJ, Handelsman DJ. Blood testosterone threshold for androgen deficiency symptoms. *The Journal of clinical endocrinology and metabolism*. 2004; 89:3813–3817. [PubMed: 15292310]
5. Zitzmann M, Faber S, Nieschlag E. Association of specific symptoms and metabolic risks with serum testosterone in older men. *The Journal of clinical endocrinology and metabolism*. 2006; 91:4335–4343. [PubMed: 16926258]
6. Araujo AB, Esche GR, Kupelian V, et al. Prevalence of symptomatic androgen deficiency in men. *The Journal of clinical endocrinology and metabolism*. 2007; 92:4241–4247. [PubMed: 17698901]
7. Wu FC, Tajar A, Beynon JM, et al. Identification of late-onset hypogonadism in middle-aged and elderly men. *The New England journal of medicine*. 2010; 363:123–135. [PubMed: 20554979]
8. Shea JL, Wongt PY, Chen Y. Free testosterone: clinical utility and important analytical aspects of measurement. *Advances in clinical chemistry*. 2014; 63:59–84. [PubMed: 24783351]
9. Cardarelli R, Singh M, Meyer J, Balyakina E, Perez O, King M. The Association of Free Testosterone Levels in Men and Lifestyle Factors and Chronic Disease Status: A North Texas Healthy Heart Study. *Journal of primary care & community health*. 2014; 5:173–179.
10. Anawalt BD, Hotaling JM, Walsh TJ, Matsumoto AM. Performance of total testosterone measurement to predict free testosterone for the biochemical evaluation of male hypogonadism. *The Journal of urology*. 2012; 187:1369–1373. [PubMed: 22341266]
11. Lackner JE, Rucklinger E, Schatzl G, Lunglmayr G, Kratzik CW. Are there symptom-specific testosterone thresholds in aging men? *BJU international*. 2011; 108:1310–1315. [PubMed: 21231990]
12. Cabral RD, Busin L, Rosito TE, Koff WJ. Performance of Massachusetts Male Aging Study (MMAS) and androgen deficiency in the aging male (ADAM) questionnaires in the prediction of free testosterone in patients aged 40 years or older treated in outpatient regimen. *The aging male : the official journal of the International Society for the Study of the Aging Male*. 2014; 17:147–154.
13. Bernie AM, Scovell JM, Ramasamy R. Comparison of questionnaires used for screening and symptom identification in hypogonadal men. *The aging male : the official journal of the International Society for the Study of the Aging Male*. 2014:1–4.

Table 1

Baseline Characteristics of hypogonadal men with serum total testosterone between 250 and 350ng/dL. Values represented are median +/- IQR.

n	231
Age	43.5 (34.96,57.60)
Total testosterone (ng/dL)	303 (269.5,323.75)
Free Testosterone	6.3 (5.27,7.395)
Estradiol (ng/dL)	2 (2,3)
SHBG (nmol/L)	27 (21,36)
qADAM (range 10-50)	32 (29,37)
ADAM (range 1-5)	4 (1,7)

Table 2

Comparison of characteristics between men with low and normal free testosterone.

	Free T < 6.5 pg/mL	Free T > 6.5 pg/mL	p-value
n	116	115	-
Age	51.08 (38.84,60.71)	37.76 (33.22,48.22)	<0.001
Total testosterone (ng/dL)	295.5 (264.25,322.25)	307 (287.5,327)	0.026
Free Testosterone	5.27 (4.89,6.01)	7.395 (6.72,8.21)	<0.001
Estradiol (ng/dL)	2 (2,2.5)	2 (2,3)	0.674
SHBG (nmol/L)	36 (30,44)	21 (18,26)	<0.001
ADAM	32.5 (29,38)	31 (28.75,36)	0.088
qADAM	4 (1,6)	4 (2,7)	0.289

Univariate and multivariable analysis of hypogonadal symptoms predicting a free testosterone (analyzed as a continuous variable). Only factors that were statistically significant ($p<0.05$) on univariate analysis were included in the multivariable analysis.

Table 3

Univariate analysis	Beta	Std Error	95% CI	P-value
Age	-0.46	0.2789	-0.057 – -0.034	<0.001
Do you have decreased libido?	0.181	0.1815	-0.175 – 0.537	0.318
Do you have a lack of energy?	0.273	0.1816	-0.083 – 0.629	0.132
Do you have a decrease in strength or endurance?	0.232	0.1753	-0.112 – 0.575	0.186
Have you noticed a decreased “enjoyment in life”?	0.427	0.1801	0.074 – 0.780	0.018
Are you sad and/or grumpy?	0.617	0.1794	0.265 – 0.968	0.001
Are your erections less strong?	0.212	0.1822	-0.145 – 0.569	0.245
Has there been a recent deterioration in your work performance?	0.772	0.2007	0.378 – 1.165	<0.001
Are you falling asleep after dinner?	0.246	0.1786	-0.103 – 0.596	0.167
Have you noticed a recent deterioration in your ability to play sports?	0.092	0.1790	-0.259 – 0.443	0.607
Have you lost height?	-0.480	0.2679	-1.009 – 0.048	0.075

Multivariable analysis	Beta	Std Error	95% CI	P-value
Age	-0.040	0.0071	-0.053 – -0.026	<0.001
Have you noticed a decreased “enjoyment in life”?	0.293	0.2106	-0.119 – 0.706	0.163
Are you sad and/or grumpy?	0.356	0.2137	0.775 – 2.782	0.095
Has there been a deterioration in your work performance?	0.167	0.2447	-0.312 – 0.467	0.494