Published in final edited form as:
Mult Scler. 2014 October ; 20(12): 1584–1592. doi:10.1177/1352458514527864.

Low testosterone is associated with disability in men with multiple sclerosis

R. Bove, MD[1,2,3], **A. Musallam, MPH**[1], **B.C. Healy, PhD**[1,2,4], **K. Raghavan, BA**[2], **B.I. Glanz, PhD**[1,2], **R. Bakshi, MD**[1,2], **H. Weiner, MD**[1,2,3], **P.L. De Jager, MD PhD**[1,2,3], **K.K. Miller, MD**[2,5], and **T. Chitnis, MD**[1,2,3]

[1]Partners Multiple Sclerosis Center, Department of Neurology, Brigham and Women's Hospital, Brookline, MA 02445

[2]Harvard Medical School, Boston, MA 02115

[3]Center for Neurologic Diseases, Harvard Medical School, 77 Avenue Louis Pasteur, NRB168, Boston, MA 02115, USA

[4]Massachusetts General Hospital Biostatistics Center, Boston, MA 02114

[5]Neuroendocrine Unit, Massachusetts General Hospital, Boston, MA 02114

Abstract

Background—Gonadal steroids may modulate disease course in multiple sclerosis (MS).

Objective—To assess the prevalence and clinical associations of hypogonadism in MS men.

Methods—Males, aged 18-65 years, with relapsing remitting MS or CIS and first symptom <10 years prior, were selected from a longitudinal clinical study, Hormones were measured in stored morning blood samples. Expanded Disability Status Scale (EDSS) scores were collected every 6 months, and Symbol Digit Modalities test (SDMT) annually.

Results—The analysis included 96 men with a mean age of 40 years, EDSS of 1.1, and disease duration of 4.6 years. Of these men, 39% were hypogonadal (total testosterone <288 ng/dL); none showed compensatory elevations in luteinizing hormone. Low testosterone levels and testosterone:estradiol ratios were negatively correlated with body mass index and leptin, and showed no correlation with 25-hydroxyvitamin D levels. In the primary cross-sectional analyses, there was a negative age-adjusted correlation between total testosterone and EDSS (p=0.044). In

Corresponding Author: Tanuja Chitnis M.D., Partners Multiple Sclerosis Center, 1 Brookline Place, Suite 602, Brookline, MA 02445, USA, Phone: 617-525-6550, Fax: 617-5-732-6466, tchitnis@rics.bwh.harvard.edu.
rbove@partners.org, amusallam@partners.org, bchealy@partners.org, kraghavan@partners.org, bglanz@partners.org, rbakshi@bwh.harvard.edu, hweiner@rics.bwh.harvard.edu, pdejager@partners.org, kkmiller@partners.org

Disclosures: Dr. Bove reports no disclosures. A. Musallam reports no disclosures. Dr. Healy has received research support from Merck Serono and Novartis. K. Raghavan reports no disclosures. Dr. Glanz has received research support from Merck Serono. Dr. Bakshi reports no disclosures. Dr. Weiner has served as a consultant for Teva Neurosciences, Novartis, Biogen Idec, EMD-Serono, and has received research support from Merck Serono. Dr. De Jager has served as a consultant for Teva Neurosciences, Merck Serono and Biogen-Idec and has received research support from Biogen-Idec. Dr. Miller reports no disclosures. Dr. Chitnis has served as a consultant for Biogen-Idec, Sanofi Aventis, Novartis, EMD-Serono, and Teva Neurosciences, and received grant support from Merck-Serono and Novartis for unrelated activities.
The authors report no competing interests.

the age-adjusted longitudinal analyses, higher baseline testosterone levels were associated with less decline in SDMT (p=0.012).

Conclusion—Men with MS may experience hypogonadotropic hypogonadism. Low testosterone levels may be associated with worse clinical outcomes. A potential neuroprotective role for testosterone warrants further investigation.

Keywords

testosterone; multiple sclerosis; leptin; BMI; cognition

INTRODUCTION

Men may be three times less likely to develop multiple sclerosis (MS) than women, but they are more likely to display progressive forms of the disease and to display brain atrophy, disability and cognitive impairment, irrespective of disease type [1, 2]. Cognitive impairment is one of the major factors associated with unemployment in persons with MS and may affect function in all spheres of daily living [3].

Gonadal hormones may mediate sex-related differences in inflammation and neurodegeneration in MS [4]. Age-related declines in male testosterone levels [5] have been hypothesized to partially explain the later onset of MS relative to women. While some studies have found decreased levels of testosterone in men with MS relative to healthy controls [6-8], these studies had important limitations, including variable definitions of hypogonadism, lack of control for confounding introduced by recent steroid use, circadian variation of testosterone levels, and the inclusion of men with advanced disease, in whom hypogonadism may be caused by chronic disease [9].

In this study, we hypothesized that a significant subgroup of men with MS have low testosterone levels and that these levels are associated with disease severity. We first characterized testosterone levels early in the disease course of a large cohort of men with MS. Second, we investigated the correlation between testosterone and other endocrine modulators of MS, both known (vitamin D) [10] and exploratory (body mass index, leptin)[11, 12]. Finally, we determined the association between baseline testosterone levels and both cross-sectional and longitudinal disability measures, including clinical disability and cognitive outcomes.

METHODS

Subjects

The subjects included in this study were male patients of the Partners MS Center, aged 18-65, enrolled in the Comprehensive Longitudinal Investigation of Multiple Sclerosis at the Brigham and Women's Hospital (CLIMB)[13]. Over 2,000 patients have enrolled since 2000, and are followed longitudinally with annual standardized clinical exams, magnetic resonance imaging scans (MRIs) and stored blood samples. Patients selected for this study met the diagnostic criteria of relapsing remitting MS (RRMS) by the 2005 McDonald

criteria [14] or clinically isolated syndrome (CIS), and had at least 2 years of clinical follow up available after a blood sample.

Standard Protocol Approvals, Registrations, and Patient Consents

Institutional Review Board approval was granted by the Partners Human Research Committee.

Hormonal and metabolic measures

Stored blood samples were selected if they had been drawn between 0830 and 1130 to minimize diurnal variability, and at least 30 days from exposure to exogenous glucocorticoids. In order to minimize the confounding caused by deleterious effects of chronic disease on testosterone levels [15], only samples drawn within 10 years of first symptom onset were included. If several samples meeting these criteria existed for a given individual, one sample per individual was selected according to the following priorities: shortest disease duration, earliest time of day, and greatest volume of specimen remaining for subsequent CLIMB analyses. According to CLIMB protocol, all samples were stored at −80° C following their collection. All samples were processed in the Harvard Catalyst Clinical laboratory.

Plasma was used for some hormonal analyses and serum for others, to maximize the volume of samples available for analysis. Testosterone, estradiol, luteinizing hormone (LH) and 25-hydroxyvitamin D were assessed from plasma samples. Plasma testosterone, estradiol and LH levels were measured using Access Chemiluminescent Immunoassay (Beckman Coulter, Fullerton, CA). The lower limit of detection of testosterone was 10 ng/dL, of estradiol was 20 pg/mL, and of LH was 0.2 mIU/mL. Testosterone levels are marginally lower in plasma than serum (manufacturer assay 95% reference range 168-758 vs. 175-781 ng/dL, both with median 384 ng/dL). Plasma 25-hydroxyvitamin D levels were measured using a radioimmunoassay (Diasorin, Inc, Sillwater, MN). The lower limit of detection was 1.5 ng/dL. Sex hormone binding globulin (SHBG) and leptin were assessed from serum samples. SHBG levels were measured using Access Chemiluminescent Immunoassay (Beckman Coulter, Fullerton, CA). The lower limit of detection was 0.33 nmol/L. Leptin levels were measured using a radioimmunoassay (Millipore, St. Charles, MO). The lower limit of detection was 0.1 ng/mL.

Free testosterone was calculated from total testosterone and SHBG using the laws of mass action [16], and free androgen index was calculated using 100(testosterone)/SHBG [16]. The ratio of testosterone to estradiol levels was calculated, as a potential measure of anti- vs. proinflammatory effects [7].

Height and weight measurements were available in a subset (N=84), and body mass index (BMI, kg/m^2) was calculated. Mean BMI for these individuals was 28.4 kg/m^2 (SD 4.8); mean BMI for a subset of 56 individuals with BMI data within 6 months of blood draw was 28.8 kg/m^2 (SD 4.5).

Clinical outcomes

Two primary outcomes were investigated. Clinical disability was measured using the Expanded Disability Status Scale (EDSS[17]). The Symbol Digit Modalities Test, a test of executive function and processing speed and a sensitive early marker of longitudinal cognitive changes in MS,[18, 19] was performed annually in a subgroup of these subjects enrolled in a detailed CLIMB quality of life and cognitive substudy. In these subjects, depression was measured using the Center for Epidemiologic Studies Depression scale (CES-D). The mean (SD) CES-D score was 8.5 (6.9) and 14% scored 16 or above (the cutoff for depression).

Follow up data were available for an average of 3.7 years for EDSS and 2.7 years for SDMT.

Statistical Analysis

Variable processing—To ensure normality, leptin values were log-transformed. To control for seasonal variability in 25-hydroxyvitamin D levels, in line with previous analyses [20], 25-hydroxyvitamin D levels were regressed on the periodic function $-\sin(2\pi X/12) - \cos(2\pi X/12)$, where X is month of sample collection. The residuals from this model were added to mean 25-hydroxyvitamin D from the entire sample to create an adjusted 25-hydroxyvitamin D measurement. When estradiol levels were less than detectable (<20pg/mL, N=10) a value of 19 pg/mL was used.

Analyses—In order to examine associations between androgen measures and other hormones, Pearson correlation coefficients were calculated, and partial Pearson correlation coefficients adjusting for age were also calculated.

The pre-specified primary cross-sectional analysis was the correlation between total testosterone and EDSS at the same visit date (using partial Spearman correlations adjusting for age). Secondarily, we examined the cross-sectional correlation between total testosterone and SDMT (using partial Pearson correlations adjusting for age). Exploratory analyses were run for the other androgen variables (free testosterone and testosterone:estradiol ratio) using similar models. Since testosterone was correlated with both leptin and BMI, in order to determine whether testosterone exerts effects on outcomes independent of its association with adiposity, thepartial correlation coefficients were also calculating adjusting for age and leptin.

The longitudinal analyses examined the association between baseline testosterone levels and longitudinal changes in EDSS and SDMT, using generalized estimating equations [21]. For each outcome and predictor pair, the mean change in each outcome was assumed to be linear with time, and the focus of the analysis was the effect of the baseline testosterone measurement on the change over time. Each model was fit assuming an identity link function and an exchangeable working correlation structure, and robust standard errors were used to calculate p-values. When the GEE model for the EDSS using the proportional odds link, the conclusions were the same as those reported below. Exploratory analyses for the

other androgen variables (free testosterone and testosterone:estradiol ratio) were performed using similar models.

RESULTS

Clinical and demographic characteristics

At the time of sampling, subjects had a mean age of 39.8 years, a mean disease duration of 4.6 years since their first symptom, and a low EDSS (mean 1.07) (Table 1). Their mean hormonal measures are summarized in Supplementary Table 1.

Testosterone levels early in MS course

When we assessed the prevalence of hypogonadism in our sample, 39% of subjects had testosterone levels below 10 nmol/L (288 ng/dL). This threshold representsthe lower limit of the normal range for total testosterone level in healthy young men (280-300 ng/dL), below which symptoms of hypogonadism are more commonly seen in clinical practice,[22,23] and a commonly used cutoff for hypogonadism in research settings.[24, 25] When partitioning subjects by age, hypogonadism was observed across all age categories (Table 2). Among the 39% of men who were hypogonadal, all had luteinizing hormone (LH) levels that were either low (22%; <1.24 mIU/mL) or normal (78%; 1.24-8.62 mIU/mL), suggesting hypogonadotropic, i.e. central, hypogonadism. When we further assessed possible confounders of testosterone levels, neither treatment status, smoking status nor depression score at time of the blood sample had a significant effect on testosterone levels ($p>0.15$ for all)

Testosterone correlations with other hormonal markers

Other hormonal (vitamin D) and metabolic (BMI, leptin) markers have been associated with MS susceptibility or outcomes in MS [10-12], and testosterone levels have been associated with these markers in healthy populations [26-28]. We therefore decided to assess the association between testosterone and these markers in our subjects with MS. We found negative partial Pearson correlations between baseline testosterone levels and both leptin ($r= -0.23$, $p=0.03$) and BMI ($r= -0.23$, $p= 0.04$). There were similar negative partial correlations between the testosterone:estradiol ratio and both leptin ($r=-0.27$, $p=0.01$) and BMI ($r=-0.33$, $p=0.003$) (Supplementary Table 2). There was no correlation between any androgen measure and 25-hydroxyvitamin D levels.

Testosterone associations with disease outcome measures

Cross-sectional outcomes—In our primary analyses assessing the cross-sectional relationship between testosterone levels and disease outcome measures, lower testosterone levels were correlated with higher EDSS (partial $r=-0.21$, $p=0.04$). When we controlled for disease duration there was no change in the significance of this association. Testosterone levels were not correlated with SDMT ($p=0.32$) (Table 3a).

Given the correlation between testosterone and adiposity measures (leptin and BMI), we further sought to determine whether the association between testosterone and EDSS was independent of adiposity, by performing additional partial Spearman correlations adjusting

both for age and for leptin. Leptin was chosen over BMI because of the larger number of individuals contributing leptin measures to the model, and because leptin is a marker of adiposity, whereas BMI can be increased both by adiposity and muscle mass. The p-value for the correlation between testosterone and EDSS in age- and leptin-adjusted models was 0.092.

In secondary analyses, there was a negative correlation between the testosterone:estradiol ratio and EDSS (age-adjusted r=−0.26 and p=0.013; age- and leptin-adjusted r=−0.22, p=0.034). The negative correlation between free testosterone and EDSS (r=−0.27, p=0.010) was only suggestive after adjusting for age (r=−0.20, p=0.051) and for age and leptin (r=−0.19, p=0.066).

Longitudinal outcomes—In our analyses assessing the relationship between baseline total testosterone and longitudinal clinical changes (EDSS and SDMT), lower baseline testosterone levels were associated with worse course in terms of SDMT (age-adjusted, p=0.012) but not in terms of EDSS (p=0.125). When we controlled for disease duration, as well as for treatment status with disease modifying therapies, there was no change in the significance of this association.

Given the cross-sectional correlation between baseline testosterone levels and EDSS, to assess whether longitudinal SDMT changes could be attributed to a higher baseline EDSS and not to lower testosterone levels, EDSS was included in an additional model, with no change in the association between baseline testosterone levels and longitudinal SDMT changes. In secondary analyses, lower free testosterone levels were also associated with greater change in SDMT (p=0.011) (Table 3b).

DISCUSSION

In this investigation of testosterone levels early in disease course in men with RRMS and CIS, there was a high prevalence of hypogonadism in our subjects (39%) across all age categories, all of whom exhibited inappropriate gonadotropic responses that suggested central hypogonadism. Clinically, lower testosterone levels were associated with both increased EDSS at baseline and greater longitudinal changes in SDMT.

The large proportion of men with MS observed to have testosterone levels below 288 ng/dL supports findings from some smaller studies [4, 6-8]. An important limitation of this paper is the lack of a healthy control reference group with testosterone levels measured using the same assay. Nonetheless, data from the literature do provide important comparisons. In contrast to the high prevalence of hypogonadism in our MS sample (over 40% of males aged 18-40 years) in a healthy reference cohort of 456 males aged 18-40 years from the Framingham Heart StudyGeneration 3, with total testosterone level measured using liquid chromatography tandem mass spectrometry, the 2.5th percentile (below which males are typically considered hypogonadal in reference populations) was 348 ng/dL, which corresponds to our mean value for all subjects in this age range of 349 ng/dL. The 1st percentile was 282 ng/dL.[15] Similarly, the reference range for healthy men aged 20-40 in another study was 10.6-31.9 nmol/L (305-919 ng/dL) [29]. In a healthy cohort of 394 much

older males, aged 70-89 years, the 5th percentile for total testosterone levels was 222 ng/dL and the 25th percentile was 314 ng/dL [30]. Studies of testosterone levels in males with other autoimmune disease such as systemic lupus erythematosis and rheumatoid arthritis, have suggested that low testosterone levels may be present even close to disease onset [9] and thus may be implicated not only as a consequence of, but also as a risk marker for, autoimmune disease. Given that testosterone levels may decline in men with chronic diseases [15], and that there may be a period of vague symptoms and high healthcare utilization prior to any formal MS symptoms or diagnosis [31], our cross-sectional data do not permit a determination of causality. In fact, it is possible that other factors such as even remote use of glucocorticoids beyond 30 days from blood draw, or MS comorbidities such as depression, suppress gonadal activity to some extent. We attempted to address this possibility by including men with early MS (mean time from symptom onset of 4.6 years), low EDSS (mean of 1), and no recent glucocorticoid administration. To more fully address the possibility that hypogonadotropic hypogonadism is a risk factor for MS, longitudinal assessments of stored testosterone levels in pre-symptomatic populations are necessary.

To our knowledge, this is the first observational study to report an association of low baseline testosterone levels with disease severity, and with longitudinal changes in cognition. Mechanistically, testosterone has both an anti-inflammatory and a neuroprotective role in animal models. Putative neuroprotective mechanisms include (1) its ability to cross the blood brain barrier in its free form and directly influence neuronal cells; (2) protection of spinal cord neurons from glutamate toxicity; (3) protection from oxidative stress in neuronal cell lines; and (4) an increase in the expression of neurotrophic factors such as brain derived neurotrophic factor (BDNF) [4]. Ongoing investigations appear to support a protective role of testosterone treatment against cognitive decline in healthy aging men. It is important to note, first, that these effects have been seen when individuals were given dihydrotestosterone, which unlike testosterone cannot be converted via aromatase to estradiol, supporting a direct androgenic effect, and second, that there may be an optimal therapeutic window.[32] In MS, due to neurodegenerative, hippocampal-dependent cognitive deficits that may be shared with normal cognitive aging [33], testosterone treatment could be hypothesized to increase hippocampal synapses and thereby slow the rate of cognitive decline. In one small pilot clinical trial of 10 men with RRMS aged <65 years, treatment with testosterone gel for 12 months was associated with improved cognition, slowing of brain atrophy [34], and changes in immunologic reactivity [35].

In secondary analyses, we also found a negative association between the testosterone:estradiol ratio and disease activity. Both testosterone and estrogens have been implicated in MS, with estrogens having carrying pro- or anti-inflamatory effects at different concentrations, and with estriol appearing to have more potent anti-inflammatory effects than estradiol [4]. Both testosterone and estradiol are associated with disease activity on MRI [7], and the testosterone:estradiol ratio may represent a ratio of anti-versus pro-inflammatory activity. Exploration of different forms of estrogens and androgens may shed light on a possible optimal balance of hormones in neuroprotection in MS.

The negative association between testosterone and adiposity in this study parallels findings from healthy populations [36]. The potential modulatory role of metabolic factors (e.g.

adolescent obesity [11]), and metabolic signaling hormones (e.g leptin, adiponectin [12]) on MS susceptibility is an area of active investigation. Leptin, an appetite signaling hormone produced primarily in adipocytes, may act as a powerful pro-inflammatory cytokine that promotes Th1 responses on one side and inhibits regulatory T cell expansion on the other, increasing MS disease risk [12]. Thus, the observed association between testosterone and adiposity markers was expected. However, the fact that inverse associations between testosterone levels and EDSS remained suggestive after controlling for leptin levels, a marker of adiposity, supports a relationship between T levels and MS disease severity independent of adiposity, i.e. that we were not simply observing an effect of increased adiposity in sedentary patients to decrease testosterone levels.

In this study, we did not find any association between vitamin D and either testosterone or the disease outcomes examined. Vitamin D deficiency has emerged as a risk factor for MS, and low vitamin D levels may influence disease course[10, 37]. An interaction between vitamin D and estradiol has been noted [38], but, to our knowledge, no studies have examined the relationship between testosterone and vitamin D in MS.

This study is the largest, to our knowledge, to explore androgen levels in men with MS, and to control for many important variables. Nonetheless, there were several important limitations. First, we were likely underpowered to detect an association between testosterone and EDSS independent of adiposity, particularly given the low EDSS scores early in disease course.Second, we believe that interpretation of our findings warrants further validation given our small sample size, the fact that our mean SDMT scores did not decline as expected from a prior CLIMB cohort study [19], and the fact that the association between testosterone and SDMT change only became significant in later years. Others have suggested that SDMT may be sensitive even in early MS [39]. Nonetheless, it is possible that individuals in this cohort manifested either strong practice effects, or greater cognitive reserve, than expected. The limited range of disability in our subjects may also have contributed. Further work is required to assess the association between testosterone and MRI outcomes in male MS patients.

In summary, our observation that men with MS have high rates of hypogonadism early in disease course, and that this hypogonadism is associated with more significant declines in cognitive function. These results suggest that testosterone treatment may potentially have a higher benefit:risk ratio in MS patients relative to a healthy population [22].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors are grateful to the patients participating in the CLIMB study for their contributions to MS research, to Mariann Polgar-Turcsanyi MS, for her role in managing the Partners MS Center research database, and to Eda Gjika for her assistance with sample preparation.

Study Funding: This research was supported by the National Multiple Sclerosis Society RG-4256A4/2 (TC), and the National Multiple Sclerosis Society/American Brain Foundation Clinician Scientist Award FAN 1761-A-1 (RB). This work was conducted with support from Harvard Catalyst | The Harvard Clinical and Translational

Science Center (National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health Award 8UL1TR000170-05 and financial contributions from Harvard University and its affiliated academic health care centers). The content is solely the responsibility of the authors and does not necessarily represent the official views of Harvard Catalyst, Harvard University and its affiliated academic health care centers, or the National Institutes of Health.

REFERENCES

1. Bove R, Chitnis T. Sexual disparities in the incidence and course of MS. Clin Immunol. 2013
2. Savettieri G, Messina D, Andreoli V, et al. Gender-related effect of clinical and genetic variables on the cognitive impairment in multiple sclerosis. J Neurol. 2004; 251:1208–14. [PubMed: 15503099]
3. Shawaryn MA, Schiaffino KM, LaRocca NG, Johnston MV. Determinants of health-related quality of life in multiple sderosis: the role of illness intrusiveness. Mult Scler. 2002; 8:310–8. [PubMed: 12166502]
4. Gold SM, Voskuhl RR. Estrogen and testosterone therapies in multiple sclerosis. Progress in brain research. 2009; 175:239–51. [PubMed: 19660660]
5. Anawalt BD, Merriam GR. Neuroendocrine aging in men. Andropause and somatopause. Endocrinol Metab Clin North Am. 2001; 30:647–69. [PubMed: 11571935]
6. Safarinejad MR. Evaluation of endocrine profile, hypothalamic-pituitary-testis axis and semen quality in multiple sclerosis. Journal of neuroendocrinology. 2008; 20:1368–75. [PubMed: 19094084]
7. Tomassini V, Onesti E, Mainero C, et al. Sex hormones modulate brain damage in multiple sclerosis: MRI evidence. J Neurol Neurosurg Psychiatry. 2005; 76:272–5. [PubMed: 15654051]
8. Wei T, Lightman SL. The neuroendocrine axis in patients with multiple sclerosis. Brain. 1997; 120(Pt 6):1067–76. [PubMed: 9217689]
9. Bove RM. Autoimmune diseases and reproductive aging. Clinical Immunology. 2013
10. Munger KL, Ascherio A. Prevention and treatment of MS: studying the effects of vitamin D. Mult Scler. 2011; 17:1405–11. [PubMed: 21998006]
11. Munger KL, Chitnis T, Ascherio A. Body size and risk of MS in two cohorts of US women. Neurology. 2009; 73:1543–50. [PubMed: 19901245]
12. Matarese G, Carrieri PB, Montella S, De Rosa V, La Cava A. Leptin as a metabolic link to multiple sclerosis. Nat Rev Neurol. 2010; 6:455–61. [PubMed: 20606678]
13. Gauthier SA, Glanz BI, Mandel M, Weiner HL. A model for the comprehensive investigation of a chronic autoimmune disease: the multiple sclerosis CLIMB study. Autoimmun Rev. 2006; 5:532–6. [PubMed: 17027888]
14. Polman CH, Reingold SC, Edan G, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the “McDonald Criteria”. Ann Neurol. 2005; 58:840–6. [PubMed: 16283615]
15. Bhasin S, Pencina M, Jasuja GK, et al. Reference ranges for testosterone in men generated using liquid chromatography tandem mass spectrometry in a community-based sample of healthy nonobese young men in the Framingham Heart Study and applied to three geographically distinct cohorts. J Clin Endocrinol Metab. 2011; 96:2430–9. [PubMed: 21697255]
16. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab. 1999; 84:3666–72. [PubMed: 10523012]
17. Kurtzke J. Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS). Neurology. 1983; 33:1444–52. [PubMed: 6685237]
18. Amato MP, Portaccio E, Goretti B, et al. Relevance of cognitive deterioration in early relapsing-remitting MS: a 3-year follow-up study. Mult Scler. 2010; 16:1474–82. [PubMed: 20729256]
19. Glanz BI, Healy BC, Hviid LE, Chitnis T, Weiner HL. Cognitive deterioration in patients with early multiple sclerosis: a 5-year study. J Neurol Neurosurg Psychiatry. 2012; 83:38–43. [PubMed: 21746743]
20. Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. Jama. 2006; 296:2832–8. [PubMed: 17179460]

21. Liang KY, Zeger SL. Longitudinal data analysis using generalized linear models. Biometrika. 1986; 73:13–22.
22. Bhasin S, Basaria S. Diagnosis and treatment of hypogonadism in men. Best Pract Res Clin Endocrinol Metab. 2011; 25:251–70. [PubMed: 21397197]
23. Petak SM, Nankin HR, Spark RF, Swerdloff RS, Rodriguez-Rigau LJ. American Association of Clinical Endocrinologists Medical Guidelines for clinical practice for the evaluation and treatment of hypogonadism in adult male patients--2002 update. Endocr Pract. 2002; 8:440–56. [PubMed: 15260010]
24. Bobjer J, Naumovska M, Giwercman YL, Giwercman A. High prevalence of androgen deficiency and abnormal lipid profile in infertile men with non-obstructive azoospermia. Int J Androl. 2012; 35:688–94. [PubMed: 22519695]
25. Carrero JJ, Qureshi AR, Nakashima A, et al. Prevalence and clinical implications of testosterone deficiency in men with end-stage renal disease. Nephrol Dial Transplant. 2011; 26:184–90. [PubMed: 20624775]
26. Nimptsch K, Platz EA, Willett WC, Giovannucci E. Association between plasma 25-OH vitamin D and testosterone levels in men. Clin Endocrinol (Oxf). 2012
27. Lee DM, Tajar A, Pye SR, et al. Association of hypogonadism with vitamin D status: the European Male Ageing Study. Eur J Endocrinol. 2012; 166:77–85. [PubMed: 22048968]
28. Michalakis K, Mintziori G, Kaprara A, Tarlatzis BC, Goulis DG. The complex interaction between obesity, metabolic syndrome and reproductive axis: a narrative review. Metabolism. 2013; 62:457–78. [PubMed: 22999785]
29. Neale SM, Hocking R, Biswas M, et al. Adult testosterone and calculated free testosterone reference ranges by tandem mass spectrometry. Ann Clin Biochem. 2013; 50:159–61. [PubMed: 23404928]
30. Yeap BB, Alfonso H, Chubb SA, et al. Reference ranges and determinants of testosterone, dihydrotestosterone, and estradiol levels measured using liquid chromatography-tandem mass spectrometry in a population-based cohort of older men. J Clin Endocrinol Metab. 2012; 97:4030–9. [PubMed: 22977273]
31. Marrie RA, Yu N, Wei Y, Elliott L, Blanchard J. High rates of physician services utilization at least five years before multiple sclerosis diagnosis. Mult Scler. 2012
32. Holland J, Bandelow S, Hogervorst E. Testosterone levels and cognition in elderly men: A review. Maturitas. 2011; 69:322–37. [PubMed: 21696899]
33. Sicotte NL, Kern KC, Giesser BS, et al. Regional hippocampal atrophy in multiple sclerosis. Brain. 2008; 131:1134–41. [PubMed: 18375977]
34. Sicotte NL, Giesser BS, Tandon V, et al. Testosterone treatment in multiple sclerosis: a pilot study. Arch Neurol. 2007; 64:683–8. [PubMed: 17502467]
35. Gold SM, Chalifoux S, Giesser BS, Voskuhl RR. Immune modulation and increased neurotrophic factor production in multiple sclerosis patients treated with testosterone. J Neuroinflammation. 2008; 5:32. [PubMed: 18671877]
36. Vermeulen A, Goemaere S, Kaufman JM. Testosterone, body composition and aging. J Endocrinol Invest. 1999; 22:110–6. [PubMed: 10442580]
37. Mowry EM. Vitamin D: evidence for its role as a prognostic factor in multiple sclerosis. J Neurol Sci. 2011; 311:19–22. [PubMed: 21762931]
38. Correale J, Ysrraelit MC, Gaitan MI. Gender differences in 1,25 dihydroxyvitamin D3 immunomodulatory effects in multiple sclerosis patients and healthy subjects. J Immunol. 2010; 185:4948–58. [PubMed: 20855882]
39. Amato MP, Goretti B, Ghezzi A, et al. Cognitive and psychosocial features in childhood and juvenile MS: two-year follow-up. Neurology. 2010; 75:1134–40. [PubMed: 20876467]

Table 1

Baseline demographic and clinical characteristics of 96 men aged 18-65 with relapsing remitting MS or CIS, within 10 years of first symptom onset

CHARACTERISTIC	VALUE
N	96
Age, years (mean (SD))	39.8 (9.3)
Race: % white	95
Ethnicity: % Hispanic [+]	3
BMI, kg/m^2 (mean (SD)) [*]	28.4 (4.8)
Disease duration, yrs (mean (SD))	4.6 (2.5)
EDSS (mean (SD)) [+]	1.07 (0.98)
Disease category (% RR)	94
Smoking history (% present smokers) [+]	19
Depression score (CES-D) [++]	
(mean (SD))	8.5 (6.9)
% above 16 (cutoff for depression)	14%
Disease modifying treatments at baseline (%) [+++]	
None	71%
Interferons (Avonex, Betaseron, Rebif)	19%
Glatiramer acetate	7%
Natalizumab	1%
Mycophenolate mofetil	1%
Cyclophosphamide	2%

Legend:

[+] Smoking history was available for N=77

[++] Depression history was available for N=64

[+++] 1 patient was missing these features

[*] 84 patients had BMI measurements

Table 2

Distribution of testosterone levels, by age

Age Group	N	Testosterone (ng/dL)			% Subjects with Testosterone <288 ng/dL
(Years)		Mean (SD)	Minimum	Maximum	
25-29	13	374 (168)	167	651	46
30-34	20	328 (99)	133	513	40
35-39	24	352 (130)	142	646	38
40-44	12	345 (108)	166	517	33
45-49	12	375 (125)	237	672	17
50-54	6	254 (159)	49	472	67
55+	9	308 (94)	126	450	44
TOTAL	96	342 (126)	49	672	39

Table 3a

Association between baseline androgens and both clinical (EDSS) and cognitive (SDMT) cross-sectional outcomes. For the EDSS correlations, Spearman's correlation coefficients are reported; for the SDMT, Pearson's correlation coefficients are reported

Cross-sectional associations between baseline androgen levels and clinical outcomes

CROSS-SECTIONAL								
		Unadjusted			Age adjusted		Age & Leptin adjusted	
	HORMONE	N	R	P	R	P	R	P
EDSS	Testosterone	95	−0.22	**0.033**	−0.21	**0.044**	−0.18	0.092
	T/E2 ratio	93	−0.27	**0.008**	−0.26	**0.013**	−0.22	**0.034**
	Free T	94	−0.27	**0.010**	−0.20	0.051	−0.19	0.066
SDMT	Testosterone	40	0.20	0.22	0.16	0.32	0.25	0.14
	T/E2 ratio	39	0.25	0.12	0.12	0.45	0.21	0.22
	Free T	40	0.28	0.077	0.14	0.39	0.20	0.24

Table 3b

Association between baseline androgens and both clinical (EDSS) and cognitive (SDMT) cross-sectional outcomes. For the EDSS correlations, Spearman's correlation coefficients are reported; for the SDMT, Pearson's correlation coefficients are reported

Association between androgen levels and longitudinal changes in clinical outcomes

LONGITUDINAL CHANGES					
	HORMONE	**N**	**EST**	**95% CL**	**P-VALUE**
EDSS	Testosterone	96	0.034	−0.01, 0.08	0.11
	T/E2 ratio	94	0.078	−0.04, 0.21	0.20
	Free T	95	1.84	−0.11, 3.78	0.065
SDMT	Testosterone	40	0.63	0.14, 1.12	**0.012**
	T/E2 ratio	39	0.72	−0.27, 1.71	0.153
	Free T	40	34.3	7.8, 60.8	**0.011**

Legend: All of these analyses included an adjustment for age on the intercept.

GEE analysis for change in yearly change in EDSS or SDMT for a 100-unit increase in baseline hormones, controlling for age. A positive estimate reflects less decline for the SDMT, and a positive estimate reflects greater decline for the EDSS.