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Original Article

Bulbocavernosus muscle area as a novel marker for hypogonadism

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Abstract *Objective:* Late-onset hypogonadism, or androgen deficiency in the aging male, is a significant cause of morbidity in older men. Many men in the low normal or equivocal range for low testosterone level exhibit signs and symptoms of hypogonadism. Serum testosterone is an imperfect maker for hypogonadism as symptoms vary greatly within the low to low normal range in addition to variations among testosterone assays. Perineal ultrasound can be effectively used to examine the bulbocavernosus muscle (BCM), an androgenized tissue that may be impacted by androgen receptor activity.
Methods: This study was a retrospective analysis of men who underwent perineal ultrasound for hypogonadism. The ultrasound data were used to calculate the area of the BCM and correlate it with indices of hypogonadismin symptomatic men including free and total testosterone and dual-energy X-ray absorptiometry (DEXA).
Results: The results demonstrate that there is a significant correlation between total and free testosterone and BCM area in hypogonadal patients. Comparison between BCM area and total testosterone showed $R^2 = 0.061$ and $p = 0.0187$ and comparison between BCM area and free testosterone showed $R^2 = 0.0957$ and $p = 0.0034$. In addition, low BCM was also correlated with DEXA results showing osteoporosis and osteopenia ($R^2 = 0.2239$, $p = 0.0027$).
Conclusion: There has been recent controversy over the safety of testosterone replacement therapy. This might be particularly important in men with hypogonadal symptoms but a low normal testosterone level. Our study investigated the use of perineal ultrasound to measure BCM as a surrogate marker for poor androgenized men presenting with hypogonadism.

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1. Introduction

Late-onset hypogonadism, or androgen deficiency in the aging male, is a significant cause of morbidity in older men. The Hypogonadism in Males Study shows a 38.7% prevalence of androgen deficiency in men 45 years or older [1]. The Massachusetts Male Aging Study (MMAS) estimates 481,000 new cases of male hypogonadism per year [2] and the Boston Area Community Health (BACH) survey reveals a prevalence of hypogonadism of 5.6% among men age 30—79 years, and increases markedly with age to 18.4% among men 70 years and older [3]. Androgen deficiency has many signs and symptoms which are improved with testosterone supplementation such as fatigue, poor mood, decreased erectile function, decreased sexual desire, decreased muscle mass, decreased bone density, increased fat mass, and lower quality of life. Androgen deficiency typically is diagnosed by symptoms and serum testosterone level. There is much debate over the lower limit of normal testosterone level and how it varies with age. Also specific testosterone assay from various labs may have some discordance. Many men in the low normal or equivocal range for low testosterone level exhibit signs and symptoms of hypogonadism.

Diagnosis and treatment of late-onset hypogonadism has come under increased scrutiny recently with the FDA black box warning that testosterone treatment may increase a patient's risk for thromboembolic events. Pharmaceutical companies have invested heavily in direct-to-consumer marketing, which has resulted in increased pressure on physicians to be both conscientious and cautious in treating patients with hypogonadism.

The bulbocavernosus muscle (BCM, also called the bulbospongiosus muscle) differs in size and action between men and women. The BCM is the muscle overlying the urethra and corpora cavernosa in the perineum. BCM requires androgen in embryological development to develop into the male phenotype and covers the bulbar urethra (Fig. 1). As a result, BCM is responsive to androgen and directly affected by androgen levels [4]. In castrated adult male rats BCM has been shown to atrophy in fewer than 10 days. Additionally, these studies showed androgenized tissue including bulbospongiosus tissue, bulbocavernosus tissue, the glans penis, seminal vesicles, and ventral prostate to increase in size and weight with androgen replacement [5]. Increased resistance of the androgen receptor to androgen, which decreases end organ responsiveness to testosterone, is associated with decreased BCM area in adult men [6]. Thus BCM area, easily measurable on perineal ultrasound, may be a surrogate for end-organ effect of testosterone and can act as a marker for testosterone deficiency.

This study investigates whether a relationship exists between BCM area as measured on perineal ultrasound and serum testosterone levels as well as other signs of hypogonadism.

2. Methods

2.1. Population

Institutional Review Board approval was obtained to collect data from adult male patients presenting to an andrology clinic with symptoms of hypogonadism who were undergoing a standard evaluation including history, physical exam, and morning serum hormone levels.

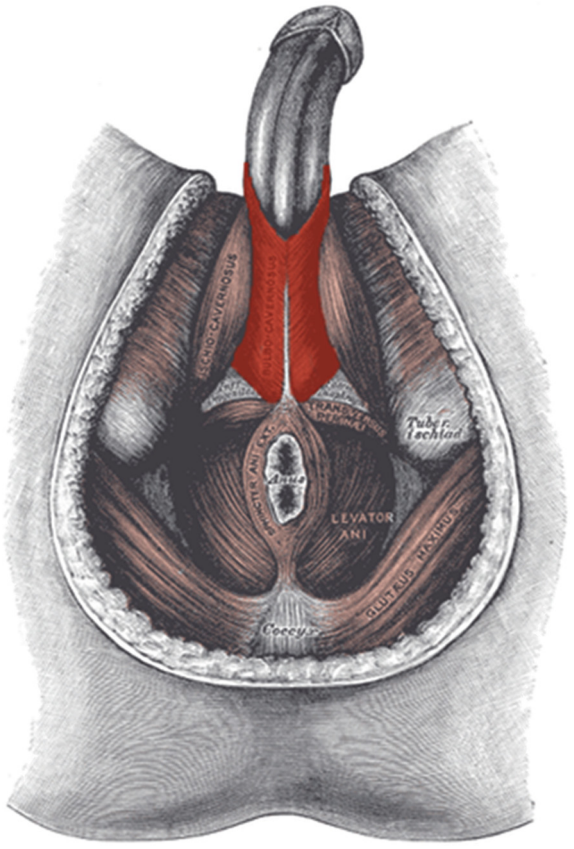

Figure 1 Bulbocavernosus muscle is outlined in red in the image above. The bulbospongiosus (aka bulbocavernosus) is located in the middle line of the perineum in front of the anus. It consists of two symmetrical parts, united along the median line by a tendinous raphe.

2.2. Data

Morning serum total and free testosterone levels were obtained via liquid chromatography/mass spectrometry assay. Patients also underwent measurement of BCM area via perineal ultrasound. In men with clinical hypogonadism, a dual-energy X-ray absorptiometry (DEXA) scan was obtained from the femoral neck, hip and spine to measure bone mineral density. The lowest T-score from any of these areas was used for data analysis. If patients complained of erectile dysfunction they also underwent evaluation with penile duplex ultrasound. Men previously treated for hypogonadism were excluded.

2.3. BCM measurement

BCM area was obtained via perineal ultrasound. With B Mode ultrasound, using a linear array high frequency transducer, the transverse image of the BCM was identified and the perimeter outlined by freehand on a static image (Figs. 2 and 3). A video demonstrating the procedure can be

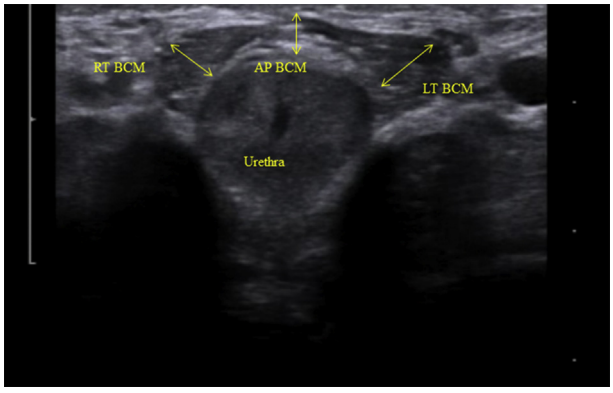

Figure 2 With B Mode ultrasound the transverse image of the BCM is imaged where the cross section of the urethra is circular. The diameter of the right (RT BCM), left (LT BCM) and mid (AP BCM) was measured. BCM, bulbocavernosus muscle.

found at https://youtu.be/79t3SvINMNE. Two separate areas adjacent to each other were obtained and the calculated area averaged. In addition to the calculation of the BCM area measurements, the width of the right (RT BCM), left (LT BCM) and mid (AP BCM) BCM was measured as well as the urethral diameter.

2.4. Statistical analysis

Microsoft Excel 2010, version 14.0 (Microsoft, Redmond, USA) was used to perform statistical analysis. Continuous variables were compared using Student's *t*-test. Trend lines were created with linear regression. A p value <0.05 was considered statistically significant.

3. Results

3.1. Population characteristics

Ninety-one men underwent evaluation with BCM area measurement. Mean age of the population was 53 years. Mean body mass index (BMI) of the population was 29.32 kg/m^2. Mean morning serum total testosterone was 323.7 ng/dL and mean morning serum free testosterone was 6.5 ng/dL. Mean BCM area as measured on ultrasound was 0.60 cm^2. No subjects had been previously treated for hypogonadism. Thirty-eight patients underwent DEXA scan. Twelve patients were diagnosed with osteopenia, two patients were diagnosed with osteoporosis. Thirty-eight patients underwent penile duplex ultrasound. Thirty-six patients had peak systolic velocity less than 35 cm/s. Eighteen patients had end diastolic velocity >5 cm/s.

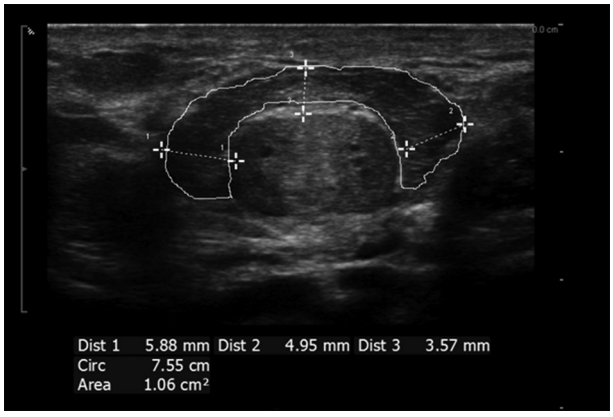

Figure 3 With B Mode ultrasound, using a linear array high frequency transducer, the transverse image of the BCM was identified and the perimeter was outlined by freehand on a static image. BCM, bulbocavernosus muscle.

3.2. BCM trends

Linear regression comparing BCM area to total testosterone and free testosterone revealed statistically significant correlation. Comparison between BCM area and total testosterone showed $R^2 = 0.061$, $p = 0.0187$ (Fig. 4A). Comparison between BCM area and free testosterone showed an $R^2 = 0.0957$, $p = 0.0034$ (Fig. 4B). Comparison between BCM and the actual DEXA T-score also yielded a significant relationship ($R^2 = 0.2239$, $p = 0.0027$) (Fig. 4C). No correlations were found between BCM area and peak systolic velocity ($R^2 = 0.0742$, $p = 0.0977$), end diastolic velocity ($R^2 = 0.0046$, $p = 0.6864$), or resistive index ($R^2 = 0.0136$, $p = 0.4857$) on penile duplex ultrasound.

3.3. BCM *vs.* testosterone

There was a statistically significant difference in BCM area at a total testosterone cutoff of 300 ng/dL. Above testosterone of 300 ng/dL mean BCM area was 0.67 cm^2, while below testosterone of 300 ng/dL mean BCM area was 0.56 cm^2 ($p = 0.039$).

4. Discussion

These data demonstrate a correlation between morning serum testosterone levels and BCM surface area as measured on ultrasound. Linear regression analysis showed an association with both total testosterone and free testosterone. BCM area shows a better fit with free testosterone likely because free testosterone is bioavailable and able to act directly on the BCM allowing growth and preventing atrophy of the muscle. Mean BCM area also showed a significant difference at a total testosterone cutoff of 300 ng/dL, which is the low-normal level at our lab; i.e., a total testosterone level in the normal range implies a higher BCM surface area and a total testosterone level below the normal range implies a lower BCM surface area.

Osteopenia and osteoporosis are known sequelae of hypogonadism. Our data showed a significant correlation between BCM and DEXA measurements. BCM and bone mineral density likely have different rates of change when related to testosterone level with BCM being more acutely affected and bone mineral density changes occurring over a longer time. Serum testosterone level change was seen almost immediately after supplementation while BCM size, from animal studies, appears to take weeks to change [5]. DEXA values likely take many months or years to change [7,8]. Only 37 patients of the 90 total included in our study underwent DEXA scan, which is less than 40% of the men

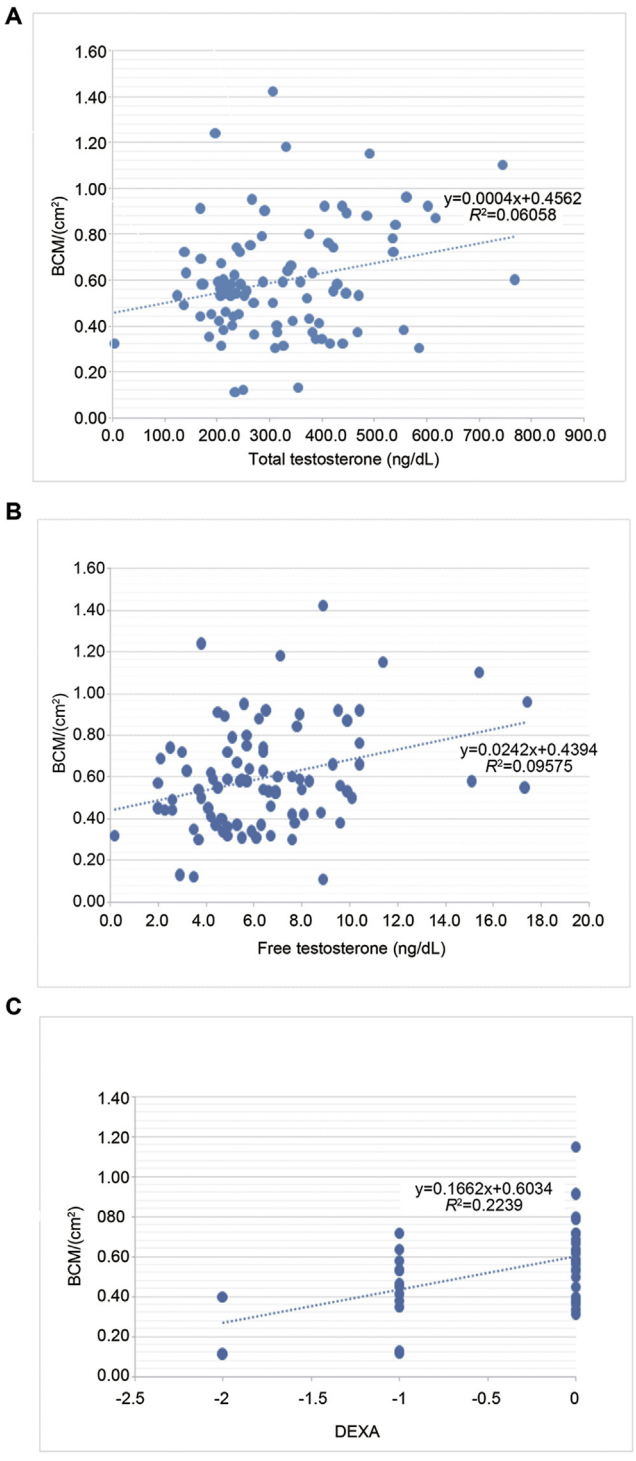

Figure 4 BCM area *vs.* (A) total testosterone, (B) free testosterone, and (C) DEXA result (−2, osteoporosis; −1, osteopenia; 0, normal).

who presented with hypogonadal symptoms. Thus, in order to fully capture this effect more data will need to be gathered on all the men who presented with hypogonadal symptoms. We eliminated from our analysis those patients that were taking prescribed drugs that are known to decrease testosterone (e.g., opioids, neuroleptics). However, it is possible that our patients did not report non-prescribed supplements or other medications that they might have been taking, that might have changed our DEXA results. Multiple studies have demonstrated that opioids, neuroleptics, and other drugs effect the hypothalamic—pituitary—gonadal axis causing abnormalities in bone turnover and testosterone production [9,10]. Osteopenia and osteoporosis are later and more severe consequences of hypogonadism and thus need to be evaluated in patients presenting with hypogonadal symptoms. It is unclear, at this time, if atrophy of the BCM in men, is an acute or chronic result of hypogonadism. The utility of using BCM area to identify hypogonadal men is to identify these men before they develop the severe sequelae such as osteoporosis. A baseline DEXA is important in the evaluation of these men and with treatment they can avoid osteopenia and osteoporosis.

Lack of correlation between BCM area and penile duplex ultrasound parameters is also not surprising. Erectile function is multifactorial and with much overlap of overall health and cardiovascular status, therefore, finding a direct correlation between eugonadism and erectile function is unlikely especially in a small sample size. Peak systolic velocity and end diastolic velocity on penile duplex ultrasound owe more to vascular integrity than androgenization. BCM area, as a marker for androgenization, would not directly correlate with erectile dysfunction.

BCM surface area is a promising marker for androgenization. The embryological development of the muscle, as it develops differently in male and female fetuses, makes it responsive to androgen by nature [4]. Animal studies have shown atrophy of the muscle in castrate rats [5]. Dabaja et al. [6] showed that BCM area in men atrophies with increased androgen receptor resistance to androgen. In the DNA coding region of the androgen receptor, an increased number of nucleotide series CAG repeats creates increased receptor resistance to androgen [11]. Dabaja et al. [6] found that an increased number of CAG repeats within the DNA coding of the androgen receptor in men was associated with decreased size of BCM area as measured by ultrasound. Thus, in men, decreased activation of the muscle by androgen causes atrophy of the muscle. As a result, decreased size of BCM can signal decreased end-organ activity of androgen, whether by androgen deficiency or androgen resistance.

Measurement of end-organ androgen activity is important as the diagnosis of late-onset hypogonadism can be difficult. The diagnosis is made with a combination of symptomatology and low serum testosterone level. However, the main symptoms of hypogonadism: fatigue poor mood, and decreased libido, are nonspecific and may be related to other conditions such as depression or anxiety. Serum testosterone level can also be difficult to interpret. Different testosterone assays exist and the different laboratories run assays distinctly. Radioimmunoassay (RIA) and enzyme immunoassays (EIA) require a very specific antibody for testosterone, however testosterone is a poor antigen. Different manufacturers of RIAs and EIAs use different sources and types of antibodies, which contribute to varied results. Overall RIAs and EIAs have good accuracy for eugonadal men but poor accuracy for testosterone levels less than 300 ng/dL. Liquid chromatography—mass spectrometry (LC—MS), while having much better accuracy for

low testosterone levels, still has significant variation between laboratories due to different instrumentation, reagents and calibrators. Results at different laboratories are difficult to compare with each other [12].

Even the definition of "normal range" testosterone varies wildly. The FDA uses testosterone values less than 300 ng/dL to define hypogonadism for clinical trial development. However, clinically, numerous societies such as the European Association of Urology (EAU), European Association of Andrology (EAA), and American Society of Andrology (ASA) recommend treatment of testosterone level below 230 ng/dL and no treatment for testosterone levels above 350 ng/dL with the in-between area as a gray zone. Without a definitive definition of hypogonadism by testosterone level, signs and symptoms of hypogonadism are equally important as serum testosterone in the diagnosis of hypogonadism [12].

Many men however, have significant signs and symptoms of hypogonadism with serum testosterone levels in the low-normal range. This makes the decision to treat with testosterone replacement therapy very complex. Evidence of end organ androgen deficiency give objective evidence to make the diagnosis; however waiting for signs such as osteoporosis before initiating testosterone replacement therapy puts men with true hypogonadism at risk for dangerous and harmful sequelae before initiating the required treatment.

The over diagnosis of late-onset hypogonadism has diluted the benefits of appropriate testosterone replacement therapy. An FDA analysis found that 28% of men newly prescribed testosterone did not have serum testosterone level measured beforehand [13]. Nguyen et al. [14] question whether "age-related hypogonadism" is a disease process that requires treatment as opposed to normal aging. They also point out the lack of studies proving benefits of testosterone in older men diagnosed with hypogonadism associated with age. Indeed, until recently, no large, randomized controlled trial existed proving the symptomatic and health benefits of testosterone against placebo for late-onset hypogonadism.

Two recently published randomized, placebo-controlled trials have shown some symptomatic benefit to testosterone replacement therapy in older men. Brock et al. [15] performed a short-term study investigating how well a 16-week course of testosterone replacement therapy improved libido and energy level compared to placebo and found an improvement in libido with some improvement in energy level. Snyder et al. [16] assessed symptomatic improvement after testosterone replacement therapy for 1 year against placebo and found increased sexual desire and sexual activity with some improvement in mood and depressive symptoms. Both of these studies found a significant improvement in libido and sexual activity, however the other symptomatic improvements were mild at best. Neither study found, nor was powered to find, an increased risk of thromboembolism with testosterone replacement therapy.

The major debate currently engulfing late-onset hypogonadism focuses on the cardiovascular risks and benefits of testosterone replacement therapy. When the FDA reviewed the literature, it ruled that a "weak signal" existed that testosterone replacement therapy caused an increased risk of thromboembolic events. This conclusion was based on a few studies in high-profile journals.

Basaria et al. [17] found an increased rate of cardiovascular-related events in elderly, relatively immobile men treated with testosterone gel. However, only two myocardial infarctions were actually reported and the remainder of the adverse events were events such as syncope and peripheral edema. These adverse effects may or may not be related to cardiovascular health, but are also known side effects of testosterone therapy and may also be due to the sudden increase in physical activity found in the treatment group. Finkle et al. [18] compared risk of myocardial infarction (MI) in a group given testosterone prescription and a group given a prescription for phosphodiesterase-5 (PDE-5) inhibitors and found an increase risk for MI in the group given a testosterone prescription. The study did not confirm that the treatment arm actually complied with testosterone therapy nor did they investigate to what extent these subjects responded to testosterone therapy. Also, PDE-5 inhibitors have known cardioprotective effects, making the PDE-5 inhibitor arm an invalid comparison. A study performed in the Veterans Administration (VA) health system investigating patients who were prescribed testosterone after a normal coronary angiogram found an increase rate of myocardial infarction and stroke [19]. However, the study excluded patients who had MI or stroke before testosterone use rather than include them in the no-treatment arm. The results of these studies, despite their inconsistencies, prompted the FDA to issue a black box warning.

There are, however, a number of studies which show no harmful cardiac effects to appropriate testosterone therapy. A second study was conducted in the VA setting by Sharma et al. [20] followed 83,000 patients with low testosterone, of whom half were treated and achieved normal serum total testosterone levels. In comparing patients treated successfully, patients treated unsuccessfully, and patients not treated at all, the study found improved all-cause mortality, risk of MI, and risk of stroke in the successfully treated population. There was no difference in all-cause mortality, risk of MI, or risk of stroke between the group unsuccessfully treated and the group not treated at all.

Two meta-analyses have found significantly more literature in support of testosterone with regard to cardiovascular risk than there is against it. Morgentaler et al. [21] reviewed literature from 1940 to 2014 and found four articles suggesting testosterone therapy presented an increased cardiovascular risk: the three studies mentioned above and one meta-analysis performed with what the authors considered to be questionable data. They found overall an inverse relationship between testosterone therapy and cardiovascular risk. Corona et al. [22] performed the largest meta-analysis to date regarding testosterone therapy and cardiovascular risk. This analysis found no increase in cardiovascular risk related to testosterone therapy and even found a protective effect of testosterone therapy on cardiovascular risk in patients with metabolic syndrome. The cardiovascular risk with testosterone therapy has likely been overstated and may be mitigated by proper patient selection and monitoring.

Claiming that testosterone replacement therapy is cardioprotective may, however, be a step too far given the

current literature. Mechanisms such as testosterone's protective effect on endothelial function have been proposed to explain a protective effect on overall cardiovascular health [23], however, no direct protective effect has been measured in a randomized-controlled trial. Testosterone does cause changes in metabolism and body parameters such as muscle mass and fat mass. Traish [24] shows that testosterone replacement given to hypogonadal men increased muscle mass and decreased fat mass. These changes may aid in treating the constellation of symptoms derived from obesity and metabolic syndrome and help spur lifestyle improvements. Thus testosterone may indirectly improve cardiovascular risk and overall health.

An additional limitation of this study is its retrospective nature. Also, all subjects included in this study were men presenting to an andrology clinic for evaluation of infertility, sexual dysfunction with hypogonadism. A study designed with increased number of patients with and without hypogonadal symptoms, may better capture a more diverse population. This would help to properly evaluate the variations in BCM on the general population. It would allow for a stronger comparison of hypogonadal men relative to the asymptomatic oreugonadal but symptomatic patients.

Overall there is a benefit to testosterone replacement therapy for hypogonadal men. The challenge remains in identifying truly hypogonadal men who can benefit from testosterone replacement therapy. Serum testosterone level and individual signs and symptoms of hypogonadism may be variable amongst patients, however, increased evidence of decreased androgenization help to confirm a true diagnosis of late-onset hypogonadism. This will allow clinicians to accurately and appropriately treat hypogonadal men that suffering acutely and allow them to prevent long-term health implications related to hypogonadism. Also, making the diagnosis of hypogonadism in symptomatic men with a low normal or mildly low serum testosterone is challenging. Measurement of BCM area by perineal ultrasound can be a marker of decreased end-organ androgen activity and thus can be useful in the evaluation of the hypogonadal male. Further study is necessary to confirm the relationship between BCM and total body androgenization, including the response of BCM area to testosterone replacement therapy.

Conflicts of interest

The authors declare no conflict of interest.

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