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Testosterone replacement therapy

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Thesis

TESTOSTERONE REPLACEMENT THERAPY

by

MICHAEL ANDREW VROLYK

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Approved by

First Reader
Gwynneth D. Offner, Ph.D.
Director, M.S. in Medical Sciences Program
Associate Professor of Medicine

Second Reader
Karen Symes, PhD
Assistant Dean of Student Affairs
Associate Professor of Biochemistry
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TESTOSTERONE REPLACEMENT THERAPY

MICHAEL ANDREW VRolyk

ABSTRACT

Physicians and scientists have suspected that the testes secrete a substance into the body that causes male secondary sexual characteristics for hundreds of years. However, testosterone was not synthesized until 1935 and it was not until the 1940’s when scientists could accurately measure the amount of this hormone in the blood. Since then, scientists have been able to make correlations between the levels of testosterone in the body and men’s health.

Scientists have long observed higher levels of testosterone to be associated with an increase in levels of Hematocrit (Hct). As a result, Testosterone Replacement Therapy (TRT) has been used to treat anemia associated with chronic diseases. In recent years, prescription sales for testosterone have sky rocketed due to new clinical uses such as androgen deficiency in older men. In fact, the rate of prescription for testosterone products has increased by over 170% in the previous five years.

Long-term data shows that the level of testosterone in the male body begins to decrease at about the age of 30. As the life expectancy of the general population continues to increase, TRT may be a viable option for older men with low testosterone to increase the quality and duration of life. However, an increase in Hct continues to be a major side effect of TRT. New research is beginning to make clear the mechanism by which testosterone affects erythropoiesis.
New research suggests TRT suppresses hepcidin and leads to an increase in the rate of iron (Fe) retention in red blood cells (RBCs). Inter-individual differences in the pharmacogenetic effects of TRT have been observed. In the future TRT could be genetically tailored based on the individuals DNA. In this case, the optimal dose of testosterone can be given to maximize benefits and reduce side effects.

Here, the risks and benefits associated with TRT and a review of the updated Clinical Guidelines for its use will be presented. The effects of TRT on erythropoiesis will be investigated via a review of the literature. The main objective of this review is to provide a general understanding of TRT and a major side effect of its use, excessive erythropoiesis.
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LIST OF ABBREVIATIONS

2,3-BPG .............................................................................. 2,3-Bisphosphoglycerate
AR ........................................................................................... Androgen Receptor
BMP ..................................................................................... Bone Morphogenetic Protein
DHEA ..................................................................................... Dehydroepiandrosterone
Fe ............................................................................................ Iron
GnRH ............................................................... Gonadotrophic Releasing Hormone
Hct ............................................................................................ Hematocrit
Hb ............................................................................................ Hemoglobin
LH ............................................................................................ Luteinizing Hormone
LOH ................................................................. Late-Onset Hypogonadism
RBC ............................................................................................ Red Blood Cell
SHBG ..................................................................................... Sex Hormone Binding Globulin
TRT ......................................................................................... Testosterone Replacement Therapy
INTRODUCTION

Early History

The primary source of testosterone and its phenotypic effects have been known for about 6,000 years when Neolithic farmers discovered castration of animals improved their domestication (Freeman et al., 2001). Figure 1 is a depiction of Neolithic farmers with domesticated animals. However, the genotypic effects of testosterone on the human body have still not been fully elucidated at present.

Figure 1. Depiction of Neolithic Farmers with Domesticated Animals

“Neolithic Settlement” Zdenek Burian, 1948
The modern era of testosterone began in 1849 when Arnold Berthold investigated the effects of castration and transplantation on roosters. Berthold found that when roosters were castrated at a young age they suffered from a lack of physical and behavioral development typical of normal roosters (Soma, 2006). When Berthold transplanted testes back into young castrated roosters, normal development was observed (Figure 2). Furthermore, Berthold discovered new vascular connections in the transplanted testes. Berthold concluded that the testes are generating something that affects behavioral and sexual characteristics (Soma, 2006).

Figure 2. Effects of Castration on Male Chick

Evolution of Endocrinology

The fountain of youth has been an age-old dream of humankind. People have long used extreme measures to prevent or reverse normal aging and senescence (Kozminski et al., 2011). In 1889 Charles Edouard Brown-Sequard (Figure 3) reported “he had increased his physical strength, mental abilities and appetite by self-injection with an extract derived from the testicles of dogs and guinea pigs” (Freeman et al., 2001). Brown-Sequard argued that “spermatic anemia” resulted in fatigue and senility that could be treated by increasing the level of sperm or testicular substance in the blood stream (Kozminski et al., 2011). This claim led to the development of “Organotherapy”, a field of research dedicated to the treatment of diseases with extracts from animal organs (Freeman et al., 2001).

Figure 3. Charles Edouard Brown-Sequard

Figures and Institutions of the Neurology Sciences in Paris from 1800 to 1950. Part II: Neurophysiology. Barbara et al., 2012
By the end of 1889, manufacturing chemists were making fortunes selling extracts from animal testes, which claimed to be the “Elixir of Life” (Figure 4), even though Brown-Sequard’s claims were never scientifically proven. In addition, the risks associated with the use of animal extracts to treat diseases were never publicized. As a result, many patients were at serious risk of infection and inflammation from its use. Nevertheless, the new medical field of organotherapy continued to advance rapidly (Freeman et al., 2001).

Figure 4. The “Elixir of Life”
Dunbar et al., 1889
In 1902 William Bayliss and Ernest Starling (Figure 5) recovered an interesting extract from the lining of the small intestine they called “secretin” because it activated the secretion of water and bicarbonate by the pancreas (Freeman et al., 2001). This discovery supported the theory of organotherapy and eventually led to the development of modern day endocrinology. The discovery of secretin suggested that the secretion of substances from one organ can be responsible for physiological occurrences seen in another organ (Freeman et al., 2001).

Figure 5. William Bayliss (bottom right) and Ernest Starling (top left)
Ernest Starling and the Discovery of Secretin. Modlin et al., 2001
Bayliss and Starling continued to ambiguously refer to these blood borne messengers as “chemical messengers” until William B. Hardy proposed the name hormone in 1905. Hardy derived the term hormone from the Greek word horman meaning “I arouse to activity,” as in “setting something in motion.” Bayliss and Starling postulated that hormones have receptors far from the site of origin that influence many functions in the body (Freeman et al., 2001).

The “Steinach Operation”

Work in the late 19th century suggested that fatigue and senility, particularly in men, could be treated with a substance found within the testicle. Accordingly surgeons implemented techniques to enhance or supplement rejuvenating material in men (Kozminski et al., 2011). The famous “Steinach Operation” was invented in 1920 by the Viennese physiologist Dr. Eugene Steinach (Figure 6). The “Steinach Operation” consisted of unilateral vasoligation of the vas deferens; essentially the operation was a vasectomy. Figure 7 shows the same person before and after the “Steinach Operation.” “Steinach argued that the sex gland contained puberty cells and reproductive cells. Steinach believed that disrupting the outflow tract of the sperm producing cells would lead to a back pressure, resulting in atrophy of this portion of the testis. As the one portion atrophied, the other had to proliferate to prevent a vacuum within the body” (Kozminski et al., 2011). This treatment for middle-aged listless males became a popular procedure until the discovery of testosterone (Freeman et al., 2001).
Figure 6. Dr. Eugen Steinach

Figure adopted from ‘Dr. Steinach Coming to Make Old Young!’: Sex Glands, Vasectomy and the Quest for Rejuvenation in the Roaring Twenties (Sengoopta, 2003)

Figure 7. Before (left) and After (right) the “Steinach Operation”

Kahn, 2007
William Butler Yeats was an Irish poet and Nobel Prize winner who underwent the “Steinach Operation” at the age of 69. In 1937, referring to the “Steinach Operation” Yeats wrote, “It revived my creative power. It revived also sexual desire; and that in all likelihood will last me until I die.” Sigmund Freud and numerous others underwent the procedure in the “belief that it would rejuvenate them physically, mentally and sexually” (Freeman et al., 2001).

The Discovery of Testosterone

By the 1930’s chemists were still attempting to capitalize on the popularity of organotherapy brought about by Brown-Sequard nearly half a century earlier. In 1935, the publication of “On Crystalline Male Hormone from Testicles (Testosterone),” identified a new hormone produced by the testes (PubChem; Figure 8). Testosterone proved to be a powerful androgenic factor, responsible for male secondary characteristics.
In 1935, just one week before Ruzicka et al published “On the Artificial Preparation of the Testicular Hormone Testosterone (Andro-sten-3-one-17-ol),” Butenandt et al published “A Method for Preparing Testosterone from Cholesterol (Freeman et al., 2001).” Ruzicka et al and Butenandt et al were both offered the 1939 Nobel Prize in chemistry however; Butenandt was forced to decline the Nobel Prize by the Nazi government (Freeman et al., 2001).
Figure 9: Pathway for Testosterone Synthesis

Erogenic Aids: Counseling the Athlete, Ahrendt, 2001

**Recent History**

After the discovery of testosterone, the effects of this hormone on the human body could be scientifically evaluated by measuring the amount of testosterone in the blood. Men’s elevated concentration of Hemoglobin (Hb) and Hct as compared to women’s has been attributed to men’s elevated levels of testosterone. Due to testosterone’s effect on Hb and Hct, it was the main pharmacologic agent used to treat patients suffering from anemia before recombinant hematopoietic growth factors became available (Rochira et al., 2008).
Recently the influence of testosterone on physical well-being has become a focus of research attention. Particularly, the risk benefit ratio of TRT in treating a variety of conditions has been studied. Demographic data indicates that the population in older age groups is increasing. Low testosterone in older men has become an interesting topic of discussion. Studies routinely indicate that testosterone levels decrease with age and that many men over the age of 60 years have testosterone levels below the lower limit of normal for men aged 20-30 years. The main question raised is whether older men with low levels of testosterone will benefit from TRT (Wang et al., 2008.)

**Effects of TRT on Erythropoeisis**

TRT is associated with increases in the rate of erythropoeisis and therefore an increase in Hct (Fernandez-Balsells, 2010). However, the mechanism by which TRT increases the rate of erythropoeisis remains poorly understood (Bachmen et al., 2010). Understanding the underlying mechanism by which TRT stimulates erythropoeisis may help to prevent an excessive increase in Hct during TRT (Guo et al., 2013). The clinical significance of these findings and the effects on patient-important outcomes such as mortality and cardiovascular events requires further investigation (Fernandez-Balsells, 2010). Clarification of factors that modify the effect of testosterone on erythropoeisis would better inform the medical field and the general public about the risks and benefits of TRT and thereby improve medication safety (Spitzer, 2013).
Aims

Therefore, the primary objective here is to present the risks and benefits associated with TRT. A review of the updated Clinical Guidelines will be presented and the effects of TRT on erythropoiesis will be investigated via a review of the literature. Lastly, a suggestion for the future of TRT will be proposed. We hope this will bring about a more general understanding of the risks and benefits of TRT in the clinical setting.
PUBLISHED STUDIES


Physiology of Testosterone

Testosterone secretion begins in utero, during an early “sensitive period”, that can be seen as a peak in testosterone concentration in the male fetus at about 12 weeks. A second peak is observed after birth. From birth up until puberty, Luteinizing Hormone (LH) is secreted in a pulsatile fashion leading to the maturity of the Leydig cells. The maturation of the Leydig cells leads to increased levels of testosterone in the body (Basaria et al., 1999). Testosterone is required for the healthy development, maintenance, and physiological functions of many organs in males throughout life (Ullah et al., 2014).

Testosterone secretion is controlled by a negative feedback mechanism; a decrease in the concentration of testosterone in the blood stream will cause the hypothalamus to release Gonadotrophic Releasing Hormone (GnRH). The increase in GnRH will then signal the pituitary to release LH and Follicle-Stimulating Hormone (FSH). LH and FSH signal the Leydig cells of the testes to secrete testosterone into the blood stream (Figure 10). Conversely, a high concentration of testosterone in the blood stream will cause a decrease in the rate of production. Testosterone signals both the hypothalamus and the pituitary to stop releasing GnRH, LH, and FSH (Basaria et al., 1999).
The average adult male produces 0.24 micromoles of testosterone daily. Only 2% of testosterone is in the blood as free testosterone; 18% has a weak bound with albumin, while 80% has a strong bound with Sex Hormone-Binding Globulin (SHGB) (Nigro et al., 2012; Basaria et al., 1999). The testosterone that is bound to SHBG is not considered usable by most target organs because it is bound too tightly. Therefore only about 20% of the testosterone concentration is considered usable by the body (Surampudi et al., 2012). Studies have shown the aging male can suffer from a 1-2% decrease of total testosterone levels per year, beginning at about the age of 30. A concomitant increase in SHBG with
aging has also been observed, such that the decline in free testosterone is even more significant in older males (Nigro et al., 2012).

**Risks Associated with Low T**

Low testosterone concentrations in older males are associated with declining physical and cognitive performance, increased cardiovascular morbidity, and all-cause mortality (Figure 11). Older men have an increased risk of anemia (Yeap et al., 2009). Anemia “is associated with an increased risk of falls and fractures, cognitive impairment, mortality, and decreased physical ability. Anemic men older than 85 years had more than twice the risk of death than men without anemia” (Paller et al., 2012).

**Fig 11: Low T is Associated with Shorter Survival**

Men with low and equivocal T levels had shorter survival than men with normal T levels. Low T = total T < 250 ng/dL or free T < .75 ng/dL

Figure adapted from Traish et al., 2010
Benefits of TRT

TRT has been shown to restore testosterone levels to within the normal range for young men. TRT has also been shown to improve mood, energy levels and patients’ sense of well-being. Studies also show improvements in sexual function, lean body mass and muscle strength (Gooren, 2010). Recent studies show short-term beneficial effects of TRT in elderly men on multiple target organs however there is little long-term data. The effects of TRT on functional benefits that may delay physical or mental frailty of older males or improve the quality of life of the elderly are also very limited.

Risks of TRT

There is a correlation in men between the amount of testosterone in the plasma and the level of Hb. Higher levels of testosterone are associated with higher levels of Hb. A recent study demonstrated a TRT dose-dependent stimulatory effect on erythropoiesis (Synder et al., 2000) (Figure 12). Higher values of Hb and Hct are associated with vascular conditions such as cerebral vascular accidents and coronary heart disease (Gooren, 2010). The Honolulu Heart Program and The Framingham Heart Study both found a high Hct to be a risk factor of death from cardiovascular disease (Paller et al., 2012). However, due to a lack of large-scale long-term data, a relation between increases in Hct as a result of TRT and the risk for any cardiovascular event in general has not been demonstrated (Gooren, 2010).
Figure 12. Mean (+/- SE) serum testosterone, Hct, and Hb values in men with previously untreated hypogonadism who were treated with TRT for 36 months (Synder et al., 2000).
Conclusion

Due to the growing interest from practitioners on TRT as a treatment for low levels of testosterone in adult men, the Clinical Guidelines Subcommittee of The Endocrine Society selected a Task Force to update the guidelines for the evaluation and treatment of low testosterone levels in men (Wang et al., 2008).
Testosterone Therapy in Men with Androgen Deficiency Syndromes: An Endocrine Society Clinical Practice Guideline. Bhasin et al., 2010.

Hypogonadism

Hypogonadism is defined as a clinical syndrome resulting from the failure of the testis to produce normal levels of testosterone and a normal number of spermatozoa due to a malfunction in the hypothalamic-pituitary-testicular axis (Bhasin et al., 2010).

Malfunctions in the hypothalamic-pituitary-testicular axis can be described as primary or secondary testicular failure. Malfunctions at the testicular level cause primary testicular failure, while abnormalities in the hypothalamus or pituitary cause secondary testicular failure. Hypogonadism can also reflect defects of both primary and secondary testicular failure (Bhasin et al., 2010).

“Primary testicular failure results in low testosterone levels, impairment of spermatogenesis, and elevated GnRH levels. Secondary testicular failure results in low testosterone levels, impairment of spermatogenesis, and low or low-normal GnRH levels. Combined primary and secondary testicular failure results in low testosterone levels, impairment of spermatogenesis, and variable GnRH levels, depending on whether primary or secondary testicular failure predominates” (Bhasin et al., 2010).
Late-Onset Hypogonadism

Late-Onset Hypogonadism (LOH) is defined as a clinical and biochemical syndrome characterized by symptoms associated with older age and testosterone levels below the reference range for young healthy adult men (Wang et al., 2008). It is believed LOH is primarily due to testicular dysfunction based on the observed decrease in number and volume of Leydig cells, impaired testicular perfusion, and impaired steroid biosynthesis in aging men (Basaria et al., 1999) however, an additional hypothalamic component is also possible (Nigro et al., 2012). It is believed LOH may result in a decrease in the quality of life and adversely affect the function of several organ systems (Wang et al., 2008).

Clinical Guidelines

The Clinical Guidelines Subcommittee of The Endocrine Society updated the guidelines for the evaluation and treatment of androgen deficiency syndromes in adult men in 2010. The Task Force suggests only making a diagnosis of hypogonadism in men with signs and symptoms suggestive of low testosterone (Table 1) and consistently low serum testosterone levels (Bhasin et al., 2010).
Table 1. Signs and Symptoms Suggestive of LOH in Men (Bahsin et al., 2010).

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<tr>
<td>Incomplete or delayed sexual development, eunuchoidism</td>
<td>Decreased energy, motivation, initiative, and self-confidence</td>
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<tr>
<td>Reduced sexual desire (libido) and activity</td>
<td>Feeling sad or blue, depressed mood, dysthymia</td>
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<tr>
<td>Decreased spontaneous erections</td>
<td>Poor concentration and memory</td>
</tr>
<tr>
<td>Breast Discomfort gynecomastia</td>
<td>Sleep Disturbance, increased sleepiness</td>
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<tr>
<td>Loss of body (axillary and pubic) hair, reduced shaving</td>
<td>Mild anemia</td>
</tr>
<tr>
<td>Very small (especially &lt;5ml) or shrinking testes</td>
<td>Reduced muscle bulk</td>
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<tr>
<td>Height loss</td>
<td>Increased body fat</td>
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The Task Force suggests taking a blood sample in the morning to determine the patients’ total testosterone level. The initial testosterone level should be confirmed by repeating a morning blood sample at least one more time. The Task Force notes that some men may have total testosterone levels that are near the lower limit of normal and still be experiencing symptoms of LOH. In these men, blood samples should be taken to determine the amount of serum SHBG. High levels of SHBG would bind a larger percentage of testosterone therefore decreasing the amount of free testosterone available in the patient. Figure 13 depicts an approach for the diagnostic evaluation of adult men with signs and symptoms suggestive of LOH. They recommend TRT for men with symptomatic androgen deficiency to induce and maintain secondary sex characteristics.
and to improve their sexual function, sense of well-being, muscle mass and strength, and bone mineral density (Bhasin et al., 2010).

Figure 13. An Approach for the Diagnostic Evaluation of Adult Men Suspected of Having Androgen Deficiency (Bhasin et al., 2010).
The Task Force recommends evaluating patients at baseline, 3 months, and 6 months after beginning TRT and then yearly to evaluate the patients’ symptoms and determine if the patient is suffering any side effects. Blood samples should be drawn at each visit, with the goal of reaching serum testosterone levels in the mid-normal range for young adult men (Bahsin et al., 2010).

The Task Force found TRT to cause a dose-dependent increase in Hct due to excessive erythrocytosis as one of the most common side effects. “Testosterone treated men were nearly four times more likely than placebo-treated men to experience Hct above 50%” (Bahsin et al., 2010). The Task Force notes more research is needed to elucidate how testosterone effects erythropoiesis and causes an increase in Hct.
Testosterone Suppresses Hepcidin in Men: A Potential Mechanism for Testosterone-Induced Erythrocytosis. Bachmen et al., 2010.

Hepcidin

Hepcidin regulates the amount of iron (Fe) available for RBC production by competing for Fe absorption sites in the small intestine. Hepcidin binds to the Fe transport protein, therefore preventing Fe from binding and being transported into the body for RBC production (Guo et al., 2013).

Bachmen et al hypothesized that testosterone increases Hct by decreasing the concentration of hepcidin. Hepcidin is a liver-derived peptide responsible for regulating the availability of Fe within the body. Increased levels of hepcidin correlate with a decrease in systemic Fe bioavailability and result in mild anemia. Conversely, decreased levels of hepcidin increase iron bioavailability and therefore lead to an increase in RBC production (Bachmen et al., 2010).

To test their hypothesis, men were stratified based on age and varying doses of TRT for 20 weeks. In order to generate stable levels of testosterone, all subjects received monthly injections of GnRH agonist to suppress endogenous testosterone production (Bachmen et al., 2010). Subjects received weekly doses of 25, 50, 125, 300, or 600 mg TRT to produce the 5 cohorts of subjects within each age group (Figure 14 and 15) (Bachmen et al., 2010). Finally, testosterone, free testosterone, Hct, Hb, serum erythropoietin, ferritin, serum iron, iron binding capacity, and percentage saturation were measured at week 0, 1, 2, 4, 8, and 20 (Bachmen et al., 2010).
Figure 14. Testosterone Dose-Dependently Suppresses Serum Hepcidin (Bachman et al., 2010).

TRT Effects on Hepcidin

Testosterone increased Hct and Hb dose-dependently. High testosterone levels results in a 60% suppression of serum hepcidin levels within one week and all dose groups experienced at least a 50% decline in hepcidin. Serum hepcidin levels were suppressed in the higher testosterone dose groups in younger men and dose-dependently suppressed in older men. Graded increases in serum testosterone were associated with graded suppression of serum hepcidin to a maximum of 60% in the 300 and 600 mg/week groups. Testosterone dose was highly correlated with suppression of hepcidin and this effect remained highly significant throughout the treatment duration. They estimate a 100 ng/dl increase in serum testosterone level was associated with a 14.9% decrease in serum hepcidin (Bachmen et al., 2010).
Figure 15. Effects of Testosterone on Hepcidin (Bachman et al., 2010)

**Conclusion**

Older men had higher baseline hepcidin levels and experienced significantly greater suppression of hepcidin levels for any dose of testosterone compared to younger men. Hepcidin levels at week four were inversely correlated with Hct levels at week 16; however, a greater level of significance was seen in older men. Therefore, men with the
greatest change in hepcidin levels also had the greatest risk of developing erythrocytosis during TRT (Bachmen et al., 2010).
Testosterone Administration Inhibits Hepcidin Transcription and is Associated with Increased Iron Incorporation into Red Blood Cells. Guo et al., 2013

TRT Effects on Hepcidin

Guo et al show that testosterone regulates the amount of hepcidin and causes an increase in Fe availability for RBC production. Gue et al were also able to demonstrate how testosterone influences hepcidin availability. It was shown that testosterone activates the Androgen Receptor (AR) and interferes with bone morphogenetic protein (BMP)/Smad signaling to reduce hepcidin transcription (Guo et al., 2013).

Adult mice were administered testosterone via subcutaneous implants. After a lag of two days, a gradual increase in Hct was observed. Significantly higher levels of Hb and Hct were seen two weeks post testosterone administration as compared to mice in the control group. Serum Fe, transferrin saturation, reticulocyte count, and reticulocyte Hb ratio were also significantly higher in the testosterone group than placebo group (Guo et al., 2013). However, total Fe binding capacity was significantly lower in the testosterone group. Therefore, it was evident that TRT stimulates erythropoeisis and increases markers of Fe bioavailability for erythropoeisis (Guo et al., 2013).

Testosterone administration was associated with nearly a 70% decrease in hepcidin availability (Guo et al., 2013). To further elucidate the effects of testosterone on hepcidin availability, mice with an over-expression of hepcidin gene (Tg+) and mice carrying a silent hepcidin gene (Tg-) were administered TRT. Hepcidin mRNA
expression was significantly higher in Tg+ than in Tg-. Therefore, Guo et al 2013 postulated that if hepcidin suppression were essential for mediating testosterone’s effects on Hb, then TRT would not increase Hb in Tg+ mice. TRT did not cause any significant increases in Hb or Hct in Tg+ mice. However, TRT-treated Tg- female mice exhibited significant increases in Hb and Hct compared to baseline. These data indicate that regulation of hepcidin by TRT is important for mediating TRT-induced increases in Hb (Guo et al., 2013).

**RBC Fe**

The Fe available for erythropoiesis is provided either from dietary intake or from the recycling of Fe in RBCs (Guo et al., 2013). The recycling of Fe from old RBCs provides the majority of Fe available for new RBC production over short durations. However, the absorption of Fe through dietary intake regulates the availability of Fe over long periods. The spleen produces proteins responsible for the recycling of RBC Fe. Ferroportin is one such protein produced by the spleen that recycles RBC Fe (Guo et al., 2013).

Guo et al demonstrated that the spleens of TRT-treated mice had significantly higher expression of ferroportin as compared to non-TRT-treated mice. Furthermore, TRT-treated mice had significantly lower Fe stores within the spleen. This result was expected, as ferroportin promotes Fe transport out of the spleen. Therefore, increased
ferroportin expression would increase the iron recycled by spleen back into blood and therefore reducing the amount of Fe in the spleen (Guo et al., 2013).

Guo et al used an intravenous injection of Fe tracers to determine the effects of TRT on Fe incorporation into RBCs. The previous findings of increased serum Fe, increased transferring saturation, and decreased splenic Fe retention were expected to favor Fe incorporation into RBCs for heme/Hb synthesis (Guo et al., 2013). Indeed, the amount of Fe available for RBCs was much larger in testosterone-treated mice than in the control group (Guo et al., 2013).

Erythroid cells incubated with either serum from testosterone-treated mice or serum from non-testosterone-treated mice were used to determine if increases in Fe availability in TRT-treated mice was associated with increased Hb synthesis (Guo et al., 2013). Incubation with testosterone-treated mice was associated with significantly greater Hb accumulation than with serum from the control group (Guo et al., 2013).

**TRT and Hypoxia**

It has been shown that TRT causes several physiological adaptions, for example increased Hb, Hct, EPO, and increased tissue capillarity that are similar to those induced by exposure to high altitude or hypoxia (Guo et al., 2013). Hypoxia-inducible factors have been shown to regulate transcription of hepcidin expression; therefore Guo et al determined whether down regulation of hepcidin by TRT is associated with the hypoxia-sensing mechanism. RBC 2,3-BPG levels were higher in testosterone-treated mice, which
would be expected to shift the oxygen/Hb dissociation curve to favor oxygen dissociation. Guo et al. found testosterone did not induce hypoxia-sensing mechanisms in the liver and kidney; instead testosterone induced adaptations that increase tissue oxygen delivery i.e. increased Hb, Hct, and 2,3-BPG (Guo et al., 2013).

**TRT and The Androgen Receptor**

BMPs and their downstream targets are important regulators of hepcidin transcription (Guo et al., 2013). In response to BMP activation, Smad1/5/8 translocates into the nucleus of the cell and binds to Smad4 (Guo et al., 2013). The Smad1/5/8-4 complex then binds to other cofactors to form a transcription factor (Guo et al., 2013). The transcription factor then binds to BMP-responsive elements located in the hepcidin gene to generate transcription (Guo et al., 2013). Inhibitors of Smad1 have been shown to decrease hepcidin expression (Guo et al., 2013).

Guo et al found TRT to increase liver AR expression however, TRT increased rather than decreased Smad1 in the liver. With the increase in AR expression after testosterone injection, the association of AR with Smad1 and Smad4 was enhanced. Guo et al concluded that AR associates with Smad1 and Smad4 via direct binding, thereby impairing hepcidin transcription. Guo et al also proved that TRT reduced the protein-DNA binding between Smad1 as well as Smad4, and the BMP/Smad-response elements in the hepcidin promoter. Therefore, TRT influenced the amount of hepcidin expression mostly at the level of gene transcription (Guo et al., 2013).
Pharmacogenetics of Testosterone Replacement Therapy. Zitzmann et al., 2007.

Introduction

TRT has been used for about 60 years with inter-individually different responses (Zitzmann, 2009). One hypothesis for the different responses seen in patients is the pharmacological effect of TRT on the AR gene. Pharmacogenetics is a new field of medicine dedicated to individual differences in genetic make-up and how that affects the response to medication. Zitzmann notes that the diagnosis of LOH has been constrained by strict definitions of thresholds for normal serum testosterone concentrations. New research into symptom specific thresholds for LOH and genetically determined degrees of testosterone action, are changing the concept of normal testosterone levels (Zitzmann, 2009). In the future, individually tailored TRT may become available to optimize the risk benefit ratio of LOH men (Zitzmann, 2009).

The AR is located within the cell and it is structurally related to other steroid hormone receptors (Zitzmann, 2009). After entering the cell, testosterone interacts with the ligand-binding pocket of the AR and activates a cascade that ultimately results in either activation or repression of target gene transcription (Zitzmann, 2007).
Clinical Implications of Androgen Receptor Polymorphisms

The normal development of males is regulated by the interaction of testosterone with the AR in target tissues (Zitzmann, 2009). A range of clinical conditions has been correlated with mutations in the AR (Zitzmann, 2009). Differences in transcriptional activity caused by the AR have been attributed to a polyglutamine stretch in the AR receptor (Zitzmann, 2009). This polyglutamine stretch is encoded by a variable number of CAG triplets in exon 1 of the AR gene (Figure 16) (Zitzmann, 2009). The normal range of CAG repeats is between 9-37 (Zitzmann, 2007).
Figure 16. Display of The X-chromosome with the AR gene (Zitzmann, 2007)
The modulatory effect on AR gene transcription appears to be linear and regulated by coactivator proteins of the polyglutamine stretch (Zitzmann, 2009). Men presenting with features of LOH may have normal testosterone levels but also have CAG repeat lengths above the normal range. A CAG repeat length longer than 25 is still considered to be within the normal range, however it is probably associated with a decrease in testosterone action (Zitzmann, 2009). This particular patient would have signs and symptoms suggestive of LOH but have testosterone levels within the normal range. Therefore, this patient would not be eligible for TRT based off of the Task Force’s recommendations for the evaluation and treatment of LOH with TRT.

Pharmacogenetic Implications of the CAG Repeat Polymorphism

In 48 hypogonadal men with Klinefelter syndrome, baseline LH and Hb concentrations were not associated with CAG repeat length (Zitzmann, 2009). However after TRT, suppression of LH concentrations and elevation of Hb concentrations were more significant in men with shorter CAG repeats (Zitzmann, 2007).

A Hypothetical Model of Androgen Action

Testosterone levels within the normal reference range will mostly saturate ARs. Studies have shown the androgenic effects of TRT reach a plateau at certain levels, most likely due to total AR saturation (Zitzmann, 2009). Significant increments in the effects of TRT caused by increasing testosterone concentrations are only seen beyond the normal
reference range (Zitzmann, 2009). It can be assumed that genetically determined functional differences in AR activity can be best observed within the normal range of testosterone (Zitzmann, 2009). However, in LOH androgenicity will be dependent on amount of testosterone available to bind with ARs (Zitzmann, 2007).

When examining the effects of testosterone, regression models are required to include both testosterone concentrations and the length of the AR CAG repeat polymorphism (Zitzmann, 2007).

During TRT of hypogonadal men, effects of testosterone will be induced by the increase in testosterone levels from the low into the normal range. TRT will also be mediated through the AR CAG repeat polymorphism (Zitzmann, 2009). The effect of the AR CAG repeat does not become a factor until normal testosterone levels have been reached (Zitzmann, 2007).

However, testosterone effects seem to follow a nonlinear pattern, when baseline total testosterone levels and AR CAG repeat length taken into account at the same time (Zitzmann et al., 2007). Zitzmann et al present a nonlinear model derived from general hormone kinetics to describe the effects of testosterone treatment on Hct (Figure 17). In agreement with previous studies, an AR CAG repeat length greater than 25 seems to require testosterone levels above the normal range (Zitzmann et al., 2007). Men with AR CAG repeat lengths larger than 25 may present with signs and symptoms suggestive of LOH but have normal testosterone levels (Zitzmann et al., 2007). According to the number of AR CAG repeats, the testosterone level may be individually tailored to achieve an optimal risk benefit ratio (Zitzmann, 2007).
Figure 17. Nonlinear Pharmacogenetic Model to Describe the Effects of T Levels and AR Polymorphism on Hct (Zitzmann, 2007)

Conclusion

Zitzmann et al concluded that a general threshold for testosterone levels in the diagnosis of LOH might not exist. They propose there may be individual thresholds of testosterone levels according to the length of the AR CAG repeat polymorphism. This could have a pivotal role when deciding whether or not to start TRT in men with LOH (Zitzmann et al., 2007).

In the future, TRT could be modified by determining the AR CAG repeat polymorphism; men with shorter AR CAG repeats might require lower doses of TRT.
while men with longer repeats might need higher doses to reach normal levels (Zitzmann et al., 2007).

Zitzmann et al believe nonlinear calculation models could be used to assess the proper dose of TRT needed to reach the androgen effect while avoiding any adverse effects (Zitzmann et al., 2007).
RESULTS

The amount of research into the role testosterone plays in the physiology and pathology of older men has progressed rapidly over the last two decades. It has become evident that there is a decline in testosterone levels that are associated with older age. New research has shown testosterone to have a large impact on functions not related to the classical male hormone actions thought of in the past (Zitzmann, 2008). Low testosterone can cause anemia, osteoporosis, decrease of lean body mass, and an increase of body fat content (Zitzmann, 2008). There are also a number of psychological complaints of low testosterone such as fatigue, aggressiveness, decrease of cognitive abilities and depression (Zitzmann, 2008). Unfortunately, TRT does have side effects, the most common being excessive erythropoeisis and an increased Hct.

Testosterone’s stimulation of erythropoeisis is considered beneficial because a low RBC count is often found in men with low testosterone (Bhasin et al., 2010). In addition, anemia may contribute to complaints of weakness and fatigue, both of which are sings and symptoms suggestive of LOH. However, TRT may increase RBC production more than is desired and cause the blood to become too viscous (Bhasin et al., 2010). Hct values greater than 52% are associated with increased blood viscosity, and there is evidence, from studies unrelated to TRT, that suggests elevated Hct can result in cerebral ischemia (Bhasin et al., 2010). However, it remains unclear whether high Hct caused by TRT has the same relevance on hemostasis. Although TRT has been shown to increase Hct, the incidence of cerebral vascular events hast not been affected as compared
to non-TRT treated men (Bhasin et al., 2010). It has also been shown that the effect of TRT on Hct is dose as well as age related (Zitzmann, 2007).

Zitzmann demonstrated that testosterone exerts its actions via ARs leading to gene transcription. The higher the number of CAG repeats, the lower the transcriptional activity of the AR (Zitzmann, 2008). It was also shown that short AR CAG repeats contribute to elevation of Hct during TRT. This mechanism impacts on both effects and side effects of TRT (Zitzmann, 2008). The AR CAG repeat polymorphism in the treatment of LOH with TRT seems to be of importance (Zitzmann, 2007). The normal length of AR CAG repeats is 9-37; longer lengths are associated with less physiological activity induced by TRT in vitro (Zitzmann, 2007).
DISCUSSION

In men, testosterone levels decrease in a slow progressive way as part of the normal aging process (Yassin, 2006). The age related decline of testosterone will rarely be manifest in men under the age of 50 years and becomes usually only quantitatively significant in men over 60 years of age (Yassin, 2006). It is still controversial whether the age related decline of plasma testosterone levels constitutes a true clinical entity and whether this condition needs to be treated via TRT (Zitzmann, 2008). Professional organizations have formulated guidelines/recommendations for the administration of testosterone to elderly men (Bahsin, 2010). This demonstrates that there is a common agreement among experts in regard for the need to define LOH in various stages of the male life (Zitzmann, 2008).

A coworker of Dr. Steinach once noted that even if the restoration of libido was considered immoral or dangerous, the rejuvenation restores ones ability to work and improves the quality of life (Sengoopta, 2003. The “Ability to work” has been a key phrase in the history of TRT. The ‘inability to work’ is a common complaint of patients’. Patients’ electing to undergo treatment frequently report ‘enhanced energy and efficiency’ the earliest improvements after treatment (Sengoopta, 2003).

In 1960 a Swiss genitourinary surgeon, Paul Niehans, published the book *Introduction to Cellular Therapy* in which he envisions the replacement of organ transplantation by the injection of viable cells. For example, under functioning organs were treated with cells of the same organ, and cells of the antagonistic organ were
injected in cases of over function. Niehan believed this technique was a method for treating the whole organism on a biological basis, capable of revitalizing the human organism with trillions of cells by bringing to it those embryonic or young cells that it needs. Niehan contented that the injection of testicular cells increased the long-term excretion of testosterone derivatives. His patients included Pope Pius XII, Bernard Baruch and Aristotle Onassis.

Low testosterone is a common but not mandatory condition in elderly men. There are numerous indications that TRT has beneficial effects. Special care must be taken concerning erythropoiesis. Following the guidelines as specified by a number of professional organizations, truly testosterone deficient elderly men can be responsibly treated via TRT (Zitzmann, 2008).
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CURRICULUM VITAE

Michael Andrew Vrolyk
345 Commonwealth Ave Apt. #3, Boston, MA 02115
drew@vroly.com
(508) 341-6120
Year of birth: 1987

Education

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Work Experience

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Certification

Emergency Medical Services, valid April 1, 2010 through April 1, 2016
CPR and AED, valid March 1, 2010 through March 1, 2016