Endocrine Society

Conference Courier Highlights Report on Hypogonadism

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Hypogonadism has a significant impact on men's overall health and well-being. This important subject was the focus of 3 symposia, a workshop, numerous posters, and a case management forum at the 92nd Annual Meeting of the Endocrine Society (ENDO 2010), in San Diego, California, June 19 to 22, 2010. One of the most common themes at ENDO 2010 was the role of circulating endogenous testosterone in vascular morbidities and the effect of testosterone therapy for hypogonadal men with or at risk for cardiovascular or cerebrovascular events. A symposium was dedicated to the topic, as were a lecture and several poster presentations.

The purpose of this Conference Courier is to review some of the fundamental information and report the newest data on hypogonadism and its management as presented at ENDO 2010.

CASE MANAGEMENT FORUM

Diagnosis and Management

Ronald S. Swerdloff, MD, briefly reviewed basic information about late-onset hypogonadism (LOH), with updates from the most recent literature, in "Diagnosis and Treatment of Borderline Hypogonadism in the Aging Male," a session supported by Auxilium Pharmaceuticals, Inc. Risk factors for LOH include age, obesity, and the presence of certain comorbidities or chronic health conditions.[1,2] The overall prevalence of LOH in the European Male Aging Study (EMAS) population was 2.1%, which rose with increasing age from 0.1% in the fifth decade to 5.1% in the eighth decade (Figure 1A), increasing obesity (Figure 1B), and number of comorbidities (Figure 1C).[3] In healthy men, aging is associated with an annual decline, after age 40 years, of about 1% to 2% in total (TT) and free testosterone (FT) levels.[4] Functional

alterations associated with aging and hypogonadism result from defects at all levels of the hypothalamic-pituitary-gonadal axis, with decreased production of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and subsequent decline in spermatogenesis.

Guidelines recommend making a diagnosis of hypogonadism only in men with unequivocally low testosterone levels and consistent characteristic signs and symptoms.[1,2] "Unequivocally low" means that testosterone levels should be outside the reference range for young, not age-matched, adult men. Peak testosterone levels in men with a normal wake-sleep cycle occur in the morning; therefore, blood for TT measurement should be drawn

Figure 1. The prevalence of LOH in the EMAS overall and stratified by age (A), BMI (B), and comorbidity expressed as the number of coexisting illnesses (C). The syndrome of late-onset hypogonadism was defined by at least 3 sexual symptoms associated with total testosterone levels of <11 nmol/L and free testosterone of <220 pmol/L. BMI, body mass index; EMAS, European Male Aging Study; LOH, late-onset hypogonadism.
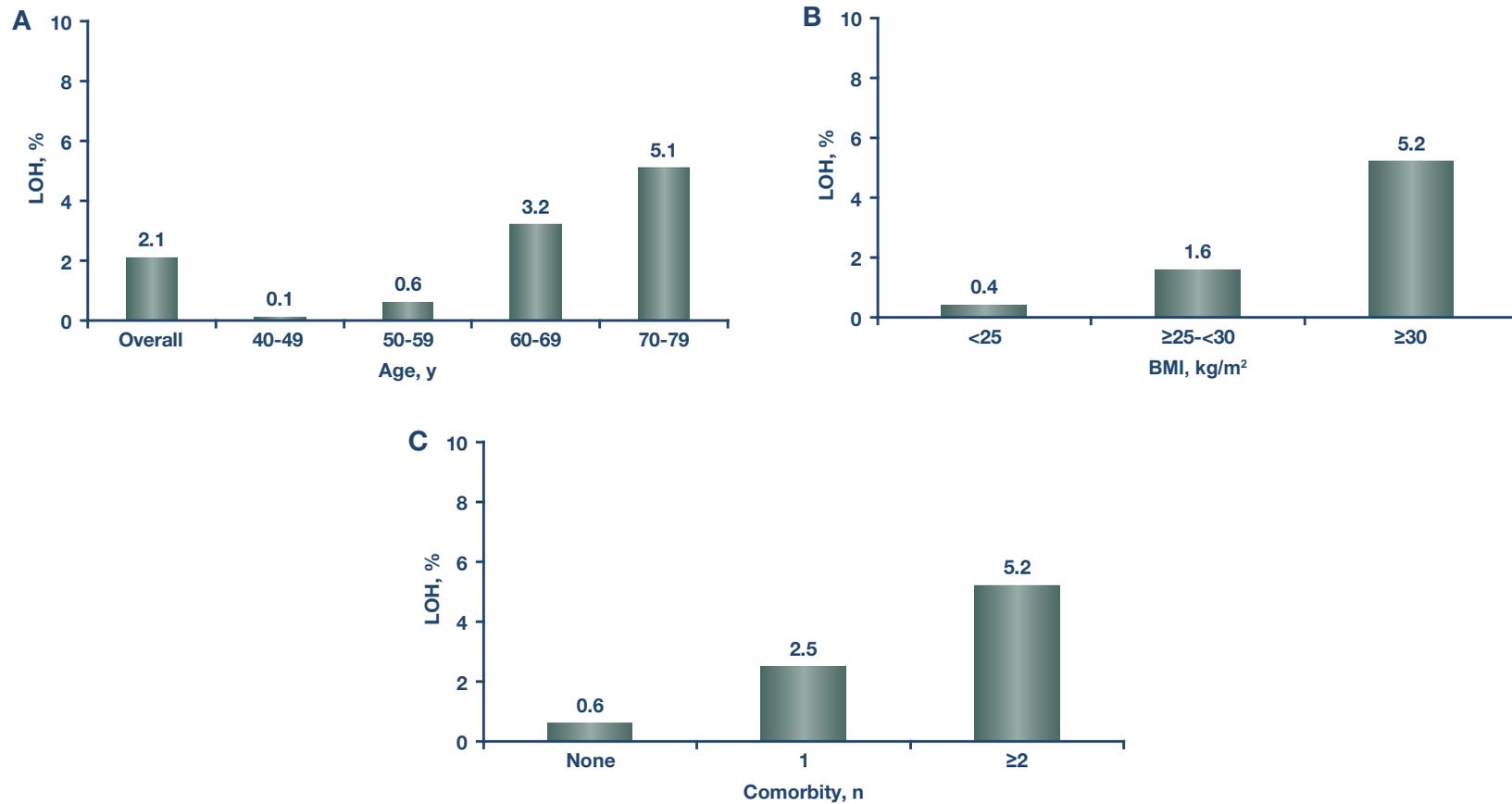
between 7:00 and 11:00 AM. The initial measurement should be TT. If the testosterone level is low or equivocal on the initial measurement, the test should be repeated. Approximately one-third of low testosterone measurements will be normal at the second measurement. If the diagnosis is still unclear, calculation of FT or direct measurement of FT by equilibrium dialysis, or calculation or measurement of bioavailable testosterone by ammonium sulfate precipitation is recommended. The currently preferred method for measuring TT is liquid chromatography followed by tandem mass spectrometry (LC-MS/MS), although many immunoassays are acceptable. Many factors cause reference ranges to vary between assays and laboratories. Always use the reference range provided by the laboratory that performed the test. If the TT level is still equivocal, FT should be measured directly by equilibrium dialysis. Calculated FT is dependent on the quality of TT and sex hormone-binding globulin (SHBG) assays and is acceptable if the formula has been well validated by correlation with equilibrium dialysis.

Symptoms are generally nonspecific and, as men age, comorbid conditions may play an increasing role. Among 3219 men from 8 European centers enrolled in the EMAS, Wu et al correlated low testosterone levels with 9 symptoms in 3 categories: sexual (reduced libido, loss of morning erections, erectile dysfunction [ED]), physical (inability to perform vigorous activity, walk >1 km, or bend, kneel, or stoop), and psychological (loss of energy, sadness or low mood, fatigue).[3] The probability of symptoms was inversely related to testosterone levels, and different symptoms had different thresholds for TT.

Examples are as follows:

- Decreased frequency of sexual thoughts: 8 nmol/L (230 ng/dL)

- ED: 8.5 nmol/L (250 ng/dL)

- Decreased frequency of morning erections: 11 nmol/L (320 ng/dL)

- Diminished vigor: 13 nmol/L (370 ng/dL)

The FT thresholds were 160, 280, 280 pmol/L (46, 81, 81 pg/mL) for the 3 sexual symptoms, respectively, and 160 pmol/L for sadness and fatigue. FT thresholds were not identified for physical symptoms nor TT thresholds for psychological symptoms.

Testosterone levels have been independently associated with reduced hemoglobin levels, and men with TT levels <10 nmol/L or FT <0.23 nmol/L have the highest likelihood of anemia.[5] Anemia commonly occurs in patients with type 2 diabetes and has been correlated with older age and the presence and severity of chronic kidney disease, systemic inflammation, and reduced iron availability. Grossmann et al suggested that erythropoiesis could be a marker for testosterone action.

Testosterone therapy for otherwise healthy hypogonadal men decreases fat mass and increases lean mass, muscle strength, and bone mineral density (BMD).[1,6-8] Randomized controlled trials in men without diabetes have also demonstrated significant improvements in waist:hip ratio,[9] physical performance,[9,10] total cholesterol,[9] low-density lipoprotein (LDL),[9] leptin,[9,10] mood, and libido[7] and increases in hemoglobin.[10]

Managing ED in hypogonadal men is more complicated. Because ED has arteriogenic, psychogenic, and neurogenic causes, men with concomitant ED and hypogonadism may benefit from combined therapy with phosphodiesterase type 5 (PDE5) inhibitors and testosterone.[11-16]

Frederick C. Wu, MD, discussed 2 cases involving situations physicians may commonly encounter.

Case 1. A 72-year-old man presented with complaints of ED, diminished libido, fatigue, and a decrease in exercise. He was taking medications for hypertension and high cholesterol. His prostate was enlarged but without nodules. An afternoon blood sample showed TT of 270 ng/dL and prostate-specific antigen (PSA) of 3.7. Repeat TT and FT measurements were normal (360 ng/dL and 68 ng/dL, respectively), as were SHBG, LH, and FSH. Despite the symptoms, most of which were nonspecific, this patient does not meet the guidelines for treatment.

Case 2. A 68-year-old man with metabolic syndrome presented with diminished libido, spontaneous erections, stamina, ability to concentrate, and memory. His initial and repeat TT levels were 260 ng/dL and 268 ng/dL, respectively. SHBG was within normal range at 16 ng/dL. The patient was started on testosterone enanthate. Unhappy with the modest improvement and with an increase in hematocrit, he discontinued treatment. The patient refused all further therapy but was determined to make major lifestyle changes. After losing 20 kg and reducing his body mass index (BMI) to 32, testosterone levels increased into the low-normal range and sexual function improved.

Discussion of Cases. Diagnosis of LOH presents a number of challenges, and guidelines for testosterone measurement were described by Dr Swerdloff. Testosterone therapy is associated with substantial benefits for the appropriate candidate but is also accompanied by potential risks, and some special precautions should be taken. The patient in Case 1 had a PSA level near the upper limit of normal. Although the risk of prostate cancer increases with increasing PSA, an elevated PSA alone provides limited information because it may have a variety of potential causes, increases normally with age, is not specific to prostate cancer, varies widely between assays, and is dichotomous. There is no absolute level that indicates whether cancer is present; rather, the results present a continuum of increasing risk.[17] PSA values overlap substantially between men who have prostate cancer and those who do not. Family history, results of digital rectal examination (DRE), and results of a prior biopsy are also risk factors that should be considered.

A metaanalysis of the effects of testosterone therapy on prostate cancer and biopsy showed no increase in prostate cancer, International Prostate Symptom Score (IPSS), lower urinary tract symptoms, or cardiovascular outcomes.[18] Although this is reassuring, no study has been powered to detect a significant increase in clinical prostate cancers; the risk must still be assessed. For men 40 years of age or older with PSA >0.6 ng/mL, the Endocrine Society guidelines recommend DRE and PSA measurements before initiating treatment, at 3 and 6 months, and then in accordance with evidence-based guidelines for prostate cancer screening by age and race of the patient.[1] Patients should be monitored for symptomatic improvement and adverse effects at 3 and 6 months and then annually. Testosterone levels should be checked at 3 and 6 months after beginning therapy. Timing of blood draws within the dosing interval depends on the formulation used. Hematocrit should be checked at 3 and 6 months and then annually. Patients should be referred for further urologic evaluation if PSA concentration increases more than 1.4 ng/mL in any 12-month period, PSA velocity is more than 0.4 ng/mL/y using PSA after 6 months of therapy as a reference, a prostatic abnormality is detected by DRE, or IPSS is >19.[1] Therapy is stopped with a hematocrit level above 54% until it decreases to a safe level, the patient is evaluated for hypoxia and sleep apnea, and then therapy is reinitiated at a reduced dose.[1]

SYMPOSIA

Testosterone and Sleep

The "[National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)] Anniversary Symposium: Testosterone and the Male Brain," chaired by Adrian S. Dobs, MD, MHS, featured 3 talks on important aspects of testosterone deficiency delivered by experts in the field.

In "Testosterone and Sleep: 20 Winks or Death by Snoring," Peter Y. Liu, MD, discussed the current data on the relationship between testosterone levels and sleep/sleep breathing. With the increasing use of testosterone therapy in older men, understanding the potential effect, if any, of testosterone on sleep is important. Impaired gonadal function, obesity, metabolic syndrome, and obstructive sleep apnea (OSA) commonly occur together in men.[19] Investigation of the relationship between hormones and sleep is confounded, however, by the effects of age and obesity, both of which decrease testosterone levels and alter sleep patterns. Slow-wave sleep (deep sleep, stages 3 and 4 of non–rapid-eye-movement (NREM) sleep increases, and stage-1 sleep (transition stage between sleep and wakefulness) decreases. Sleep-disordered breathing and OSA also increase and are more common in men than in women. Among a subset of men 65 years of age and older participating in the Osteoporotic Fractures in Men Study (MrOS; N=1312), total sleep duration was associated with adiposity but not with age. Sleep efficiency and awakenings after the onset of sleep were significantly reduced for the lowest testosterone quartile (<250 ng/dL) compared with all other quartiles, but sleep duration, time in slow-wave or rapid-eye-movement (REM) sleep, and arousal frequency did not differ across quartiles of testosterone.[20]

Induced sleep disturbances in younger men can cause hormone changes similar to those seen with aging, suggesting an effect of sleep on hormone levels. An observational study of 12 healthy men 64 to 74 years of age found that a large part of the interindividual variability in morning TT and FT levels is explained by the amount of nighttime sleep.[21] Sleep-disordered breathing can suppress gonadal function, and gonadal hormones can, in turn, acutely alter sleep breathing.[19,22] OSA with hypoxia causes reversible neuroendocrine dysfunction, resulting in decreased levels of LH, followed by reductions in testosterone during the night and suppressed morning testosterone levels.[23,24] Baseline severity of OSA has been correlated with decreased testosterone.[23,25] Studies on the effect of nasal continuous positive airway pressure on LH and testosterone levels have had inconsistent results.

Although the diurnal rhythm in testosterone is maintained in healthy men well into the seventh decade, aging significantly blunts the diurnal variation in testosterone levels.[26] In healthy young men, testosterone secretion and levels increase during sleeping and decrease during waking hours, and circadian effects are small.[27] Thus, blood draws to establish testosterone levels for a night worker should be done after awakening, not in the morning. The normal nocturnal rise in testosterone levels occurs just before the onset of REM sleep but is attenuated by preventing REM sleep.[28] At night, in middle-aged men, it has been demonstrated that less pulsatile testosterone and more LH are secreted compared with younger men, and the association between testosterone rhythm and REM sleep is disrupted.[29] Consequently,

dysfunction of the pituitary-gonadal axis has been implicated as a risk factor in men with OSA.[30]

The effect of testosterone therapy on sleep and sleep-disordered breathing is not well defined. Most of the studies have been small, not randomized or placebo-controlled, had short durations of treatment, and were conducted in young men using testosterone enanthate, and results have been inconsistent. One randomized controlled trial crossover study in 16 older men (mean age, 68 y) found that short-term (3 wk) treatment with injections of high-dose (500, 250, and 250 mg) mixed testosterone esters significantly shortened sleep and worsened sleep apnea compared with placebo, despite improvement in body composition.[31] The changes did not appear related to airway narrowing. No data were available, however, on long-term therapy at lower doses. Liu and colleagues conducted a randomized controlled trial in obese

men with OSA using lower doses of testosterone. Preliminary analysis shows that testosterone increased and LH decreased in the treated group, but weight and BMI did not differ, because both groups were on a weight-loss program (unpublished data). Sleep time and REM:NREM sleep were also similar. Hypoxia increased at 6 weeks but normalized with continued therapy. The augmentation index for arterial stiffness and the ratio of peripheral to central pulse pressure increased.

Liu et al concluded that very high-dose exogenous testosterone in older men impairs sleep and sleep breathing. Lower doses have an early adverse effect on sleep and sleep breathing, but it appears to reverse with continued long-term therapy. Thus, the short- and long-term effects of testosterone therapy on sleep and sleep breathing may differ substantially. Larger, randomized, placebo-controlled trials are needed to clarify the effects of testosterone therapy on sleep and sleep breathing.

Testosterone and Sexual Function

Giovanni Corona, MD, PhD, discussed "Testosterone and Sexual Function," citing his own and colleagues' previous and as-yet-unpublished data on the association between low TT levels and the organic, intrapsychic, and relationship domains of ED.

Using two new instruments—the 13-item Structured Interview on Sexual Dysfunction (SEIDY) and a structured interview to detect hypogonadism in men with ED (ANDROTEST)—Corona and colleagues demonstrated

that low testosterone levels are associated with decreased autoeroticism,[32] infidelity, and reduced frequency of sexual intercourse.[33,34] Testosterone levels correlated negatively with symptoms of depression and anxiety.[35] When data were analyzed by personality type, the histrionic/hysterical personality was associated with better sexual function; greater androgenization, indicated by a higher TT and FT level, greater testicular volume, and fewer signs and symptoms of testosterone deficiency; and more satisfying sexual relationships. Thus,

a good relationship, satisfying sexual activity, and higher testosterone levels appear to be interrelated, with bidirectional effects.

Testosterone appears to facilitate the control of penile ejaculation. Older men (age, 55 to 70 y; n=121) with delayed ejaculation had significantly lower TT and FT levels and a higher prevalence (26%) of hypogonadism than those with premature ejaculation (n=714) or no ejaculatory dysfunction (n=1602), even after adjustment for age, libido, and other confounders.[36] The data suggest that premature and delayed ejaculation/anejaculation are two ends of a continuum influenced by endocrine hormones.[37] Prolactin and thyroid-stimulating hormone increased along the continuum, whereas testosterone decreased significantly and independently of age, psychopathology, and use of selective serotonin reuptake inhibitor antidepressants. Clinical studies are ongoing to further define the role of TT in the control of ejaculatory reflex and determine whether testosterone therapy can reverse delayed ejaculation.

The effect of testosterone on erection is modest and primarily involved in the timing of erection as a function of sexual desire.[38] This may be explained, at least in part, by the fact that erections start with an increase in nitrous oxide and a decrease in RhoA/ROCK signaling and end with an increase in PDE5. Because testosterone controls both processes, the net effect of testosterone on erectile function will be modest. However, testosterone increases the effect of PDE5 inhibitors in hypogonadal subjects with ED.[39,40]

Disturbances in the relational domain (SEIDY-2) were associated with less frequent sexual intercourse, severe ED, lower dynamic peak systolic velocity on penile Doppler ultrasound, and symptomatic testosterone deficiency, even after adjusting for risk factors such as age, waist circumference, and smoking.[33] Multiple-regression analysis of the variables found that low penile blood flow and decreased intercourse were bidirectionally linked to a poor relational domain but indirectly associated with hypogonadism via sexual hypoactivity or inertia.[33]

Hypogonadism, ED, obesity, insulin resistance, and metabolic syndrome often coexist in patients, predisposing them to cardiovascular disease (CVD) and type 2 diabetes.[41] In the organic domain, low testosterone may be the link between cardiovascular risk and ED.[42] An observational study of 1687 men with ED and baseline testosterone levels were interviewed using the SEIDY and ANDROTEST and followed for a mean of 4.3 (2.6) years.[43,44] After adjustment for age and chronic diseases in a Cox regression model, fatal major adverse cardiac events (MACE) were significantly associated only with testosterone level below 8 nmol/L (230 ng/dL).[43] In addition, lower penile blood flow, measured by color Doppler ultrasound both before and after prostaglandin E1 stimulation (flaccid and dynamic conditions, respectively), was significantly associated with greater risk for MACE.[44] It is notable that high sexual interest in the partner plus low sexual interest in the patient protected against MACE.

In summary, testosterone directly or indirectly controls male sexual function both centrally and peripherally. Data obtained by Corona et al support the concept that testosterone in men both helps to determine and reflects changes in all 3 dimensions of ED: organic (eg, physical illness), intrapsychic (eg, reaction to stress), and relational (eg, difficulties in couple relationships).[38,42]

Testosterone and Endothelium Function

Cerebrovascular Risk

The third talk in the NIDDK anniversary symposium, "Testosterone: Cause of [Cerebrovascular Accident] or Stroke of Fortune?" was given by Bu Beng Yeap, MBBS, PhD. Most studies that have evaluated this relationship have found that low testosterone levels were associated with carotid, aortic, and peripheral atherosclerosis[45-48] and increased mortality,[49-54] although some studies were contradictory.[52,55,56] Results of studies evaluating the role of testosterone in predicting vascular events, particularly cerebrovascular events, have been inconsistent, suggesting that testosterone may be just a robust biomarker.

To determine whether lower serum testosterone levels are an independent risk factor for symptomatic cerebrovascular events in older men, 3443 men with no history of stroke and age at least 70 years were identified and followed for 3.5 years in the large Health in Men (HIM) study. After adjustment for known cerebrovascular risk factors, an increased incidence of stroke or transient ischemic attack (TIA) was predicted by lower TT and FT level (hazard ratio [HR], 1.99 and 1.69, respectively) but not by SHBG or LH.[57] Men in the lowest quartile (< 8 nmol/L) testosterone level had the most events, but even men with low-normal testosterone levels (8 to 11.7 mmol/L; n=664) had a significantly increased risk (HR, 2.08).[58] After Cox regression analysis, TT <11.7 nmol/L (n=836) predicted stroke or TIA. Follow-up is ongoing.

Definitive randomized, controlled trials are still needed, and it is unknown whether testosterone therapy administered to normalize testosterone levels will reduce the morbidity and mortality risks. The guidelines make no mention of cerebrovascular disease.[1,2,59] Some experts believe that the threshold for low testosterone may be lower in older men than in younger men. The Endocrine Society guidelines recommend treating older men only with caution and when the potential benefits greatly outweigh the risks and aiming for a TT level in the lower part of the normal range for young men (400 to 500 ng/dL).[1]

In men with low to normal baseline testosterone levels, testosterone therapy increases circulating testosterone levels, increases lean mass, reduces fat mass, and improves cardiovascular dysfunction.[1,2] It has been shown to improve glucose metabolism in some studies[60,61] but not others[62] and to improve metabolic syndrome[61] and other cardiovascular endpoints.[63-67] However, the data are not robust, because many of the studies were small and results were inconsistent. Data are inconclusive regarding whether testosterone therapy for hypogonadal older men would reduce the risk of cerebrovascular events.

Yeap et al concluded that, although good interventional studies are needed to determine whether normalizing testosterone levels will prevent morbidity and mortality from cerebrovascular disease, the data linking low testosterone levels to cerebrovascular events are compelling, and testosterone therapy should be offered to men diagnosed with hypogonadism.

Cardiovascular Risk

As indicated by the title, the symposium "Testosterone and the Male Cardiovascular System: HearTy Fare or Vile PoTion?" focused entirely on the effects of testosterone—both endogenous and exogenously administered—on the male cardiovascular system.

In the introduction to his talk, "FaulTy Heart: What Are the Effects of [Testosterone] on [Congestive Heart Failure (CHF)] and Ischemia?," Thomas Hugh Jones, MD, MB, ChB, BSc, MRCP, FRCP, reviewed evidence that men with coronary artery disease (CAD) have lower androgen levels and a higher prevalence of hypogonadism than men without CAD.[68] Whether these men can be safely treated with testosterone has been controversial, and few studies were conducted until the most recent decade.

"There is no cure for angina pectoris (heart attack), which afflicts hundreds of thousands in the US, but its agonizing pains have been relieved in a number of cases by injections of testosterone propionate."[69] So said *Time* in 1942, reporting on a paper in *Journal of Clinical Endocrinology and Metabolism*.[69,70] Following Huggins and Hodges linkage of testosterone to prostate cancer,[71] case reports continued to be published for several years. Nothing further was published until 1977, when Jaffee reported significant reduction of postexercise ST segment depression by testosterone therapy compared with placebo.[72] Another hiatus lasted until 1993, when Wu and Weng reported relief of angina pain and improvement in myocardial ischemia with no adverse effect in 12 parameters of cardiac function in 62 elderly men with coronary heart disease (CHD).[73] In 1999, Webb and coworkers reported a dose-related increase in coronary diameter up to 4.5% and coronary blood flow up to 17.4% with testosterone therapy in men with CAD.[63] Significant further research has been performed in this area during the past 10 years, but many of the studies were small and of short duration.[74]

Testosterone therapy for men with low or low-normal testosterone levels and stable CHF increased exercise tolerance (Figure 2)[75-77] and muscle/grip strength,[75,77] and improved New York Heart Association heart failure class by ≥1 class in 35% of subjects receiving treatment versus 8% of those receiving placebo (P=.01).[75] Blood pressure (BP) and echocardiogram results remained stable with testosterone but worsened with placebo.[75]

Key evidence for the acute hemodynamic effects of exogenous testosterone in men with CHF was provided by Pugh et al.[78] Administration of 60 mg buccal testosterone to 12 men with stable CHF significantly increased cardiac output and reduced systemic vascular resistance. The investigators suggested that increased cardiac output occurs via reduction of left ventricular overload. Other than one case of facial flushing, no adverse effect related to testosterone administration was noted.

Testosterone has a known vasodilator effect in many vascular beds. The first study to evaluate the effect of testosterone on human pulmonary vasculature in vitro found significant vasodilation at physiologic concentrations only in males, only in pulmonary resistance arteries, and only in pulmonary arteries with good endothelial function.[79]

The effect of lifestyle factors on testosterone[80-82] and the importance of a healthy lifestyle cannot be overemphasized. Cross-sectional analysis of data from men participating in the HIM study showed that a high lifestyle score based on 8 healthy behaviors predicted higher TT and SHBG levels up to 6 years later.[83] The relationship appeared cumulative and independent of BMI, suggesting a possible interaction between lifestyle and insulin

Figure 2. Change in walking distance on the ISWT. The mean change in distance walked was significantly greater for patients taking testosterone than for those taking placebo (P=.006, ANOVA). ANOVA, analysis of variance; ISWT, incremental shuttle walking test.
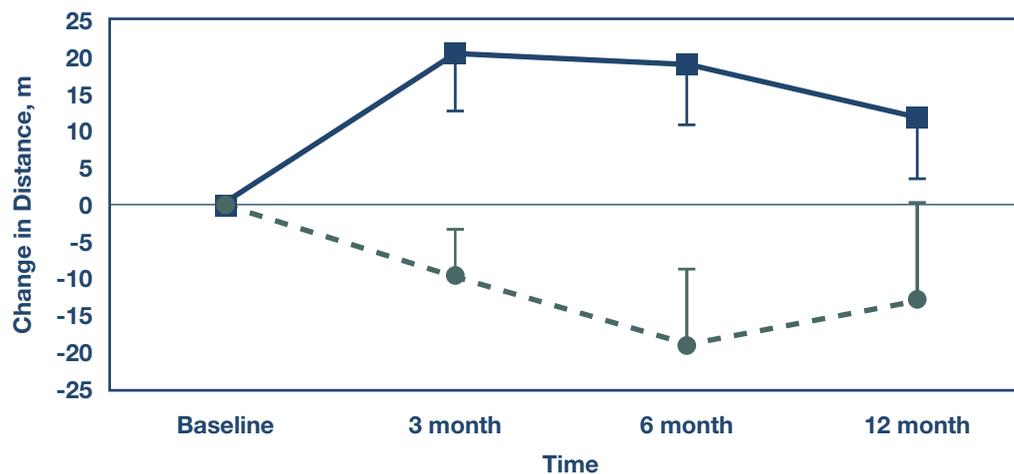
■ **Testosterone**

● **Placebo**

Adapted with permission.[75]

sensitivity. A similar study of nearly 8000 men without CVD found that scores ≤4 increased the risk of dying within 5 years (HR, 1.3) compared with scores ≥5.[84] Therefore, a sustained healthy lifestyle in older men may slow the age-related decline in testosterone levels and prolong survival.

Insulin resistance occurs commonly and is often severe in men with CHF.[85] Significant improvement in fasting insulin sensitivity, according to the homeostasis model assessment of insulin resistance (HOMA-IR),

accompanied by decreased fat mass after two injections of mixed testosterone esters to 13 men with moderately severe CHF suggests a beneficial effect on the metabolic component of CHF.[86]

Long-acting injectable testosterone undecanoate (TU) but not placebo improved glucose metabolism and baroreflex sensitivity in men (median age, 70 y) with moderately severe CHF.[77] Baseline peak oxygen consumption (VO_2) directly related to endogenous testosterone levels, and testosterone

concentrations during treatment correlated with improvement in peak VO$_2$ and maximum voluntary contraction.

A particular concern with use of testosterone in CHF is the potential for water retention, although this has occurred primarily with injectable testosterone esters. In men with hypogonadism and growth hormone (GH) deficiency, injection of testosterone enanthate increased extracellular water and decreased aldosterone.[87] The effects of testosterone therapy were magnified by coadministration with GH. No effect was seen on renin or atrial natriuretic peptide, and the investigators proposed that the mechanism of fluid retention is exerted on the renal tubules. No adverse effects or clinical signs or symptoms of excess fluid retention were observed during the study.

A metaanalysis of 19 studies involving 651 men treated with testosterone and 433 with placebo found that the frequency of cardiovascular events and death did not differ significantly between the two groups (Figure 3).[88] In fact, men with below-normal testosterone levels appear to have a reduced life expectancy (Figure 4).[49,52] In the fourth Tromsø study (1994-1995), the risk of death from any cause for 1568 men with FT in the lowest quartile was significantly higher than for those in the higher quartiles but not independently associated with TT levels.[52] The association of low testosterone levels with death from CVD or ischemic heart disease (IHD) is unclear. A review of noninterventional studies indicates that about half associated low testosterone with an increased risk of death from CVD or IHD and half found no association. Results of two studies presented at ENDO 2010 also addressed this issue:

• With an average follow-up of 3.9 years, higher endogenous TT and FT levels were associated with an increased risk of CHD in 697 elderly men participating in the MrOS study in the United States; survival data are not available[89]

• Among 587 men with type 2 diabetes, cardiovascular and all-cause mortality significantly increased in the cohort of men with TT <8 mmol/L compared with those having TT ≥8 mmol/L after a mean follow-up of 5.8 years[90]

Baseline endothelial-dependent flow-mediated dilatation of the brachial artery (FMD-BA) was found to be significantly higher in 36 newly diagnosed hypogonadal men (symptomatic, TT <12 nmol/L; mean age, 37.5 y) than in 20 normal control subjects, and testosterone levels were inversely associated with FMD-BA in the hypogonadal but not in the eugonadal range.[91] Normalization of circulating testosterone levels was accompanied by a significant decrease in FMD-BA. In light of the benefits of therapy, the investigators concluded that these results are not sufficient to withdraw or prevent testosterone therapy for hypogonadal men but that other risk factors for atherosclerosis should be eliminated or strictly controlled during treatment.

Zitzmann et al concluded that testosterone therapy is likely to have a positive effect on cardiovascular risk factors for hypogonadal men and may benefit subsets of otherwise healthy men. The effects may be adverse, however, and testosterone therapy should be undertaken cautiously for men with comorbidities.

The current prescribing information for all formulations indicates that testosterone should be prescribed with caution in men with CVD and is contraindicated in men with CHF because of the fluid-retention issue. Studies conducted to date, however, have found evidence of clinical benefit without any adverse effect of testosterone on CHF. Larger trials to further study the benefits and establish the safety of testosterone in CHF are warranted.

Figure 3. Forest plots illustrating confidence intervals for odds ratios for all cardiovascular events.
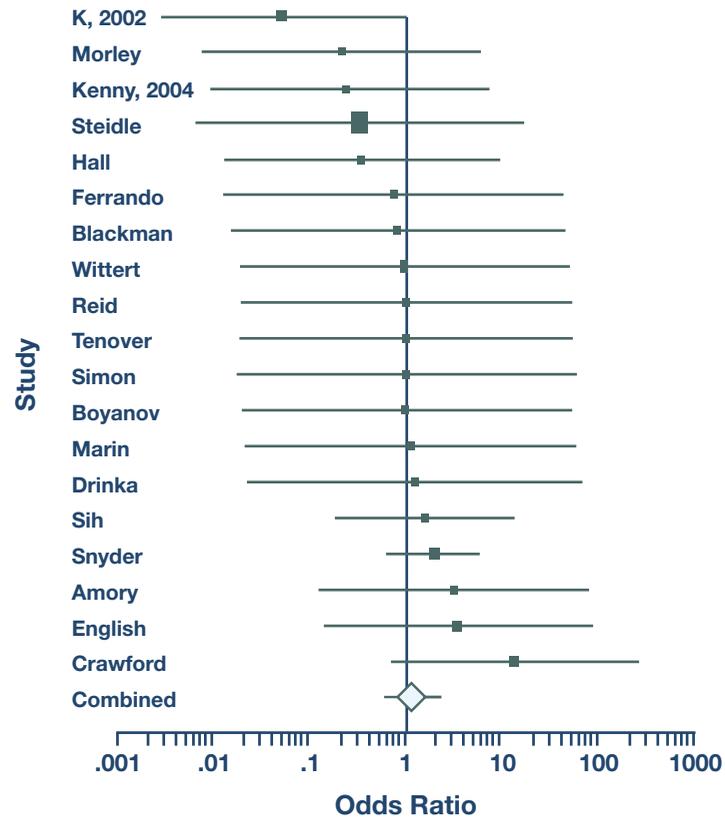
Adapted with permission.[88]

Figure 4. Unadjusted Kaplan-Meier survival curves for the 3 testosterone groups. Men with low and equivocal testosterone levels had a significantly shorter survival than men with normal testosterone levels (log-rank test; χ^2=14.4; *P*=.001).
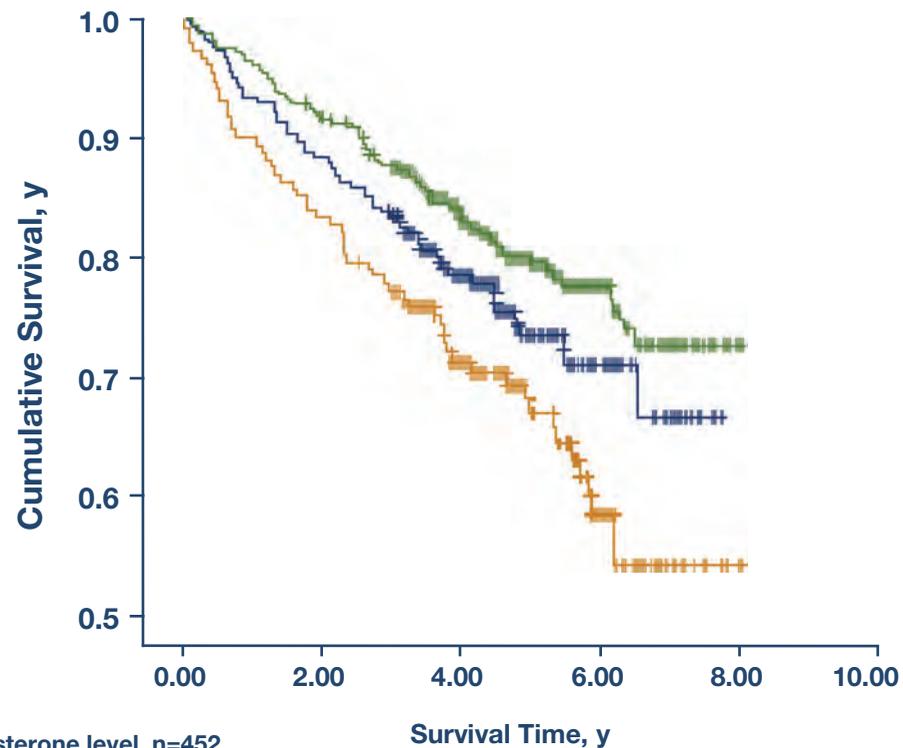
Men with a normal testosterone level, n=452
Men with an equivocal testosterone level, n=240
Men with a low testosterone level, n=166

Adapted with permission.[49]

Factors Contributing to Endothelium Dysfunction

A growing body of evidence indicates that testosterone has broad physiologic effects and that testosterone deficiency is related to increasing comorbidity and death. Though testosterone therapy provides benefits for hypogonadal men, its cardiovascular safety has been a concern. In "Testosterone and Male Blood Vessels: Ship of Fortune or Titanic Wreck?" Michael Zitzmann, MD, PhD, assessed the available data, the current understanding of the effects of testosterone on cardiovascular risk factors, and the relevance of testosterone therapy in hypogonadal men.[92]

Many factors contribute to atherogenesis and the progression of arterial blockage. Because men die of CVD at a higher rate than women,[93] it was long thought that testosterone was the culprit. It is now widely accepted, however, that below-normal levels of testosterone are associated with a variety of symptoms, including many factors also associated with CVD. Among men aged 45 years or older visiting primary care practices in the United States, the risk of being hypogonadal was significantly higher in men with "the deadly four": obesity (odds ratio [OR], 2.38), diabetes (OR, 2.09), hypertension (OR, 1.84), and hyperlipidemia (OR, 1.47) as well as asthma or chronic obstructive pulmonary disease (OR, 1.40) and prostate disease (OR, 1.29).[94] Studies have reported an inverse relationship between low testosterone levels and the development or progression of atherosclerosis. Serum FT levels were associated with progression of intima media thickness of the common carotid artery independent of cardiovascular risk factors (BMI, waist:hip ratio, history of hypertension or diabetes, history of smoking, and serum cholesterol).[48] The intima media thickness should be <1 mm; increasing thickness predicts progression of atherosclerosis and declining cardiovascular health.[95] Proinflammatory cytokines mediate the development of atheromatous plaque and associated complications. A randomized placebo-controlled crossover study found that injections of mixed testosterone esters on days 0, 14, and 28 significantly reduced day 30 inflammatory markers tumor necrosis factor-α, interleukin (IL)-1β, and total cholesterol and increased the antiinflammatory marker IL-10 in 27 older hypogonadal men (mean age, 62 y).[96]

Factor analysis of multiple variables in 113 men showed that testosterone was significantly associated with insulin, leptin, body fat mass, triglycerides, and LDL but not high-density lipoprotein (HDL).[97] Adipose tissue plays a central role in the development of hypogonadism and metabolic syndrome, and the bidirectional interplay between all 3 helps to maintain these conditions, ultimately resulting in hyperinsulinemia and disturbed endothelial function, type 2 diabetes, cardiovascular and cerebrovascular events, and ED (Figure 5).[98]

Adiponectin is an antiatherogenic, antiinflammatory, and antidiabetic protein secreted by adipocytes and believed to influence insulin sensitivity. Serum levels of adiponectin, which are inversely correlated with obesity, abdominal fat mass, and BMI, can be reduced by chemically or surgically induced testosterone deficiency and were found to be significantly higher in hypogonadal versus eugonadal men. Adiponectin levels are reduced to normal eugonadal levels by testosterone therapy.[99,100]

Figure 5. Self-perpetuating pathogenic circle between adverse metabolic parameters, with visceral adipose tissue as a pivotal component. Inflammatory cytokines are shed by adipocytes and cause dysfunctions of the hypothalamic-pituitary-testicular axis, leading to clinically relevant end points and, potentially, increased mortality. The lightning bolt symbols indicate disturbance of function.
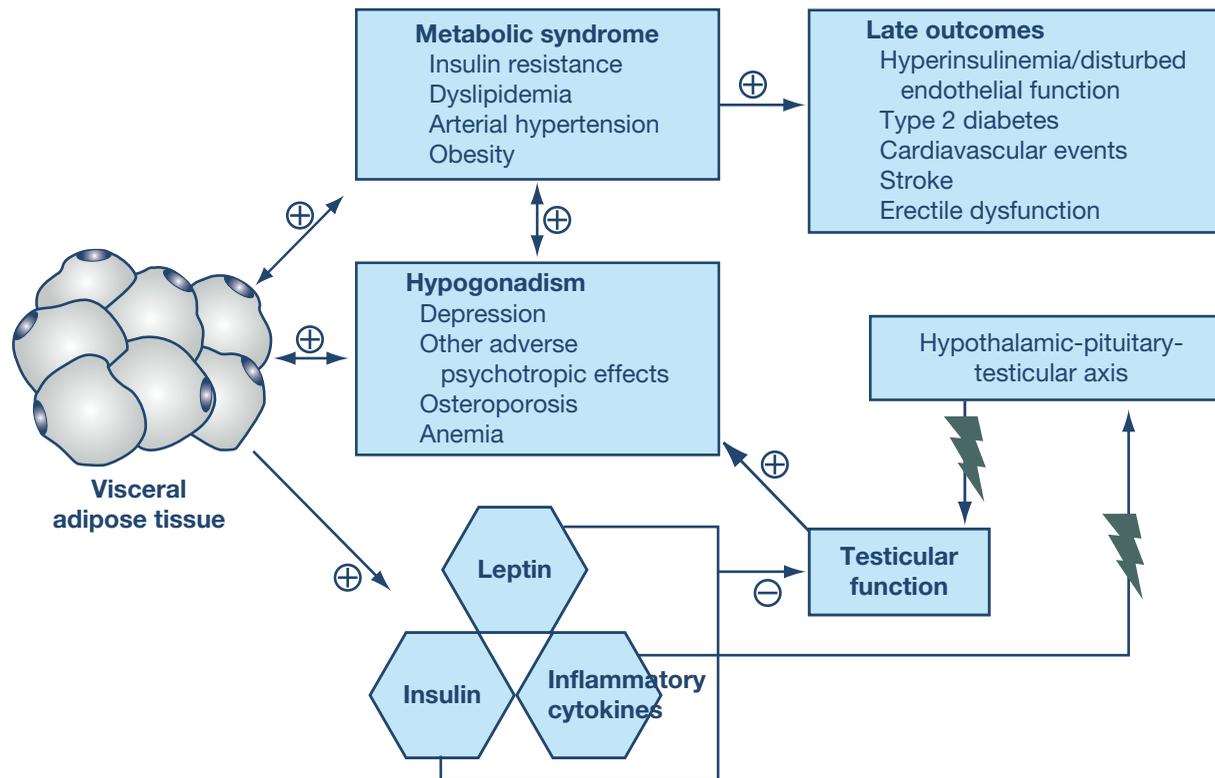
Adapted with permission.[98]

Testosterone and Insulin Resistance

To answer the title question, "How Sweet Is T: Is Type 2 Diabetes an Androgen-Deficient State?" Mathis Grossman, MD, PhD, reviewed evidence for the association of testosterone with type 2 diabetes and metabolic syndrome and the ramifications of testosterone therapy in hypogonadal men with type 2 diabetes.

Low testosterone levels are common in men with type 2 diabetes. The Centers for Disease Control and Prevention (CDC) estimated the prevalence of diabetes in adults 20 years of age or older using glycosylated hemoglobin (A1C) criteria to be 9.6%, of whom 19% were undiagnosed and an additional 3.5% were at high risk for diabetes.[101] Of men with type 2 diabetes, approximately 40% and 60% have low TT or FT levels, respectively.[102] Plasma testosterone levels are significantly lower in men with type 2 diabetes than in healthy men, and men in the upper half of testosterone levels (TT, 449.6 to 605.2 ng/dL) have a 42% lower risk (relative risk [RR], 0.58) of developing type 2 diabetes than those in the lower half (TT, 213.0 to 446.7 ng/dL).[103]

The association of low testosterone and obesity is well established. The prevalence of low testosterone levels is significantly higher in obese nondiabetic or diabetic men (40% and 50%, respectively) compared with lean nondiabetic and diabetic men (26% and 44%, respectively).[104] FT was also significantly higher in diabetic than nondiabetic men and was negatively and significantly related to age, BMI, and SHBG. Low TT levels predict development of diabetes[105-107] and metabolic syndrome.[107,108] The inverse association of testosterone and insulin resistance is not direct but is mediated through visceral fat.[109,110] The relationship between fat and testosterone deficiency is bidirectional and ultimately promotes insulin resistance.[98,111] A study presented at ENDO 2010 showed that accumulation of visceral abdominal fat and reduced BMD occur in men with prostate cancer undergoing androgen deprivation therapy.[112]

Low FT has been associated with measures of insulin resistance and type 2 diabetes in some, but not all, studies.[111] The ability of TT to predict type 2 diabetes and metabolic syndrome appears to be stronger than that of FT, which may result from a role of SHGB. SHBG levels are independently and inversely associated with the risk of type 2 diabetes[105-107,113] and metabolic syndrome.[107,108] Study results suggest that low SHBG levels are strongly associated with higher risk of type 2 diabetes and play an important and perhaps causal role in type 2 diabetes development.[113] Analyses conducted with a cohort of men from the Massachusetts Male Aging Study (MMAS; n=1156) with 7 to 10 years of follow-up, for example, found ORs of 1.58 and 1.89 for each standard deviation decrease in testosterone and SHBG, respectively (*P*<.02 for each).[105] After adjusting for age and BMI, SHBG levels were not associated with insulin resistance but were significantly associated with poor glycemic control.[102] Both circulating SHGB levels and the RR of type 2 diabetes appear to be determined or influenced by polymorphisms of the SHBG gene.[113]

It is not yet clear whether endogenous testosterone therapy is correlated with insulin resistance, because of inconsistent study results[1]; this is exemplified by two randomized controlled crossover trials with similar

designs, outcomes, and study populations. After intramuscular testosterone 200 mg every 2 weeks for 3 months, one study showed reduced HOMA-IR, A1C, fasting blood glucose, waist circumference, waist:hip ratio, and total cholesterol,[60] whereas the other reported no effect on any primary or secondary outcome.[114] A recent, much larger study, the TIMES2 study by Jones and colleagues, reported significantly greater reductions in HOMA-IR, A1C, and lipoprotein α levels at 6 and 12 months and significantly reduced waist circumference at 12 months in men with metabolic syndrome with or without type 2 diabetes compared with placebo.[115]

Testosterone therapy may improve insulin sensitivity. Among 44 young men (age, 20 to 23 y) fasting plasma glucose, fasting plasma insulin, and HOMA-IR were significantly higher and quantitative insulin sensitivity check index (QUICKI) significantly lower in the 24 men with untreated idiopathic hypogonadotropic hypogonadism compared with the normal control subjects. Despite similar low weight (62 to 63 kg) and BMI (21), fat mass was significantly higher and lean mass significantly lower in the hypogonadal group. After 6 months of testosterone therapy, body weight, BMI, lean mass, and QUICKI increased significantly, whereas fat mass, fasting plasma insulin, fasting plasma glucose, and HOMA-IR decreased significantly ($P<.0001$ for all comparisons).[116]

Low testosterone levels are associated with aging alone but can be accelerated by many health and lifestyle changes.[117] The reduction in testosterone levels resulting from a 4- to 5-kg weight gain or the loss of a spouse, for example, was equivalent to approximately 10 years of aging. Adverse lifestyle or health changes, such as development of type 2 diabetes, were associated with crossing the threshold into the hypogonadal range (RR, 2.61). These results suggest that the decline may be slowed by a healthy lifestyle and management of health issues. Testosterone therapy may enhance the beneficial effects of a healthy lifestyle. In a 52-week trial of supervised medical nutrition therapy (MNT) and physical activity with or without testosterone for 32 men with newly diagnosed type 2 diabetes and metabolic syndrome, addition of testosterone significantly enhanced the benefit of supervised MNT and physical activity alone on waist circumference, triglycerides, HDL, BP, HOMA-IR (Figure 6A) and conversion from metabolic syndrome to no metabolic syndrome (Figure 6B) as well as adiponectin and C-reactive protein.[61]

In summary, whether testosterone therapy for men with type 2 diabetes decreases insulin resistance directly or only indirectly via its fat-reducing effect is not known. Additional studies in hypogonadal men with type 2 diabetes are needed to compare testosterone therapy with lifestyle modifications aimed at weight loss and testosterone therapy with insulin-sensitizing agents. At present, the first response to age and obesity in men with type 2 diabetes and low-normal testosterone should be lifestyle management. Testosterone therapy in clinical practice should be prescribed only for men with unequivocal hypogonadism.

Figure 6. (A) Percentage of patients achieving A1C values <7.0% and <6.5% and (B) metabolic syndrome conversion rate after 52-week treatment with supervised MNT and physical activity alone or in combination with transdermal testosterone. Data are mean (SE). A1C, glycosylated hemoglobin; ATP III: Adult Treatment Panel III; IDF: International Diabetes Federation; MNT, medical nutrition therapy.

A

B
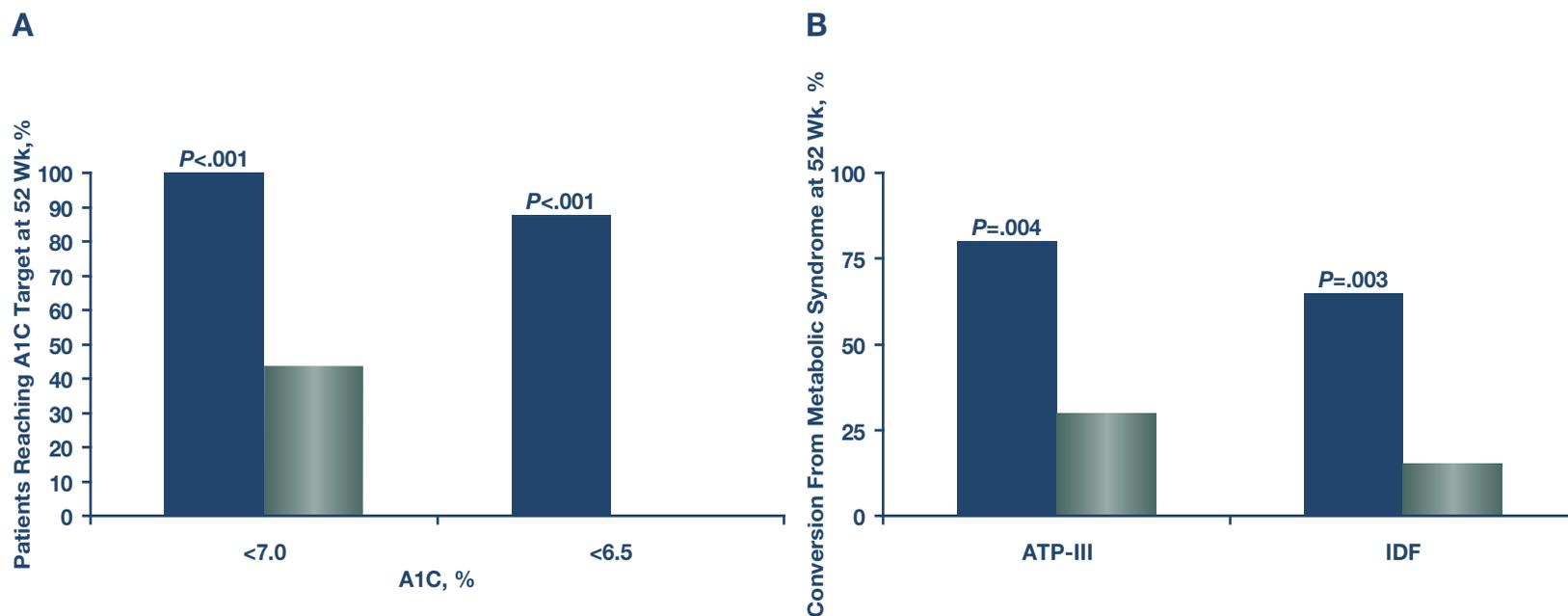
■ Supervised MNT and physical activity plus transdermal testosterone
■ Supervised MNT and physical activity alone

Adapted with permission.[61]

Standardization of Testosterone Testing

In the "Excellence in Testosterone Testing" workshop chaired by Christina Wang, MD, experts discussed the importance of standardized testing for testosterone and failures in that regard.

In his presentation, "Population-Based Reference Ranges for Testosterone in Framingham Heart Study [(FHS)] Using LC-MS/MS," Shalender Bhasin, MD, explained the urgent need for reference ranges. Population-based reference ranges have not been available, and the reference ranges in use vary widely. The historical approaches to determining reference samples have serious flaws: For example, healthy men from convenience samples or case-control studies may not be representative of the general population, and hospital- or clinic-based samples may contain too many men with conditions that alter hormone levels. Accordingly, Boston University and the CDC collaborated to create statistical ranges for endogenous testosterone levels of men aged 19 to 40 years from the third generation of the FHS, to validate and correlate the testosterone levels with outcomes, and to generate clinical guidelines for the appropriate use of reference ranges.

For biological validation, testosterone in the FHS was assayed by LC-MS/MS, which has high recovery and functional sensitivity and is accurate and precise even at low circulating concentrations. Fasting testosterone samples (N=456) were taken early in the morning. Population samples from geographically distinct areas were used for validation: subjects in the National Heart Lung and Blood Institute's FHS from the Boston area, subjects in the EMAS from 8 European centers,[118] and subjects in the MrOS population from centers in California, Oregon, Alabama, Minnesota, and Pennsylvania.[119]

The statistical distribution of TT and FT concentrations in the FHS reference sample showed a mean of 724 ng/dL and 142 ng/dL (median, 699 and 134), respectively. The lowest group had mean TT and FT levels <348 ng/dL and <70 ng/dL, respectively, which are considerably higher than the level currently considered in the hypogonadal range. The age-related rate of decline by decades in TT levels was similar to that reported in the EMAS studies, although the men in the FHS were generally healthier. In multivariate analysis, levels of testosterone correlated with smoking, alcohol consumption, BMI, and comorbidities.

TT and FT levels were validated with outcomes of sexual and physical function and metabolism (type 2 diabetes). In the FHS broad sample of community-dwelling older men, lower levels of FT were associated with a 57% increased risk of incident mobility limitation (P=.03) and 68% higher risk of worsening mobility limitation (P=.007) and significantly increased risk of incident or worsening limitation in mobility.[120] In all 3 studies, low testosterone levels were associated with prevalent diabetes and a twofold greater risk of diabetes.

Several potential limitations and caveats apply to these results:

• A T-score rather than a Z-score approach was used

• Further validation of the reference will be required in longitudinal analysis of outcomes data from clinical trials

- Potential calibration and assay differences may exist

- Epidemiologic studies can show only association, not causality

- These results apply only to men, and similar ranges are needed for women and children

In 2007, the Endocrine Society issued a position statement on the utilities, limitations, and pitfalls in testosterone testing after College of American Pathologists (CAP) proficiency testing demonstrated huge variations in test results in clinical assays for TT.[121] Testing of a sample, for example, by the same methodology but different laboratories resulted in coefficients of variation (CVs) ranging from 13% to 32%. These results were comparable to those obtained by other studies.[121-123] Inaccuracy was particularly apparent in the lower ranges. Thus, though hypogonadal men can be distinguished from eugonadal men, the severity of hypogonadism may be difficult to determine, and accurate identification of levels in healthy women and prepubertal patients is problematic.[122] Rosner et al noted that "the variation between labs is currently so high that you might just as well toss a coin." The primary conclusion of the position paper was that "laboratory proficiency testing should be based on the ability to measure accurately and precisely samples containing known concentrations of testosterone…. When such standardization is in place, normative values for TT and FT should be established for both genders and children, taking into account the many variables that influence serum testosterone concentration."

Responding to such concerns and the obvious need for standardization, the Division of Laboratory Sciences (DLS), of the CDC's National Center for Environmental Health, started a project to standardize laboratory measures of hormones with the ultimate goal of improving diagnosis, prevention, and treatment.[124] The DLS convened a workshop March 17 and 18, 2008, to discuss current needs and problems in steroid hormone testing, focusing on testosterone and estradiol (E_2). The conference was attended by more than 60 experts from the National Institutes of Health (NIH), the Endocrine Society, the American Society of Clinical Endocrinologists, the American Association of Clinical Chemistry, and the American Society for Reproductive Medicine, pharmaceutical companies, assay manufacturers, testing laboratories, and the clinical and research communities. A second consensus conference was convened by the Endocrine Society and the CDC February 18 and 19, 2010, to identify key recommendations from the scientific and medical communities on ways to enhance the use of accuracy-based testosterone assays.

William Rosner, MD, began his "Review of Recommendations of the Consensus Conference on Testosterone Measurement" with a review of assays for TT and their advantages and disadvantages.[121] Though immunoassay after extraction and chromatography has some benefits, it is labor-intensive, cumbersome, costly, and imprecise (measurement must be corrected for recovery); requires a high degree of technical expertise; uses organic solvents requiring special facilities and waste disposal; is susceptible to matrix effects; and generates radioactive waste. Direct immunoassays (radioimmunoassay [RIA], enzyme linked immunosorbent assay [ELISA], or chemiluminescence immunoassay [CLIA]) are the most commonly used methods, which are relatively inexpensive, rapid, and technically easy to perform but also have shortcomings: lack of standardization, method-dependent results and reference intervals, limited accuracy at low levels of testosterone (<300 ng/dL), poorly documented reference intervals in different populations, and, for RIA, generation of radioactive waste.

More reliable, mass spectrometry after extraction and liquid or gas chromatography methods have recently evolved. Although highly accurate

when properly validated and allowing multiple samples to be assessed from the same aliquot, it is relatively expensive, standardization is still lacking, throughput is limited (comparable to RIA after extraction and chromatography), additional error may be introduced by derivatization steps, and use of organic solvents requires special facilities and waste disposal.

Briefly, the consensus conference acknowledged the importance of assay standardization and made the following recommendations:

- With the CDC, plan a program for standardization of assays and reference calibration

- Define performance criteria and reference intervals for testosterone levels in adults and children

- Develop guidelines to ensure uniform sample preparation and handling of assays

- Encourage third-party payers and healthcare organizations to promote the use of standardized assays

- Inform the NIH of the wisdom and necessity of funding grants

- Enlist journals to insist on proper methodologies for submitted papers

- Encourage manufacturers and laboratories to develop new approaches

Hubert W. Vesper, PhD, described the "CDC Testosterone Standardization Project: Standardization Procedures and Tools for Laboratories and Assay Manufacturers," which is well under way. The project was conducted in two steps. Step 1 was to establish metrologic traceability by standardizing procedures for reference measurement and routine measurement. Step 2 was to verify and monitor the consistency of calibration and value assignment (traceability) across individual assays.

The CDC developed a reference method with high accuracy (0.7%) and precision (CV, <2%). Accurate laboratories and assays will earn certification and CDC website listing. The program will be open to manufacturers and laboratories. Enrollment, which will occur on a quarterly basis, started in January 2010. By June 2010, 10 participants, including commercial and research laboratories and immunoassay manufacturers, had enrolled. Additional functions of the program include:

- Assign CAP reference values

- Produce guidelines on mass spectroscopy at the Clinical Laboratory Standards Institute

- Assess new reference and proficiency testing materials

- Provide quality assurance

- Offer assistance to assay and instrument manufacturers and laboratories in developing assays and solving problems

ORIGINAL RESEARCH

Testosterone Therapy for Hypogonadism

Intramuscular Testosterone Undecanoate 1000 mg in Daily Clinical Practice: Preliminary Results. Of the target accrual of 1500 men, 937 men (mean age, 49 y; range, 36 to 63 y) have been enrolled to date in an ongoing study from 18 countries, comprising the largest worldwide sample of hypogonadal men ever studied. Testing after the fifth injection (~50 to 70 wk) showed marked and significant improvements in scores of mental acuity, mood, and sexual function/satisfaction (Table 1). Waist circumference decreased significantly from 100.5 cm to 95.6 cm (*P*<.0001). The mean PSA and hematocrit both increased but stayed well within

normal and acceptable limits (1.0 to 1.3 and 43.7% to 45.8%, respectively).[125]

Safety of Intramuscular Testosterone Undecanoate 1000 mg Over 42 Months. In 122 hypogonadal men (testosterone 5.9 to 12.1 nmol/L; mean age, 59.5 [6.0] y), prostate size increased slowly but steadily, but PSA did not increase by similar magnitude. Residual bladder volume and IPSS scores decreased over the first 24 months and then stabilized. PSA rose slightly over the first 24 to 36 months, stabilized at 5% to 10% higher than

Table 1. Preliminary Results of Large (N=937) Ongoing International Surveillance Study of Intramuscular TU 1000 mg in Daily Clinical Practice[125]

Parameter	Patients, %			
	Baseline	Injection 3	Injection 4	Injection 5
High overall vitality	11	44	56	60
High/very high response to PDE5 inhibitor	37	62	59	59
High or very high sexual desire/libido	11	44	53	59
Good/very good ability to concentrate	30	60	67	72
Positive/very positive overall mood	23	62	70	77
Severe ED	36	9	10	10
No or mild ED	38	71	76	75

ED, erectile dysfunction; PDE5, phosphodiesterase type 5; TU, testosterone undecanoate.

baseline, and then rose again after 42 months but never exceeded 4.0 ng/mL. Hematocrit increased significantly but reached maximum value after 12 months; hematocrit >52% occurred in 9 patients over the 42-month study period but never occurred twice in the same patient, and no specific measures were taken.[126]

Effect of Intramuscular Testosterone Undecanoate 1000 mg on Cardiovascular Risk Factors. In this retrospective study of hypogonadism, 120 younger men (mean age, 48 [16] y) were treated in routine clinical practice from 2005 to 2009. Among the 117 patients not taking lipid-lowering medications, statistically significant improvements were seen after 1 year of treatment in total and calculated LDL; modest increases in HDL did not reach statistical significance. A1C did not change in the 23 diabetic patients with no increases in diabetic medications or surgery for obesity. Treatment had no effect, beneficial or adverse, on body weight or BP. The presenter speculated that the lack of significant effect in some parameters may be due to the high proportion of subjects (82%) who previously had been treated with other testosterone formulations.[127]

Testosterone Therapy and Metabolic Syndrome

A retrospective analysis of data in the Testim Registry in the United States (TRiUS) showed that 47% of the 577 hypogonadal men with sufficient data for analysis had metabolic syndrome according to Adult Treatment Panel III (ATP III) criteria (Table 2). A diagnosis of metabolic syndrome was significantly associated with baseline testosterone levels <250 ng/dL, independent of age (RR, 1.25; 95% confidence interval, 1.1, 1.5; *P*<.005). Mean testosterone levels increased significantly in all subjects treated with Testim 1% gel for 6 months (n=229) or 12 months (n=218). In men with metabolic syndrome, increases in mean testosterone levels from 263 to 491 ng/dL were accompanied by statistically significant improvements in fasting serum glucose, waist circumference, and systolic and diastolic BP after 12 months (Table 3). Improvements in HDL and plasma triglycerides did not reach statistical significance.[128] Readers should note that the data provided here, taken from the poster, are substantially different from those in the published abstract.

Hypogonadal men (TT <12.0 nmol/L or calculated FT <225 pmol/L) aged 35 to 70 years received intramuscular TU 1000 mg (n=88) or placebo (n=57) for the 30-week blinded study period, and then all men received TU for an additional 33 weeks. In the treatment group, BMI, waist circumference, and insulin and glucose levels improved progressively over 66 weeks of treatment, and maximal improvements in cholesterol (total, LDL, HDL) and triglycerides occurred by 30 weeks. Men who switched to TU for the open-treatment phase had improvements in all study variables and eventually matched the other group's measurements.[129]

Table 2. ATP-III Criteria Features of Metabolic Syndrome in Hypogonadal Men at Baseline[128]

Variable	Metabolic Syndrome -		Metabolic Syndrome +		P
	Mean (SD)	n	Mean (SD)	n	
Fasting serum glucose, mg/dL	95.4 (30.8)	194	115.0 (39.2)	237	<.001
Waist circumference, in	36.8 (5.0)	305	44.2 (6.5)	267	<.001
Plasma triglycerides, mg/dL	1.28 (91.9)	179	208.1 (129.9)	263	<.001
HDL, mg/dL	49.2 (14.5)	180	39.3 (8.9)	259	<.001
Systolic BP, mm Hg	121.6 (11.7)	304	133.3 (16.1)	269	<.001
Diastolic BP, mm Hg	75.8 (7.9)	303	81.9 (9.8)	269	<.001

ATP-III, Adult Treatment Panel III; BP, blood pressure; HDL, high-density lipoprotein.

Table 3. Changes in Features of Metabolic Syndrome From Baseline After 12 Months of Therapy With Testim 1% Gel[128]

Variable	Metabolic Syndrome -		Metabolic Syndrome +	
	Mean (SD)	P	Mean (SD)	P
Fasting serum glucose, mg/dL	-0.6 (4.6)	0.9	-14.7 (4.0)	<.001
Waist circumference, in	0.21 (0.2)	0.3	-0.7 (0.2)	<.001
Plasma triglycerides, mg/dL	-2.0 (81.9)	0.9	-25.7 (114.3)	.09
HDL, mg/dL	-0.3 (7.8)	0.8	-1.8 (8.2)	.19
Systolic BP, mm Hg	2.9 (1.4)	0.05	-3.5 (1.4)	.004
Diastolic BP, mm Hg	0.73 (0.91)	0.42	-2.8 (0.87)	.001

BP, blood pressure; HDL, high-density lipoprotein.

Effect of Testosterone Therapy in Obese, Aging Men

In a double-blind, randomized, controlled trial, 35 men aged 40 to 70 years (mean, 53.3 [7.4] y) with a mean BMI of 34 kg/m^2 and mean waist circumference of 113 cm received either intramuscular TU 1000 mg (n=19) at weeks 0 and 6 and then at 10-week intervals or placebo (n=16). All men had ongoing weight loss advice (MNT and physical activity). Serum testosterone levels increased significantly with treatment but not with placebo. Total body weight, BMI, and waist circumference did not change, but total body fat and fat mass declined significantly in the treatment group. Subcutaneous abdominal and thigh fat decreased significantly with testosterone; visceral fat did not change. Lean and skeletal muscle mass increased significantly with therapy but not with placebo. Total cholesterol and LDL decreased significantly, but triglycerides, HDL, glucose, insulin, and the HOMA-IR index did not change. A significant increase was seen in hemoglobin (14.8 to 16.3 g/dL) and hematocrit (43% to 47%), but PSA and sleep parameters were not affected.[130]

Male Functional Hypogonadotropic Hypogonadism: A Distinct Clinical Entity?

This small study aimed to determine whether male functional hypogonadotropic hypogonadism (MFHH) is a distinct clinical entity in the manner of hypothalamic amenorrhea (HA) in women. Seven men with symptoms of hypogonadism and at least one of the predisposing factors for HA (excessive exercise, n=4; weight loss, n=3; psychological stress, n=3) discontinued treatment and underwent detailed genotyping and phenotyping. Results were compared to 35 healthy control subjects. No mutations in known loci associated with gonadotropin-releasing hormone deficiency were found. All 7 men had hypogonadotropic hypogonadism (HH) with significantly lower levels of testosterone, BMI, body fat mass, E$_2$, LH, and inhibin levels. The authors concluded that HH in men can occur in the setting of energy deficits and psychological stress and that it represents a distinct clinical entity akin to HA in women. A follow-up study is under way to assess reversibility and establish the clinical diagnosis for MFHH.[131]

Is Total Testosterone a Definitive Diagnostic Test?

Anawalt et al evaluated the sensitivity and specificity of TT levels for predicting low calculated FT in a cohort of 3672 men who were evaluated for hypogonadism at the Puget Sound Veterans' Administration from 1997 to 2007.[132] FT was calculated using the Vermuelen formula, which correlates well with equilibrium dialysis followed by LC-MS/MS.[133] TT levels <280 ng/dL were fairly sensitive for calculating FT, but specificity was low until TT was <150 ng/dL. Thus, TT >450 to 500 ng/dL excludes low calculated FT in virtually all men, but low TT lacks specificity for the biochemical diagnosis of hypogonadism.

Safety of Long-term Testosterone Therapy

No Increased Risk of Prostate Cancer. In an observational study, 154 hypogonadal patients (mean age, 58 [1.7] y) taking intramuscular TU 1000 mg and 160 eugonadal matched control subjects were followed for up to 5 years.[134] Transrectal ultrasound-guided prostate biopsies were performed when PSA velocity >0.75 mg/L or PSA >4 mg/L. Initial biopsies included 10 2.2-cm cores; repeat saturating biopsies, when indicated, included 24 to 32 2.2-cm cores. Baseline prostate volumes and PSA levels were lower in hypogonadal than eugonadal subjects. No abnormalities were found on rectal palpation in either group, and the incidence of prostate cancer was not higher in the treated group (Table 4). In addition, of subjects in whom prostate cancer developed, those in the treatment group had smaller tumors with better differentiated cells.

Overall Safety and Effect on Metabolic Syndrome. During up to 12.4 years of treatment with intramuscular TU 1000 mg every 10 to 14 weeks, testosterone levels were sustained in the low-normal range in 227 younger hypogonadal men (mean age, 38 y [range, 15 to 71 y]; 117 primary, 79 secondary, 31 LOH). The prevalence of diagnosed metabolic syndrome fell from 86% to 45% (P=.001), with significant improvements in nearly all features: waist circumference, from 106 cm to 95 cm within 8 injections (P<.001); and lipoproteins (LDL, HDL, total cholesterol, triglycerides), resting systolic and diastolic BP, and fasting blood glucose levels. Hematocrit and PSA increased significantly. Hematocrit never went higher than 54.4%, however, and PSA never exceeded 4.0 except in two cases of prostatitis. The investigators concluded that long-term testosterone therapy with intramuscular TU 1000 mg is safe, effective, and feasible.[135]

Table 4. Prostate Health Parameters in 154 Hypogonadal Men at Baseline and After 5 Years of Intramuscular TU 1000 mg Versus a Cohort of 160 Matched Control Subjects[134]

| Variable	Baseline		After 5 Years	
	Hypogonadal	Eugonadal	Hypogonadal	Eugonadal
PSA, mg/L	0.77	2.15	2.0	3.0
Prostate volume, mL	27.6	39.5	43.0	44.0
TZV, mL	11.6	14.6	14.7	19.0
IPSS	14.2	13.3	11.9	15.9
Residual post-voiding volume	31.5	32.0	24.4	36.4
AUR surgical intervention	—	—	0/0	1/3
Pathology				
Biopsies, n	—	—	22	39
Prostate cancer, n	—	—	5	16
Unilateral, n	—	—	3	2
Bilateral, n	—	—	2	14
Tumor cells in core, %	—	—	10	Up to 80
Gleason score	—	—	3+2/3+3	3+3 to 5+4
High-grade PIN	—	—	2	7

AUR, acute urinary retention; IPSS, International Prostate Symptom Score; PIN, prostatic intraepithelial neoplasia; PSA, prostate-specific antigen; TZV, transition-zone volume.

Novel Testosterone 1.62% Gel Formulation

Results of a Phase 3 Trial. Of 274 subjects enrolled, 196 (168 gel; 28 placebo) completed the phase 3 trial.[136,137] Gel was applied to the upper arm/shoulder or abdomen. After a 42-day titration period, 17 patients received 1.25 g/day, 66 received 3.75 g/day, and 91 received 5 g/d. On evaluation day 14, 66% of treated patients had levels of testosterone in the eugonadal range; on days 56, 112, and 182, mean serum testosterone concentration within the eugonadal range in 82% of treated versus ≤37% of placebo subjects (*P*<.0001). At some point during the study, 10 patients had 11 "rare, brief, inconsistent, and unsustained" testosterone levels >2500 ng/dL believed to result from some form of contamination by venipuncture artifact (n=5) or subject- and site-specific circumstances giving the appearance of acute increases in systemic absorption (n=5).[136] Significant decreases in LH, FSH, and IL-10 levels were noted on days 84 and 182 among treated but not placebo subjects. Treatment-emergent adverse events (TEAEs) were reported by 56% of treated patients and 38% of placebo patients. The incidence of serious TEAEs was similar in both groups (treatment, 2.1%; placebo, 2.5%). Twenty-five patients in the treatment group discontinued treatment for an adverse event, 17 for an increased PSA, and 1 for hematocrit >54% as specified in the protocol. One each discontinued for diarrhea, fatigue, increased BP, pituitary tumor, dizziness, pollakiuria, erythema, skin nodule, vasovagal syncope, and diabetes. The investigators concluded that, because the new formulation can safely and effectively deliver eugonadal levels of testosterone with a lower mass of gel covering a smaller skin surface area than currently available gels, patient adherence to the prescribed treatment regimen may improve.

Single- and Multiple-Dose Pharmacokinetics of Testosterone With 1.62% Gel. Testosterone 1.62% gel at 1.25 g, 2.50 g, 3.7 g, 5.0 g, and 6.25 g was administered over a 14-day treatment period to 51 otherwise healthy men 18 to 75 years of age with BMI 20 to 35 kg/m^2 and TT <300 ng/dL.[138] After single doses, mean testosterone concentrations increased continuously up to 8 hours and then remained consistent and within the eugonadal range for the remainder of the 24-hour dosing interval (Figure 7). On day 14 after multiple dosing, mean testosterone concentrations were relatively consistent and within the eugonadal range over the dosing interval for all doses. The mean average concentration (C_{avg}) was within the eugonadal range with all doses on days 1 and 14 after single and multiple doses, respectively. The mean maximum concentration (C_{max}) exceeded the physiologic range on day 14 after multiple dosing at 5.0 g and 6.25 g. Dose accumulation was negligible at the 2 lower doses and less than twofold at the 3 higher doses. Maximum serum concentration of testosterone occurred faster after multiple dosing than after a single dose (median time to maximum plasma concentration (T_{max}) <1 to 8 h vs 10 to 16 h). Steady state appeared on graphic evaluation to be achieved by day 2, but statistical assessment was inconclusive. Similarly, dose proportionality could not be statistically concluded, but mean area under the serum concentration-time curve, 0-24 hours (AUC_{0-24}) and C_{max} appeared in baseline-adjusted box plots to be generally dose-proportional with a linear increase over the dose range. Seventy-seven percent of patients reported at least one TEAE. No adverse events of severe intensity, no serious adverse events, no deaths, and no trends or clinically significant changes in laboratory data, vital signs, application-site assessments, or electrocardiogram occurred during the study.

Figure 7. Mean concentration-time profiles of testosterone on day 1 after single-dose and day 14 after multiple-dose applications of topical testosterone 1.62% gel.[138]
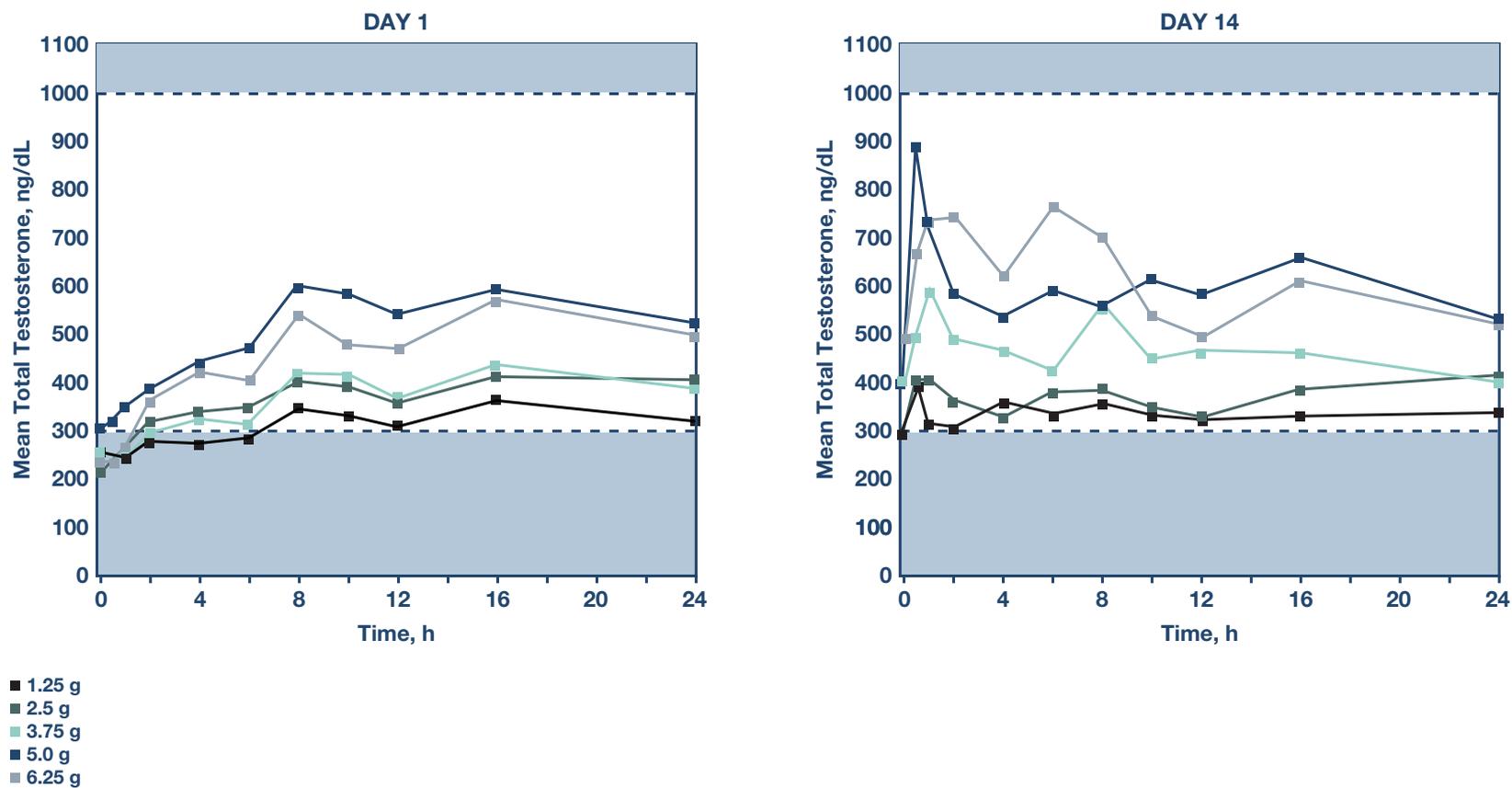
Additional findings with 1.62% gel were:

- Abdominal application provided approximately 30% to 40% lower bioavailability of testosterone compared to application on the upper arm/shoulder; however, an alternating schedule of upper arm/shoulder and abdominal application did not adversely affect the achievement of eugonadal levels of testosterone[139]

- Application of moisturizer 1 hour after application increased the AUC_{0-24} by 14% and the C_{max} by 17% compared with gel alone; application of sunscreen had no effect on AUC_{0-24} but increased C_{max} by 13% compared with gel alone. Individual and mean C_{avg} and C_{max} values remained within the eugonadal range, and increases were considered not clinically relevant[140]

- Showering or bathing 2 hours or more after application of 1.62% has minimal or no effect on systemic absorption while reducing the amount of testosterone on the skin by >80%, which may reduce the potential for transfer of testosterone to others[141]

New Oral Formulation of Testosterone Undecanoate

An oral testosterone formulation is highly desirable and eagerly awaited, but the only oral formulation to date is highly lipophilic, and its bioavailability is adversely affected by dietary fat content. An oral formulation of TU that is not affected by moderate amounts of fat intake is in late stages of development. The proprietary formulation increases bioavailability by increasing intestinal lymphatic absorption. In an open-label, 2-center, 5-way crossover-design pharmacokinetic study, the oral TU 300 mg was given to 16 hypogonadal men (TT, 7.1 [0.88] nmol/L) 30 minutes after an 800-calorie meal with varying fat content. Physiologic concentrations of testosterone were achieved for a substantial portion of subjects with meals with fat content from 10% to 50% (Table 4). Even in men in a fasted state, substantial levels of testosterone were achieved. The proportion of time with testosterone levels in the reference range was highest with food containing 10% to 30% dietary fat. More than 80% of subjects had testosterone in the reference range after taking the drug with meals containing 10% to 30% fat; increasing numbers were still acceptable. According to co-inventor Robert Dudley, the US Food and Drug Administration is satisfied with the fat–absorption data, and no dietary restrictions will be necessary.

Testosterone, dihydrotestosterone (DHT), TU, and dihydrotestosterone undecanoate (DHTU) peaked within 4 hours; testosterone, TU, and DHTU fell to near baseline at approximately 12 hours, while DHT remained higher. According to the presenter, DHT is higher than with some formulations but not excessive. Twice-daily dosing will be necessary to keep serum testosterone in the eugonadal range over 24 hours and prevent supraphysiologic levels. Though twice-daily dosing is not ideal, interest in having an oral medication is so high that physicians who have been consulted believe their patients will be accepting. A phase 3 clinical trial is slated to begin in late 2010.[142]

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