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EFFECTS OF TESTOSTERONE ADMINISTRATION ON PHYSICAL CHARACTERISTICS AND MUSCLE FUNCTION IN MEN WITH LOW TO LOW-NORMAL TESTOSTERONE CONCENTRATIONS: A META-ANALYSIS

By

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ABSTRACT

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Major Professor: Dr. Zsolt Murlasits

Acknowledgments: Dr. Jeffery Berman, Dr. Stephan Schoech

Objective: This meta-analysis uses data from clinical studies of older men with low to low-normal concentrations of testosterone (T) that were treated with exogenous T to assess treatment efficacy on: lean mass, fat mass, strength, and mobility.

Methods: The data investigated are from published and referred journals. The Cohen's *d* was used to compute effect sizes for both pre-treatment and post-treatment scores.

Results: The analysis revealed that T resulted in an overall significant ($p = 0.037$) improvement in physical characteristics and muscle function. After accounting for the variance, significance was strengthened ($p = 0.006$) for the overall effect. When the dependent variables were considered independently, the largest contribution came from a decrease in fat mass ($p = 0.003$), followed by a non-significant trend ($p = 0.06$) for an increase in lean mass. After accounting for the variance for the dependent variables independently, lean mass ($p = 0.0003$), fat mass ($p = 0.005$), and strength ($p = 0.02$) were all found to be significant contributors.

Conclusion: T administration can be considered a viable treatment for both the prevention and rehabilitation of decreased muscle mass and muscle strength seen in older men with low to low-normal T concentrations.

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Introduction

Diminished concentrations of total testosterone (T) levels have been positively correlated with decreased muscle strength (1) and impaired mobility in men (2, 3). In addition, insufficient concentrations of total and bio-available T have been associated with an increased risk of mortality, independent of multiple risk factors and pre-existing conditions in men (4). Some evidence indicates that life-style factors, such as smoking, alcohol consumption, and exercise may affect T levels, but the impact of these factors has been inconsistent (5-7). However, age-related decreases in total and bio-available T concentrations have been reported in numerous investigations (8-14). There is also a high prevalence of low total T concentrations in men with muscle wasting conditions, such as: HIV infection, chronic obstructive pulmonary disease (COPD), end-stage renal disease, malignancy, coronary atherosclerosis, diabetes mellitus, and endocrine disorders (e.g., hypogonadism) (15-21). It has been hypothesized that T (or its esters) administration to older men with diminished total T concentrations, will result in anabolic effects on the male musculature, with an end-result of improving body composition, increasing muscle strength, and promoting functional mobility, all of which result in an improvement in quality of life.

Numerous investigations have shown beneficial effects of T administration on body composition and strength in aging men with decreased T concentrations (8, 22-26), in frail older men (27, 28), and in men with muscle wasting conditions due to disease (29-32). Testosterone administration is thought to produce beneficial effects because administration has been found to promote muscular hypertrophy and muscle protein synthesis in healthy men, younger and older men, as well as, sarcopenic and cachexic

men (33-39). Testosterone administration has also been found to increase muscle volume and skeletal muscle fiber cross-sectional area in men (40), along with reports of increases in the number of myonuclei and satellite cells in male musculature (38, 41).

Normal T concentrations vary markedly among individuals, with a conservative range for adult males from 250 ng/dL to 800 ng/dL (11, 42, 43). The medical community defines low testosterone concentrations as those that fall below the normal range for adult males (i.e., less than 250 ng/dL) (44). A consensus has been established that total and bio-availiable T decrease annually by approximately 0.5 - 2.0% in males starting around the age of 30 (9, 45-48). There are likely many factors that cause the decrease in T with age; perhaps most important are changes at the level of the testes where there are declines in both the number of Leydig cells and the activity of T-producing enzymes (49-52). There is also a possibility that age-related declines in hypothalamo-pituitary-adrenal (HPA) axis function contributes to decreased T production in aging men (49-52). This is because T production is regulated via a negative feedback mechanism. When T becomes too low, there is an increase in gonadotropin-releasing hormone (GnRH) from the hypothalamus. This increase in GnRH activates the pituitary to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (42, 43) which helps produce approximately 0.24 μM/day of T (53). A gradual failure of the HPA axis to respond appropriately to the decline in T, likely due to decreased sensitivity of the pituitary to GnRH, will result in decreased T production (49, 54, 55). Other than aging, body mass index (BMI), smoking, general health status, and decreased physical activity may also contribute to alterations in T concentrations (13).

Injectable Testosterone Esters
Testosterone Enanthate ($C_{26}H_{40}O_3$)
Testosterone Cypionate ($C_{27}H_{40}O_3$)
Sustanon™ (combination of 3 esters)
Sustanon 250™ (combination of 4 esters)
Testosterone Propionate ($C_{22}H_{32}O_3$)
Testosterone Phenylpropionate ($C_{28}H_{36}O_3$)
Omnadren™ (combination of 4 esters)
Aqueous Testosterone Suspension (non-esterified)
Testosterone Decanoate
Testosterone Hexanoate
Testosterone Caproate
Testosterone Isocaproate
Testosterone Acetate

Trandermal Testosterone
Androderm™ (patch)
Testoderm TTS™ (patch)
Androgel™ (gel)
Testim™ (gel)

Oral Testosterone
Methyltestosterone (C-17α-methylated testosterone)
Testosterone Undecanoate

Sublingual/Buccal Testosterone
Dissolvable tablet under the tongue or near gums

Subcutaneous Testosterone Pellet
Crystalline Testosterone under the skin

Figure. 1 – Different Forms of T and Administration.

A common demographic of males who have been administered with T are those conflicted with T deficiencies, due to hypogonadism. According to Research and Markets, the T replacement therapy industry grew with a compound annual growth rate of 24.3% from 2005 to 2009, with approximately $838 million in sales. The United States is the largest consumer of T replacement therapy, accounting for 96% of the total sales value in 2009 (56). Testosterone administration is also used therapeutically to increase muscle mass and strength for individuals diagnosed with chronic diseases and disorders that induce cachexic states. Cachexia occurs when a chronic disease or disorder has progressively resulted in muscle wasting (57), or more specifically, decreased muscle mass and strength, which results in a reduced quality of life. The use of T administration in the studies investigated in this analysis is solely for the purpose of improving muscle mass and strength, should not be administered to patients in severe disease states, but only to patients in stabilized disease states with associated wasting conditions. The well documented anabolic effects of T upon male musculature are not intended to cure the disease, but may help elicit increased muscle mass and strength; therefore, increasing functional mobility and quality of life.

This meta-analysis uses data from clinical studies of men with low to low-normal concentrations of T that were treated with exogenous T to assess treatment efficacy on the following: (1) lean body mass, (2) fat mass, (3) muscle strength, and (4) functional mobility. If T administration improves body composition, muscle strength, and physical function, it can be considered an effective agent in the prevention and rehabilitation of

frailty, dependency, and the impaired quality of life for men inflicted with varying severities of sarcopenic or cachexic states.

Methods

Search strategy and data extraction

This study is comprised of a meta-analysis of both English and non-English journal articles related to T replacement therapy, as well as its esters, in both sarcopenic and cachexic men. The data investigated are from published and referred journals. Computer searches were conducted with PUBMED, MEDLINE, and Google Scholar. Key words used in the search were testosterone, steroids, sex-hormones, androgens, aged, aging, adult, elderly, older, geriatric, physical function, muscle strength, body composition, lean body mass, fat mass, functional mobility, sarcopenic, cachexic, hypogonadal, and men. References from the papers used were also examined to locate other relevant articles. The search covered articles published between 1970 and 2011. This study was approved by The University of Memphis Institutional Review Board.

Statistical methods

One rater reviewed each of the nineteen studies and independently pulled data and completed the coding sheet. The Cohen's *d* was used to compute effect sizes for both pre-treatment and post-treatment scores using a Microsoft Excel calculator designed specifically for Cohen's *d* calculations. Cohen proposed a commonly-used definition for his calculation as follows: a small treatment effect size is considered to be about 0.2, a medium effect size to be about 0.5, and a large effect size to be about 0.8 (58). Once Cohen's *d* scores are collected, investigators were able to determine if T (or its esters) administration will provide meaningful outcomes in physical characteristics and muscle

function in older men with low to low-normal T concentrations. Additionally, investigators were able to determine which dependent variable (lean body mass, fat mass, muscle strength, or functional mobility) contributed to the greatest extent. One-sample *t*-tests were used to determine significance ($p < 0.05$) for all of the variables combined, and each dependent variable independently. Using One-Way Analysis of Covariance, the form of administration and study type were analyzed to determine if these had a significant effect on resultant physical characteristics and muscle function. Using Pearson's correlation measure, the duration of treatment, initial T concentrations, year of publication, and the age of participants were also analyzed to determine if these variables had significant effects on resultant physical characteristics and muscle function. All statistical analyses will be conducted using SPSS v. 20.

Eligible studies

This investigation included nineteen clinical trials in twenty publications (see Table 1). Studies used were from randomized, double-blind, controlled trials of men who received treatment with T or one of its ester groups, against a 'placebo' or 'no-treatment' control group. All of the trials met the inclusion criteria: middle-aged or older men ≥ 45 years, men with low or low-normal T levels, use of T or its esters in replacement doses, and the inclusion of older men without acute illness. Clinical trials compared one treatment group with a placebo group, with the exception of four reports (29, 31, 59, 60) that compared a treatment group with a no-treatment control group (matched and randomized, but did not receive a placebo). There was one trial (61) included in the analysis that had two separate treatment groups, one group receiving T and one group receiving nandrolone. These two treatment groups had their Cohen's *d* pre-treatment and

post-treatment scores averaged together to obtain one treatment group measure for the analysis. It is also important to mention that this trial (61) included participants that had been receiving glucocorticoid therapy daily for at least six months. There was one trial (24) that had two separate treatment groups, one group receiving T and one group receiving T in combination with finasteride (used to inhibit dihydrotestosterone (DHT) production for prostate safety purposes) (24). The data from the treatment group that received T with finasteride were removed from the analysis. Another trial (62) in the analysis included a total of four groups: one group receiving T with a growth hormone placebo, another receiving growth hormone with a T placebo, a third group receiving both T and growth hormone, and a fourth group receiving both a T placebo and a growth hormone placebo. Only the data from the T with a growth hormone placebo and the double placebo control group's were retained for analysis (62). There was one trial (63) that had a total of four groups: one group receiving T, another receiving a placebo, a third group receiving T in combination with an exercise routine, and a fourth group receiving a placebo in combination with an exercise routine. The data from the two groups which included exercise routines in combination with the T or the placebo were excluded from this analysis (63). There is one trial (64) in the analysis that included two treatment groups. Both groups receiving T, but administration was altered, with one group receiving T weekly and the other group receiving T switched with a placebo on a monthly basis. The data from the group receiving T switched with a placebo on a monthly basis was removed from the analysis (64). Some investigations did not present their post-treatment standard deviation in the published literature. In these circumstances; the pre-treatment standard deviation were reused for the post-treatment

standard deviation to calculate the Cohen's *d* effect size (24, 61, 64, 65). There were four investigations where additional measurements were collected during the original investigation that would have been considered relevant to this analysis, but the data were not included in the published literature (29, 31, 59, 60). This is important to recognize, as the data may have not been published because it was not statistically significant, which would alter the results found in this analysis. Using this inclusion criteria, twenty-five studies were identified for analysis, however six of these were excluded upon further review (23, 30, 50, 66-68), because the data was not sufficiently presented or extractable in the published literature.

A total of 1,304 older men (677 in the treatment groups and 627 in the placebo and control groups) participated in the 19 studies included in the analysis. The mean age of the participants was 68.67 years (69.19 in the treatment groups and 68.16 in the placebo and no-treatment control groups). The years of publication ranged from 1992 to 2011.

Ineligible studies

Exclusion criteria included: uncontrolled investigations, cross-over designs, observational studies, interventions of supra-physiological doses of T, and interventions including exercise routines. Studies were also excluded if they recruited participants with unstable disease conditions. Studies of HIV-infected men were excluded due to the trials not meeting eligibility criteria.

Table 1. Investigation Characteristics.

	Author and Year	Study Type and Participants	Average Age (years)	Initial Testosterone (nmol/l)	Study Duration (days)	Administration Form
1	Marin et al., 1992 (69)	Treatment (n=11)	51.9	16	244	oral
		Placebo (n=12)	49.9	16.8		
2	Morley et al., 1993 (60)	Treatment (n=8)	77.6	NA	92	injection
		Control (n=6)	76.0	NA		
3	Ferreira et al., 1998 (29)	Treatment (n=10)	70.3	14.4	189	oral
		Control (n=7)	66.1	17.2		
4	Snyder et al., 1999 (25)	Treatment (n=54)	73.1	12.7	1095	transdermal
		Placebo (n=54)	73.1	12.8		
5	Clague et al., 1999 (22)	Treatment (n=7)	68.1	11.3	84	injection
		Placebo (n=7)	65.3	11.6		
6	Kenny et al., 2001 (70)	Treatment (n=24)	76.0	13.5	365	oral
		Placebo (n=20)	75.0	13.5		
7	Blackmen et al., 2002 (62)	Treatment (n=21)	70.0	14.2	182	injection
		Placebo (n=17)	70.0	13.6		
8	Boyanov et al., 2003 (59)	Treatment (n=24)	57.5	9.56	92	oral
		No Treatment (n=24)	57.5	10.76		
9	Crawford et al., 2003 (61)	Treatment 1 & 2 (n=35)	60.7	13.8	365	injection
		Placebo (n=16)	59.9	15.7		
10	Wittert et al., 2003 (26)	Treatment (n=39)	69.0	17.0	365	oral
		Placebo (n=37)	68.0	15.6		
11	Casaburi et al., 2004 (63)	Treatment (n=12)	66.6	10.5	70	injection
		Placebo (n=12)	67.7	10.5		
12	Svartberg et al., 2004 (31)	Treatment (n=15)	64.5	21.6	182	injection
		Control (n=14)	67.5	20.5		
13	Page et al., 2005 (24)	Treatment 1 (n=24)	71.0	9.9	1095	injection
		Placebo (n=24)	71.0	10.5		
14	Schroeder et al., 2005 (65, 71)	Treatment (n=20)	72.8	12.8	84	oral
		Placebo (n=12)	71.5	12.4		
15	Emmelot-Vonk et al., 2008 (8)	Treatment (n=113)	67.1	11	183	oral
		Placebo (n=110)	67.4	10.4		
16	Kenny et al., 2010 (27)	Treatment (n=53)	77.9	13.8	365	transdermal
		Placebo (n=46)	76.3	14.2		
17	Srinivas-Shankar et al., 2010 (28)	Treatment (n=130)	73.7	11.0	183	transdermal
		Placebo (n=132)	73.9	10.9		
18	Travison et al., 2011 (72)	Treatment (n=69)	73.8	8.7	183	transdermal

		Placebo (n=69)	73.9	8.0		
19	Sheffield-Moore et al., 2011 (64)	Treatment 1 (n=8)	73.0	11.8	153	injection
		Placebo (n=8)	65.0	11.9		

Results

Overall results

The Cohen's *d* pre-treatment scores from all of the variables from all of the investigations were calculated and averaged together in an attempt to determine if the treatment and control group measures did not differ from each other prior to the administration of T, with a combined average Cohen's *d* score of -0.02 calculated. Using a one-sample *t*-test, it was determined that the combined averaged Cohen's *d* scores from each investigation at pre-treatment were not significantly different from zero, $t_{(18)} = 0.35$, $p = 0.7$, confirming that the combined treatment and control group measures from all of the variables did not significantly differ from each other prior to administration of T.

To determine if there was an effect of T, the post-treatment scores from all the trials were calculated and averaged together with a combined average Cohen's *d* score of 0.15, indicating that the administration of T had a small beneficial effect on physical characteristics and muscle function. Using a one-sample *t*-test, it was determined that the combined averaged Cohen's *d* scores from each investigation at completion were significantly different from zero, $t_{(18)} = 2.25$, $p = 0.04$, confirming that the administration of T had a beneficial effect on physical characteristics and muscle function in older men with low to low-normal T concentrations.

The difference in the combined averaged Cohen's *d* measures from pre-treatment to post-treatment was 0.17, further indicating a small beneficial effect on physical characteristics and muscle function of men receiving T. In an attempt to test the difference between the combined Cohen's *d* scores of all the dependent variables from

pre-treatment to post-treatment, assuming all participants had the same value at pretreatment, an analysis of covariance was conducted, which found an adjusted mean *d* of 0.17 and that the post-treatment Cohen's *d* scores were significantly different from zero, $F_{(1, 17)} = 9.79$, $p = 0.006$.

Lean body mass

The Cohen's *d* pre-treatment scores representing the lean body mass measures, from the investigations in which it was present, were calculated and averaged together to determine if the treatment and control group measures did not differ from each other prior to the administration of T with an averaged Cohen's *d* score of -0.03 calculated. Using a one-sample *t*-test, it was determined that the lean body mass scores from participants were not significantly different from zero at pre-treatment, $t_{(14)} = 0.29$, $p = 0.8$, confirming that the treatment and control group measures for lean body mass did not significantly differ from each other prior to administration of T.

When looking at the averaged Cohen's *d* scores represented for lean body mass, a post-treatment score of 0.19 was calculated, indicating a small beneficial effect on lean body mass for participants receiving T administration. To determine significance of the post-treatment scores calculated from the investigations looking at lean body mass, a one-sample *t*-test was conducted determining that the lean body mass scores from participants did not significantly differ from zero at post-treatment, $t_{(14)} = 2.06$, $p = 0.06$.

The difference in the combined averaged Cohen's *d* measures from pre-treatment to post-treatment for lean body mass was 0.22, further indicating a small beneficial effect on lean body mass in men receiving T administration. In an attempt to test the difference between the combined Cohen's *d* scores of the lean body mass variable from pre-

treatment to post-treatment, assuming all participants had the same value at pre-treatment, an analysis of covariance was conducted, which found an adjusted mean *d* of 0.21 and that the post-treatment Cohen's *d* scores were significantly different from zero, $F_{(1, 13)} = 24.2$, $p = 0.0003$.

Fat mass

The Cohen's *d* pre-treatment scores representing the fat mass measures, from the investigations in which it was present, were calculated and averaged together to determine if the treatment and control group measures did not differ from each other prior to the administration of T with an averaged Cohen's *d* score of 0.18 being calculated, indicating a measureable difference between the treatment and control groups prior to the administration of T. Using a one-sample *t*-test, it was determined that the lean body mass scores from participants were significantly different from zero at pre-treatment, $t_{(11)} = 2.34$, $p = 0.04$, confirming that the treatment and control group measures for fat mass were significantly different from each other prior to the administration of T.

When looking at the averaged Cohen's *d* scores represented for fat mass, a post-treatment score of 0.34 was calculated, indicating a small beneficial decrease in fat mass for participants receiving T administration. To determine significance of the post-treatment scores calculated from the investigations looking at fat mass, a one-sample *t*-test was conducted determining that the fat mass scores from participants did significantly differ from zero at post-treatment, $t_{(11)} = 3.88$, $p = 0.003$.

The difference in the combined averaged Cohen's *d* measures from pre-treatment to post-treatment for fat mass was 0.16, further indicating a small beneficial effect on fat mass in men receiving T administration. In an attempt to test the difference between the

combined Cohen's *d* scores of the fat mass variable from pre-treatment to post-treatment, assuming all participants had the same value at pre-treatment, an analysis of covariance was conducted, which found an adjusted mean *d* of 0.15 and that the post-treatment Cohen's *d* scores were significantly different from zero, $F_{(1,10)} = 12.8$, $p = 0.005$.

Muscle strength

The Cohen's *d* pre-treatment scores representing the muscle strength measures, from the investigations in which it was present, were calculated and averaged together to determine if the treatment and control group measures did not differ from each other prior to the administration of T with an averaged Cohen's *d* score of -0.03 calculated. Using a one-sample *t*-test, it was determined that the muscle strength scores from participants were not significantly different from zero at pre-treatment, $t_{(10)} = 0.29$, $p = 0.8$, confirming that the treatment and control group measures for muscle strength did not significantly differ from each other prior to administration of T.

When looking at the averaged Cohen's *d* scores represented for muscle strength, a post-treatment score of 0.15 was calculated, indicating a small beneficial effect on muscle strength for participants receiving T administration. To determine significance of the post-treatment scores calculated from the investigations looking at muscle strength, a one-sample *t*-test was conducted determining that the strength scores from participants did not significantly differ from zero at post-treatment, $t_{(11)} = 1.25$, $p = 0.2$.

The difference in the combined averaged Cohen's *d* measures from pre-treatment to post-treatment for muscle strength was 0.18, further indicating a small beneficial effect on muscle strength in men receiving T administration. In an attempt to test the difference between the combined Cohen's *d* scores of the muscle strength variable from pre-

treatment to post-treatment, assuming all participants has the same value at pre-treatment, an analysis of covariance was conducted, which found an adjusted mean *d* of 0.18 and that the post-treatment Cohen's *d* scores were significantly different from zero, $F_{(1, 9)} = 7.8$, $p = 0.02$.

Functional mobility

The Cohen's *d* pre-treatment scores representing functional mobility measures, from the investigations in which it was present, were calculated and averaged together to determine if the treatment and control group measures did not differ from each other prior to the administration of T with an averaged Cohen's *d* score of -0.15 calculated, indicating measurable differences between the treatment and control groups prior to the administration of T. Using a one-sample *t*-test, it was determined that the functional mobility scores from participants were not significantly different from zero at pre-treatment, $t_{(8)} = 1.48$, $p = 0.2$, confirming that the treatment and control group measures for functional mobility did not significantly differ from each other prior to the administration of T, even though the Cohen's *d* scores indicated otherwise.

When looking at the averaged Cohen's *d* scores represented for functional mobility, a post-treatment score of -0.02 was calculated, indicating that there was not an effect on functional mobility for participants receiving T administration. To determine significance of the post-treatment scores calculated from the investigations looking at functional mobility, a one-sample *t*-test was conducted determining that the functional mobility scores from participants did not significantly differ from zero at post-treatment, $t_{(8)} = 0.16$, $p = 0.9$.

The difference in the combined averaged Cohen's *d* measures from pre-treatment to post-treatment for functional mobility was 0.13, which is close to indicating a small beneficial effect on functional mobility in men receiving T administration. In an attempt to test the difference between the combined Cohen's *d* scores of the functional mobility variable from pre-treatment to post-treatment, assuming all participants had the same value at pre-treatment, an analysis of covariance was conducted, which found an adjusted mean *d* of 0.09 and that the post-treatment Cohen's *d* scores were not significantly different from zero, $F_{(1, 7)} = 0.51$, $p = 0.5$.

Other considerations

For the purpose of determining if the form of administration had an effect on the Cohen's *d* measures at post-treatment, a one-way ANOVA was conducted comparing transdermal, oral, or injectable administration of T on the combined averaged Cohen's *d* measures from each investigation. The one-way ANOVA determined that the form of administration did not have a significant effect on the outcome measures seen at post-treatment, $F_{(2, 18)} = 0.25$, $p = 0.8$. Using the LSD post hoc comparisons, it was also determined that there was not a significant difference between the three forms of administration when comparing them against each other. In an additional attempt to determine if there was a difference in post-treatment Cohen's *d* measures based on the type of study (placebo controlled or no-treatment controlled) a one-way ANOVA was conducted, determining that the type of study did not have a significant effect on the post-treatment Cohen's *d* scores found from the investigations, $F_{(1,18)} = 0.67$, $p = 0.4$.

For the purpose of determining if the duration of treatment, average age in the treatment group, initial T levels in the treatment group, or the year of publication had a

significant effect on the post-treatment Cohen's *d* measures collected from all of the investigations reporting the above variables, Pearson's correlations were conducted. To determine if the duration of treatment of T had an effect on the combined post-treatment Cohen's *d* measures collected from all of the investigations, a Pearson's correlation was conducted which found that the duration of treatment did not have a significant effect on the post-treatment measures collected, $r_{(17)} = 0.19$, $p = 0.4$. To determine if the initial T concentrations of the participants in the treatment groups had an effect on the combined post-treatment Cohen's *d* measures collected from all of the investigations, a Pearson's correlation was conducted which found that the initial T concentrations did not have a significant effect on the post-treatment measures collected, $r_{(16)} = 0.15$, $p = 0.5$. To determine if the average age of the participants in the treatment groups had an effect on the combined post-treatment Cohen's *d* measures collected from all of the investigations, a Pearson's correlation was conducted which found that the average age of the participants did not have a significant effect on the post-treatment measures collected, $r_{(17)} = 0.02$, $p = 0.9$. To determine if the year of publication of the investigations had an effect on the combined post-treatment Cohen's *d* measures collected from all the investigations, a Pearson's correlation was conducted which found that the year of publication did not have a significant effect on the post-treatment measures collected, $r_{(17)} = 0.05$, $p = 0.8$.

Table 2. Cohen's *d* scores and significance.

Author and Year	dLBM pre	dLBM post	dFM pre	dFM post	dStr pre	dStr post	dMob pre	dMob post	dAve pre	dAve post
Marin et al., 1992 (69)	0.4337	0.4249	0.4802	0.4133					0.45695	0.4191
Morley et al., 1993 (60)							0.0205	0.6313	0.0205	0.6313
Ferreira et al., 1998 (29)							-0.2765	-0.686	-0.2765	-0.686
Snyder et al., 1999 (25)	0.0535	0.453	0.0451	0.3433	0.0945	0.0714	0.0888	0.1719	0.070475	0.2599
Clague et al., 1999 (22)	0.6163	0.4966			0.3151	0.3329	-0.4441	-0.3726	0.162433	0.1523
Kenny et al., 2001 (70)	0.3282	0.4833			-0.0734	0.1479			0.1274	0.3156
Blackmen et al., 2002 (62)	-0.986	-0.7448	0.2599	0.4095	-0.6807	-0.552			-0.46893	-0.29577
Boyanov et al., 2003 (59)	0.1294	0.0608	0.1535	0.2654					0.14145	0.1631
Crawford et al., 2003 (61)	-0.1995	0.1427	-0.0773	0.2284					-0.1384	0.18555
Wittert et al., 2003 (26)	-0.2782	-0.0596	0.1576	0.2679	-0.1275	-0.0673			-0.0827	0.047
Casaburi et al., 2004 (63)	0.3986	0.7435	-0.0735	0.0377	0.0749	0.3009			0.133333	0.3607
Svartberg et al., 2004 (31)							0.1409	0.0696	0.1409	0.0696
Page et al., 2005 (24)							-0.7602	-0.1789	-0.7602	-0.1789
Schroeder et al., 2005 (65, 71)	-0.3151	0.1926	0.0299	0.1338	0.6213	1.0939			0.112033	0.473433
Emmelot-Vonk et al., 2008 (8)	0.0833	0.2834	-0.0397	0.0787	-0.1627	-0.0818	0	0.0813	-0.02978	0.0904
Kenny et al., 2010 (27)	0.0603	0.1671			0.1326	0.1351	-0.2006	-0.0287	-0.00257	0.091167
Srinivas-Shankar et al., 2010 (28)	0.0548	0.2282	0.0523	0.1216	-0.0611	0.1142	0.094	0.1321	0.035	0.149025
Travison et al., 2011 (72)	-0.3623	-0.1167	0.3615	0.6273					-0.0004	0.2553
Sheffield-Moore et al., 2011 (64)	-0.4851	0.0255	0.8458	1.1406	-0.4703	0.1419			-0.03653	0.436
Average Cohen's *d*	-0.03121	0.185367	0.182942	0.338958	-0.03066	0.148827	-0.14858	-0.02	-0.02082	0.154674
One-Sample *t* tests	$p = 0.775$	$p = 0.058$	$p = 0.039$*	$p = 0.003$*	$p = 0.779$	$p = 0.238$	$p = 0.178$	$p = 0.875$	*p = 0.732*	p = *0.037**

dLBM pre – Cohen's *d* for lean body mass pretreatment. dLBM post – Cohen's *d* for lean body mass posttreatment. dFM pre – Cohen's *d* for fat mass pretreatment. dFM post – Cohen's *d* for fat mass posttreatment. dStr pre – Cohen's *d* for strength pretreatment. dStr post – Cohen's *d* for strength posttreatment. dMob pre – Cohen's *d* for function mobility pretreatment. dMob post – Cohen's *d* for function mobility posttreatment. dAve pre – Averaged Cohen's *d* from investigation pretreatment. dAve post – Averaged Cohen's *d* from investigation posttreatment. *Indicates significance.

Discussion

This meta-analysis, which pooled data from 19 randomized controlled trials, examined whether T administration, or its esters, improved physical characteristics and muscle function in men aged 45 and older with low to low-normal T concentrations by combining the effects on four variables: (1) lean body mass, (2) fat mass, (3) muscle strength, and (4) functional mobility. The analysis revealed that T administration resulted in an overall significant ($p = 0.037$) improvement in physical characteristics and muscle function when combining all of the variables investigated. In addition, after accounting for the variance between and within the treatment and control groups for all of the combined variables, significance was strengthened ($p = 0.006$). When the dependent variables were considered independently, the largest contribution came from a decrease in fat mass ($p = 0.003$), followed by a non-significant trend ($p = 0.06$) for an increase in lean body mass. While the strength ($p = 0.2$) and functional mobility ($p = 0.9$) measures were not found to be significant contributors to the overall beneficial effect of T, both of these non-significant variables were shown to have slight improvements in the expected direction. After accounting for the variance between and within the treatment and control groups for the dependent variables independently, lean body mass ($p = 0.0003$), fat mass ($p = 0.005$), and muscle strength ($p = 0.02$) were all found to be significant contributors, however, functional mobility ($p = 0.5$) scores were still not found to be significant. Therefore, T administration to men with low to low-normal testosterone concentrations can be considered a legitimate intervention for decreasing fat mass and eliciting modest

improvements in lean body mass and muscle strength. However, T administration does not solely need to improve body composition and muscle strength in men to be considered a successful intervention. T administration can also be considered a viable option in eliminating the deleterious effects of decreased T concentrations if it can maintain a male's lean body mass and muscle function as one ages. Therefore, the results from this investigation demonstrate that T administration can be considered a justifiable treatment to both maintain and improve body composition and muscle strength in men. Based on results from the Pearson's correlations, the magnitude of the effects of T administration were similar between the age of treatment group at baseline, the initial T concentrations at baseline, the type of T preparation, and the type of study or year the study was published.

How does testosterone elicit these beneficial effects on fat mass?

T administration has long been reported to have beneficial effects on body composition by increasing lean body mass and decreasing fat mass. Numerous investigations have found reductions in fat mass in sarcopenic and cachexic men with low to low-normal testosterone concentrations following T administration (8, 23, 25-28, 31, 34, 59, 61, 69-71, 73, 74). Reductions in fat mass may be caused by inhibition of lipid uptake and lipoprotein lipase activity in adipocytes (75), and the stimulation of catecholamine-induced lipolysis, resulting in the increase in the number of lipolytic beta-adrenergic receptors (1, 75-77). Sih et al. (50) observed significant decreases in serum leptin after T administration, therefore suggesting that alterations in the hormone leptin may be a mechanism responsible for changes in body composition with aging (50). This is because leptin, a protein released by adipocytes, has recently been identified as an

obese gene (78) and has been postulated to regulate weight and adipose tissue by signaling satiety, energy expenditure, and energy conservation (1, 76, 79). Leptin has also been identified to prevent fat accumulation in non-adipose tissue by increasing mitochondrial oxidation of fatty acids (80).

How does testosterone elicit these beneficial effects on lean body mass?

Numerous investigations have also found that T administration to sarcopenic and cachexic men with low to low-normal T concentrations increases lean body mass (8, 24-31, 34, 35, 40, 63, 65, 67, 70, 73, 74, 81, 82). The increases in muscle mass are likely due to the anabolic effect of T which has been found to increase muscular hypertrophy, protein synthesis (33, 34, 38, 39, 83), and muscle cross-sectional area (41). Specifically, Ferrando et al. (35) investigated the effects of intravenous T enanthate in seven healthy men. They assessed effects five days after administration and found a two-fold increase in the rates of protein synthesis and fractional synthesis, while the rates of protein breakdown and fractional breakdown remained unchanged (35). These positive changes in body composition due to T administration have been found to be just as effective in older men as younger, suggesting that effects on skeletal muscle and body composition are not age dependent. In addition, both older and younger men exhibited beneficial body composition changes with T that was positively correlated with dosage (84). In healthy young men with graded doses of intramuscular T administration, Bhasin et al. (41) found that muscle volume and cross-sectional areas (measured by magnetic resonance imaging) increased proportionately with dose and T concentration (41). Further, an increase in the number of myonuclei and satellite cells also resulted following T administration (38, 41). This is important because muscle growth and regeneration are

dependent on the addition of myonuclei to muscle fibers. Nuclei within the muscle fibers are postmitotic, which means that satellite cells are required for the formation of new myonuclei. Therefore, an increase in satellite cells could potentially increase the number of myonuclei, leading to muscular hypertrophy (41, 85-87).

How does testosterone elicit these beneficial effects on muscle strength?

Administration of T also increases muscle strength (35, 38, 50, 60, 61, 70). While it is difficult to predict whether a given pharmacological agent alone will improve muscle performance, different resistance exercises (concentric, eccentric, and isometric) have been used to measure the strength of specific muscle groups or joints (46, 61, 66, 69, 70, 88) following the administration of T. Ottenbacher et al. (89) used meta-analytical procedures and found that T therapy produced a moderate increase in muscle strength in men in both lower and upper extremity muscle groups (89). The underlying molecular mechanisms whereby the anabolic effects of T are realized are poorly understood. However, decades of research have given scientists numerous avenues to pursue in the search for how T increases lean body mass and how this may subsequently increase muscle strength. For instance, the myotrophic actions of T induce the retention of nitrogen (90). This effect subsequently results in an individual being in a positive nitrogen balance, a major anabolic effect of T (90). A number of investigations have found that T replacement results in muscular hypertrophy of type I and type II muscle cells by increasing fractional muscle protein synthesis (33, 34, 37-39). Schroeder et al. (67), found an increase in synthesis of myofibrillar proteins following oral androgen administration to older men (67). Satellite cell cultures from the vastus lateralis of healthy males were examined by Sinha-Hikim et al. (91), before and after administration

of a supra-physiological dose of T enanthate in an attempt to indentify androgen receptor expression. They found that androgen receptor expression primarily occurred in satellite cells and the myonuclei. However, expression was also noted in other sites, such as fibroblasts, CD34+ precursor cells, vascular endothelium, smooth muscle cells, and mast cells (91). Recently, Atkinson et al. (92) investigated the effects of transdermal T administration on the muscles of intermediate-frail and frail elderly men. The investigators hypothesized that decreases in T concentrations in aging males may cause a waning of anabolic maintenance over the years, and that this might be a factor in the atrophy of skeletal muscles in sarcopenic men. After six months, the gastrocnemius medialis in the men treated with T, showed a preservation of muscle thickness compared to a decline in muscle thickness in the placebo group, but there was not a significant change between groups in fascicle length or pennation angle of the muscle (92). The preservation of muscle thickness may indicate that T administration has the ability to maintain muscle size and strength, possibly maintaining a male's ability to stay physically capable and active. Both Urban et al. (39) and Ferrnado et al. (66) suggest that the anabolic effects of T are mediated through the up-regulation of insulin-like growth factor 1 (IGF-1) expression and the down-regulation of IGF binding protein-4 in skeletal muscle (39, 66). The IGF system in skeletal muscle is involved in the synthesis of muscle protein, and T has been found to increase peripheral IGF-1 concentrations (39, 76, 93, 94). This effect of T on IGF-1 concentrations might result in the increased synthesis of both contractile and non-contractile proteins, along with increased intramuscular concentrations of mRNA for IGF-1 in skeletal muscle (39). Mauras et al. (88) looked at T in a novel way; by using a long-acting GnRH agonist in healthy males to inhibit

endogenous T production. When this was done, in addition to decreases in protein synthesis, lean body mass, and muscle strength; investigators found decreased rates of 12C-leucine appearance in the blood, a measure of proteolysis, along with a significant decrease in nonoxidative leucine disappearance, a marker for whole-body protein synthesis. These changes in physiological function support the hypothesized role of T in the maintenance of normal body composition and specifically testosterones role in the maintenance of muscle tissue (88).

The results obtained from this meta-analysis show that T administration produced beneficial alterations in physical characteristics and muscle function. Reductions in fat mass and increases in lean body mass were the biggest contributors to these improvements, both of which concur with results from the investigations described above. What remains unclear is why there were not muscular strength improvements in conjunction with the improvements in lean body mass. Clearly, increases in lean body mass do not guarantee proportional improvements in strength. It's important to note; however, that the individuals included in these investigations did not participate in any forms of physical activity outside of their normal daily activities. This lack of physical activity could have played a role in why strength gains were not as prevalent as the improvements in body composition. Peterson et al. (95) used meta-analytical procedures, to examine the effectiveness of resistance exercise routines in eliciting strength improvement in aging persons. This analysis found improvements in each of the strength outcomes investigated, with a regression analysis revealing that higher intensity exercise programs were associated with greater improvements (95). Additionally, Peterson et al. (96) used meta-analytical procedures to determine if resistance training also improved

lean body mass. This investigation also revealed that resistance training can increase lean body mass in aging adults, with better results coming from higher volume resistance training programs (96). If resistance training increases both lean body mass and strength, it can be hypothesized that resistance training in conjunction with T administration would elicit further improvements in body composition and strength.

Although speculative, it may be that combined effects of androgens and exercise are due to the ability of T to increase protein synthesis (33, 34, 37-39). Casaburi et al. (63) examined the effects of T and resistance training in men with COPD that had low to low-normal T concentrations. Individuals that combined treatments (i.e., T and exercise) had greater average increases in lean body mass then either of the two treatments alone (exercise or T treatments). In addition, muscle strength, measured by a one-repetition leg press, on average increased 17.2% in men who only received T, 17.4% in men who only performed resistance training, and 26.8% with the men who received T in conjunction with resistance training (63). While further studies are needed to solidify consistent results from T in unison with resistance training as a form of rehabilitation for decreased lean body mass and muscle strength in men with low to low-normal T concentrations, the study of Casaburi and colleagues clearly demonstrated the potential of a combined treatment.

Limitations

It is important to remember that despite decades of anecdotal evidence about the benefits of T use, large, randomized, placebo-controlled clinical trials studying the effects of T administration are lacking. Most investigations use different forms of T and its esters, with different dosages, duration of treatment, and methods of administration. An

example of the difficulties in interpretation of findings that can result from methodological differences is exemplified by the differences in absorption rates among intramuscular, transdermal, and oral administration of T. The latter is especially problematic because a portion of the steroid is metabolized in the liver during its first pass through the portal vein. Another potential problem comes from differences in the specific form of T that is used in a given study (e.g., propionate, decanoate, undecanoate). While all of the forms used are meant to induce similar effects, not all will result in equivalent T concentrations following administration. It is necessary to make clear that while most investigations assess similar effects on male musculature, the changes in methods as our knowledge of endocrine function advances makes drawing definitive conclusions about the best methods difficult. Unfortunately, given the limitations in the number of suitable studies that were incorporated into this meta-analysis, combination of studies with disparate methods was inevitable. For example, there exist differences in T dosage, the frequency of administration (multiple times per day, daily, weekly, or bi-weekly), and forms of T and its esters administered. Regardless of the limitations, this meta-analysis provides evidence of the beneficial effects of T administration on male physical characteristics and muscle function to help the medical community determine if these benefits outweigh potential complications.

Future directions

Since the beginning of initial studies, it has been proposed that one could disassociate the anabolic and androgenic effects of androgens. More than 600 molecules and structures, mostly derived from T, have been synthesized in the search for a compound with purely anabolic actions (90, 97). The concerns regarding the risks of

long-term use of T has driven the pharmaceutical industry to try and create a derivative that promotes beneficial anabolic effects on target tissues without adverse effects on the prostate or cardiovascular system. These adverse effects have been well described by Calof et al. (98) and Haddad et al. (99) who both used meta-analytical procedures to investigate T's effects in middle aged and older men (98, 99). Subsequently, the development of selective androgen receptor modulators (SARMs) are being created and tested. SARMs are designed to bind an androgen to specific androgen receptor (AR) on target tissues. The AR is a member of the steroid and nuclear receptor superfamily, and a popular target for pharmaceuticals. AR ligands are classified as agonists (androgens) and antagonists (anti-androgens), based on their ability to either activate or inhibit the transcription of AR target genes (100). The differing interactions of steroidal and non-steroidal compounds with androgen receptors could be unique to its individual pharmacological action. In the future, compounds that assist with the maintenance or production of bone and muscle may be available, while hypothetically avoiding adverse effects on the cardiovascular system and the prostate. Unlike T, which is converted to active metabolites such as DHT, non-sterodial SARMs do not undergo aromatization or 5α-reduction. SARMs also act as agonists in muscle and bone, but only as partial agonists in the prostate and seminal vesicles (101). The first generation of SARM pharmacophores can be classified into four categories: aryl propionamide, bicyclic hydantoin, quinoline, and tetrahydroquinoline analogs. These are all non-steroidal SARMs that are orally available, and are all metabolized and eliminated solely through hepatic metabolism (100-102).

Conclusion

After the synthesis of the 19 randomized controlled trials, the analysis revealed a significant improvement in physical characteristics and muscle function when combining all the variables. The greatest contribution to the significance found from the combined variables came from a decrease in fat mass, followed by a trend for an increase in lean body mass when looking at the dependent variables independently. While the strength and functional mobility measures were not found to be significant contributors to the overall beneficial effect of T administration on physical characteristics and muscle function, all four variables were shown to have slight improvements in the expected direction. After accounting for variance, the combined effect from all the variables, as well as, the lean body mass, fat mass, and muscle strength variables were all found to be significant contributors, however, functional mobility scores still did not reach significance. What is important to remember is that T administration does not solely need to improve body composition and muscle strength in men with low to low-normal T concentrations to be considered a successful intervention. T administration can also be considered a viable option in eliminating the deleterious effects of decreased T concentrations if it can maintain a male's lean body mass and muscle function as one ages. Therefore, the results from this investigation demonstrate that T administration can be considered a justifiable treatment to both maintain and improve body composition and muscle strength in men.

The slow pace of clinical development of T agents as anabolic therapies illustrates the legal, regulatory, and conceptual barriers that have hindered this form of preventative or rehabilitative medicine for decades. A lack of consensus on how to define the concept of symptomatic physical impairment has made it difficult to develop criteria for subject selection. Once a subject selection criterion has been established, a strict dose relationship curve is needed for all measurable side effects of T administration. The optimal formulation of T for administration, the benefits and risks of long-term continual use, and the durability of measured improvements are all still issues that need to be resolved. In addition to improvements in muscle mass and strength, it would be necessary to demonstrate gains in physical function and health-related outcomes to suggest T therapy as being a responsible tool to improve the quality of life in older men with low to low-normal T concentrations that have associated reductions in muscle mass and muscle strength. Because T administration has been found to improve body composition and slightly improve muscle strength, it can be rationalized that T administration in conjunction with an exercise routine may allow for larger beneficial changes in body composition and muscle strength, possibly even resulting both statistically and clinically significant improvements in functional mobility. If T administration can continue to show promise in improving male physical characteristics and muscle function, it may be considered a viable option in both the prevention and rehabilitation of decreased muscle mass, muscle strength, and functional mobility seen in men with low to low-normal T concentrations.

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APPENDIX A
EXTENDED LITERATURE REVIEW

Androgens

Testosterone (T) is the most abundant and important androgen in the human male. In a healthy male, endogenous T production rates are approximately 7_{mg} per day (1). The majority of plasma T in men is produced by the Leydig cells of the testes, under the control of the pituitary luteinizing hormone (LH) (2, 3, 4, 5, 6). LH affects Leydig cells directly, inducing the up-regulation of T production, or by allowing the conversion of T to 5-α-dihydrotestosterone (DHT) or estradiol (7). T is converted to DHT via the intracellular enzyme 5-α reductase (6). T acts directly on the androgen receptors of target organs, such as: muscles, bones, and the testis. However, in peripheral tissues, such as the external genitalia, accessory sex organs, and the skin, T is converted to DHT before acting on the androgen receptor. In adipose and brain tissue, T is aromatized to estradiol which acts through estrogen receptors (6, 7).

The action of androgens on tissue depends highly on the developmental age of a male. During the development of the fetus, T causes differentiation and development of the seminal vesicles, epididymis, and vas deferens from the Wolffian ducts. While the development of the prostate, penis, and scrotum depends on the conversion of T to DHT. During puberty, androgens cause nitrogen retention, stimulate pubertal growth, laryngeal and phallic enlargement, and the development of secondary sex characteristics. In the

adult male, androgens are required for normal sexual function as well as the maintenance of secondary sex characteristics, normal spermatogenesis, normal haematopoiesis, and muscle and bone (3, 7).

T production is regulated via a negative feedback mechanism. When T becomes too low, there is an increase in gonadotropin-releasing hormone (GnRH) from the hypothalamus. This increase in GnRH activates the pituitary to release LH and follicle-stimulating hormone (FSH) (6, 7) which helps produce approximately 0.24 μM/day of T (2). Once T concentrations reach an individual's baseline level, GnRH secretion is inhibited, as well as LH and FSH secretion. Normal T concentrations highly vary among individuals and range from 250 ng/dL to 800 ng/dL (3, 6, 7). The second largest producer of T in the body comes from the adrenal cortex, which produces approximately 0.002 μM/day of androgens, mainly androstenedione (2, 8).

The majority of T circulating in the blood is bound to one of two proteins: albumin or sex hormone-binding globulin (SHBG) (8). The small fraction of T not bound to SHBG or albumin is considered free T. Free T is not chemically attached to any proteins and is considered the "active" form of T, because it is readily available to bind to androgen receptor sites on target organs and cells (2, 5, 6). However, this previous definition has caused disagreement in the scientific community due to the fact that T is a lipophilic compound, which means that T cannot be free in plasma without being bound to different proteins or fats while in circulation. Therefore, new endocytic pathways responsible for the delivery of steroids and vitamins to renal and gonadal tissues are being identified, offering an alternative to the "free hormone hypothesis." (9).

The combined fraction of albumin-bound and free T is commonly termed "bio-available T." This is because T's affinity for albumin is weak, and therefore making it readily available for tissues when needed, similar to free T. On the other hand, T has a strong affinity for SHBG, being tightly bound to its receptor, and not free for other processes (2, 5, 6). While T concentrations have been shown to decrease with age, the opposite is true for SHBG. In many of the same investigations that found decreased T with age, SHBG was consistently shown to increase with age (10-13). The reason for the increase in SHBG concentrations with age remains unclear. A consensus seems to have been established that total T and bio-available T have an annual decrease by approximately 0.5 - 2.0% in males starting around the age of 30 (10, 14-17).

There are likely many factors which cause the decrease in T with age including predominant changes which appear at the level of the testes, a decline in the number of Leydig cells and a decline in the activity of enzymes involved with the metabolic pathways related to T production (5, 18-20). There is also a possibility that age-related declines in hypothalamo-pituitary-adrenal (HPA) function may contribute to decreased T production in aging men (5, 18-20). The concept revolves around the idea that there is a gradual failure of the HPA axis to respond appropriately to the decline in T, likely due to decreased sensitivity of the pituitary to GnRH (18, 21, 22). Other than aging, body mass index (BMI), smoking, general health status, and physical activity may also contribute to alterations in T concentrations (12). An example of decreased T concentrations due to BMI is that accumulation of abdominal body fat results in increased activity of the HPA axis (23), causing an imbalance between corticosteroid and gonadal sex steroid hormone concentrations (24).

Anabolic effects

T is characterized to have two dominating effects on target organs: anabolic effects and androgenic effects. The androgenic effects are responsible for producing masculine secondary sex characteristics such as facial hair growth, deepening of the voice, increased body hair growth, and increased muscle development. The anabolic effects of T on a molecular level are still highly unknown and poorly understood. However, decades of research has given scientists numerous avenues to venture. One of the longer standing effects of T is the myotrophic action on musculature which is specifically responsible for the retention of nitrogen, putting an individual in a positive nitrogen balance, which has been typically identified as the prominent anabolic effect of T (25). A number of investigations have found that T replacement results in muscular hypertrophy of type I and type II muscle cells by increasing fractional muscle protein synthesis (26-30). Schroeder et al. (31), found an increase in synthesis of myofibrillar proteins following oral androgen administration to older, community-dwelling men (31). In healthy young men with graded doses of intramuscular T administration, Bhasin et al. (32) found that muscle volume and cross-sectional areas (measured by magnetic resonance imaging) increased proportionately to administration dose and T concentrations (32). An increase in the number of myonuclei and satellite cells has also been measured following T administration (29, 32). This is important because muscle growth and regeneration are dependent on the addition of myonuclei to muscle fibers. Nuclei within the muscle fibers are postmitotic, which means that satellite cells are required for new myonuclei. Therefore, an increase in satellite cells could potentially increase the number of myonuclei, leading to muscular hypertrophy (32-35). In 1998,

Ferrando et al. (36) investigated the effects of intravenous T enanthate in 7 healthy men before and 5 days following administration. A two-fold increase in the rates of protein synthesis and fractional synthesis were observed, while the rates of protein breakdown and fractional breakdown remained were unchanged (36).

Satellite cell cultures from the vastus lateralis of healthy males were examined by Sinha-Hikim et al. (37), before and after administration of a supra-physiological dose of T enanthate in an attempt to indentify androgen receptor expression. Following T administration, investigators found that the predominant sites of androgen receptor expression appeared in satellite cells and the myonuclei, but they were also identified in numerous other sites, such as: fibroblasts, CD34+ precursor cells, vascular endothelial, smooth muscle cells, and mast cells (37). Recently in 2010, Atkinson et al. (38) investigated the effects of transdermal T administration on the muscles of intermediate-frail and frail elderly men. The investigators hypothesized that decreases in T concentrations seen in the aging males may cause a waning of anabolic maintenance over the years, which might be a factor in the atrophy of skeletal muscles in sarcopenic men. After six months, the gastrocnemius medialis in the men treated with T, showed a preservation of muscle thickness compared to a decline in muscle thickness seen in the placebo group, but there was not a significant change between groups in fascicle length or pennation angle of the muscle (38). The preservation of muscle thickness may indicate that T administration has the ability to maintain muscle size and strength, possibly maintaining a male's ability to stay physically productive and physically active.

Both Urban et al. (30) and Ferrnado et al. (39) suggest that a potential mechanism of the anabolic effects of T may be the stimulation of insulin-like growth factor 1 (IGF-1)

expression and the down regulation of IGF binding protein-4 in skeletal muscle (30, 39). This might result in the increased synthesis of both contractile and non-contractile proteins, along with increased intramuscular concentrations of mRNA for IGF-1 in skeletal muscle (30). This reaction to androgens may be related to anabolic hormones stimulating ribosomal activity and RNA polymerase synthesis, by stimulating mitosis in myoblast cells (2, 30, 40). Some investigators looked at T in a novel way; by using a long-acting GnRH agonist in healthy males to inhibit endogenous T production. When this was done, investigators found decreased rates of 12C-leucine appearance in the blood, a measure of proteolysis; and a significant decrease in nonoxidative leucine disappearance, a marker for whole-body protein synthesis (41).

T administration has also been identified for promoting the differentiation of mesenchymal multipotent cells into the myogenic lineage and inhibiting their differentiation into the adipogenic lineage (42). Androgens have been noted to regulate mesenchymal multipotent cell differentiation by binding to androgen receptors, and promoting the association of the androgen receptors to β-catenin and the translocation of the new androgen receptor β-catenin complex into the cell nucleus, activating the T-cell-specific transcription factor 4 (TCF-4) (32). Activation of TCF-4 has been found to modulate a number of WNT-regulated genes that promote myogenic differentiation and inhibit adipogenic differentiation (43).

Delivery systems

Most of the T that is prescribed for the purposes of hormone therapy is in the form of T esters. There are a number of different esters of T, including: modern enanthate and cypionate, as well others such as: acetate, propionate, phenylpropionate, isocaproate,

caproate, decanoate, and undecanoate. Each of these esters is a molecular chain composed of carbon, hydrogen, and oxygen atoms. The main difference between the esters is how many carbon and hydrogen atoms make up the chain (44, 45).

The oral forms of T and alkylated androgen compounds are generally not recommended for T administration because of its ability to produce deleterious effects on the male body, including hepatotoxicity and alterations in the lipid profile (3). This is because orally administered T is almost completely metabolized and inactivated during its first pass through the liver. T undecanoate is considered a more acceptable orally administered T because it is absorbed from the gastrointestinal tract into the lymphatic system due to its lipophilic side chain (3).

There are several T formulations that can be delivered through intramuscular injections. T enanthate and cypionate are examples of T esters that are available in oil suspension preparations for intramuscular injection. Esterification of T is performed in order to improve the solubility of T in oil, which in turn slows the release of the T from the site where it enters the body. T, in its free, non-esterified form, has poor solubility in both oil and water, but it can be suspended in water. On the other hand, non-esterified T is available in aqueous injectable forms. However, this form of T stays active in the body for a very short period of time and must be injected on a daily basis in order to maintain a continuous level of increased T concentrations. For this reason, aqueous injectable formulations are rarely used for T replacement therapy, especially in clinical settings (3, 44, 45).

When a particular T ester is deemed "fast acting" or "slow acting," it is referring to the partition coefficient/solubility in oil. As described above, esters with more carbon

atoms will generally be more soluble in oil, making them often referred to as "slow-acting" esters because they will stay active longer in the blood. Esters that are less soluble in oil are often referred to as "fast-acting" forms of T, as they are more quickly available to bind with target androgen receptors. The quicker availability is due to fewer numbers of carbons on the ester chain which quickly releases the T in the bloodstream (44, 45).

Transdermal delivery of T, via a patch or gel, allows for absorption directly into systemic circulation at a controlled rate, decreasing the fluctuations compared against injectable forms of T. Transbuccal delivery systems and subcutaneous pellets are relatively new forms of delivery systems that also allow for slow absorption rates (3, 45).

Adverse effects

T replacement therapy has long been criticized for its adverse effects on the cardiovascular system. One of the more serious problems is a decrease in circulating high density lipoprotein (HDL) cholesterol, which may assist in the prevalence of cardiovascular disease (CVD). This is due to the androgens suppressing hepatic endothelial triglyceride lipase activity, the enzyme primarily responsible for the clearance of HDL-cholesterol. Almost all serious hepatic abnormalities are associated with the orally active 17α-alkylated androgens, such as methyltestosterone, methandrostenolone, oxandrolone, and stanozolol (8, 46). In 1992, Marin et al.(24), found that low to moderate doses of T absorbed without direct expose to the liver, to obese, middle-aged men, was followed by improvements in insulin resistance and decreases in blood glucose, serum cholesterol concentrations, and blood pressure (61). Both Haddad et al. (47) and Calof et al. (48), using meta-analytical procedures, could not find significant differences in cardiovascular disease event rates between T-treated and placebo-treated men (47, 48).

It is important to note that both Haddad and Calof suggest large randomized trials are still needed for more accurate results.

Another possible complication of T replacement therapy is polycythemia, which may lead to hyperviscosity syndrome, a condition known to be associated with heart failure, stroke, and other cardiovascular impairments. Investigations have noted that T administration increases the mass of red blood cells (2, 49, 50), possibly by having stimulatory effects on erythropoeisis by enhancing erythropoietin production via receptor-mediated transcription within bone marrow (2, 51). Androgen receptors have been found on cultured erythroblasts, which seemingly direct stimulatory effects on bone marrow stem cells by exogenous T (2, 52, 53). T has also been found to enhance the production of heme and globin (2, 54-56) and increase 2,3, diphosphoglycerate concentrations in the blood, which has been found to enhance oxygen delivery to tissues and organs (2, 57). Referring back to Calof et al. (48), using meta-analytical procedures, found that an increase in hematocrit over 50% was the most frequently seen adverse event related to T administration. The analysis found that the T-treated men were 3.67 times more likely to have hematocrit above 50% than placebo-treated men (48).

The last major concern associated with T administration is its effect on the prostate. Gerstenbluth et al. (58) looked at the effects of intramuscular injections of T cypionate on prostate-specific androgens (PSA) changes in hypogonadal men. Researchers found that out of the 54 patients, 6 (11.1%) required a prostate biopsy because of a rise in PSA above 4.0 ng/mL, and of those 6, 1 patient was diagnosed with prostate cancer (58). Wallace et al. (46) did a similar investigation, looking at the effects of intramuscular injections of T enanthate on prostate function, size, and PSA levels.

Investigators did not find a detectable change in prostate size or a significant change in PSA concentrations (46). However, according to the Calof et al. (48) meta-analytical results, T-treated men were 1.8 times more likely to have a prostate related event than men who had been administered a placebo (48). Observance of the prostate and PSA concentrations are extremely important during clinical investigations of T administration to combat the increased risk of prostate cancer. Although, recently the U.S. Preventive Services Task Force, which advises the government on health prevention measures, downgraded its recommendation on prostate cancer screening to a "D," discouraging against the service because "there is moderate or high certainty that the service has no net benefit or that the harm outweighs the benefits" (59). Andrew Vickers of Memorial Sloan-Kettering Cancer Center in New York analyzed how effective PSA velocity (a spike in PSA) is as a tool for predicting prostate cancer. The team studied more than 5,000 men over 55 who were participating in a study of Merck & Co Inc's finasteride sold as ProscarTM and PropeciaTM, a drug commonly used to treat enlargement of the prostate gland. The team focused on men in the placebo group who were followed with yearly PSA tests. After seven years, all men in the study underwent a prostate biopsy. Based on these tests, investigators couldn't find an important link between a sudden rise in PSA levels and prostate cancer, noting that an elevated PSA level was a much better way to predict prostate cancer (59). Regardless if PSA screening is an effective predictor of prostate related disease, it must continue to be closely monitored in relation to T administration and T's anabolic effects on prostate tissue.

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APPENDIX B
INSTITUTIONAL REVIEW BOARD APPROVAL

THE UNIVERSITY OF MEMPHIS

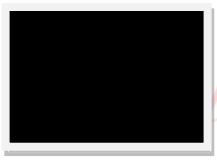
Institutional Review Board

To:	Michael Oliver, Jeffrey Berman, and Stephan Schoech Health and Sport Sciences
From:	Chair or Designee, Institutional Review Board For the Protection of Human Subjects irb@memphis.edu
Subject:	The Effects of Testosterone Replacement on Physical Function, Hand Grip Strength, Lean Body Mass and Fat Mass: A Meta-Analysis (112911-1006)
Approval Date:	December 21, 2011

This is to notify you that the Institutional Review Board has designated the above referenced protocol as exempt from the full federal regulations. This project was reviewed in accordance with all applicable statuses and regulations as well as ethical principles.

When the project is finished or terminated, please submit a Human Subjects Research Completion Form (COMP) to the Board via e-mail at irbforms@memphis.edu. This form can be obtained on our website at http://www.memphis.edu/irb/forms.php.

Approval for this protocol does not expire. However, any change to the protocol must be reviewed and approved by the board prior to implementing the change.



Digitally signed by Jacqueline Y. Reid
DN: cn=Jacqueline Y. Reid, o, ou=Institutional Review Board Administrator, email=jreid@memphis.edu, c=US
Date: 2011.12.21 14:36:23 -06'00'

Chair or Designee, Institutional Review Board
The University of Memphis

Cc: Dr. Zsolt Murlasits