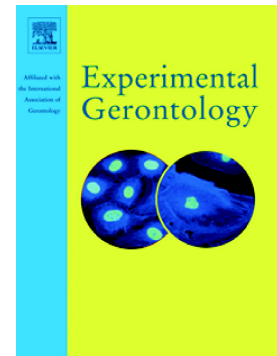


Effect of testosterone supplementation on sarcopenic components in middle-aged and elderly men: A systematic review and meta-analysis

Suena Medeiros Parahiba, Édina Caroline Ternus Ribeiro, Camila Corrêa, Patrícia Bieger, Ingrid Schweigert Perry, Gabriela Corrêa Souza



PII: S0531-5565(20)30454-X

DOI: <https://doi.org/10.1016/j.exger.2020.111106>

Reference: EXG 111106

To appear in: *Experimental Gerontology*

Received date: 4 June 2020

Revised date: 30 September 2020

Accepted date: 1 October 2020

Please cite this article as: S.M. Parahiba, É.C.T. Ribeiro, C. Corrêa, et al., Effect of testosterone supplementation on sarcopenic components in middle-aged and elderly men: A systematic review and meta-analysis, *Experimental Gerontology* (2018), <https://doi.org/10.1016/j.exger.2020.111106>

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

EFFECT OF TESTOSTERONE SUPPLEMENTATION ON SARCOPENIC COMPONENTS IN MIDDLE-AGED AND ELDERLY MEN: A SYSTEMATIC REVIEW AND META-ANALYSIS

Suena Medeiros Parahiba^{a,b}, Édina Caroline Ternus Ribeiro^{a,b}, Camila Corrêa^{ac},
Patrícia Bieger^b, Ingrid Schweigert Perry^d, Gabriela Corrêa Souza^{a,b}

^aHospital de Clínicas de Porto Alegre, 2350 Ramiro Barcelos Street, Porto Alegre, Rio Grande do Sul, Brazil.

^bGraduate Program in Food, Nutrition and Health, Medical School, Federal University of Rio Grande do Sul, Brazil- ppgans@ufrgs.br, 2400 Ramiro Barcelos Street - 2nd floor, Porto Alegre, Rio Grande do Sul, Brazil.

^cGraduate Program in Medical Sciences: Endocrinology, Medical School, Federal University of Rio Grande do Sul, Brazil – ppgendo@ufrgs.br, 2400 Ramiro Barcelos Street - 2nd floor, Porto Alegre, Rio Grande do Sul, Brazil.

^dFood and Nutrition Research Center, Hospital de Clínicas de Porto Alegre/Federal University of Rio Grande do Sul- cesar@ufrgs.br, 2350 Ramiro Barcelos Street, 3rd floor of the Clinical Research Center, Porto Alegre, Rio Grande do Sul, Brazil.

Corresponding Author: ggabriela.c.souza@gmail.com (G.C. Souza) - 2350 Ramiro Barcelos Street, 2nd floor, Porto Alegre, Rio Grande do Sul, Brazil.

E-mail addresses: s.parahiba@hcpa.edu.br (S.M. Parahiba); edinaternus@gmail.com (É.C.T. Ribeiro); camila_nutri@yahoo.com.br (C. Corrêa); pati_bieger@hotmail.com (P. Bieger); atputp@gmail.com (I.S. Perry); ggabrielacsouza@gmail.com (G.C. Souza).

ABSTRACT

The aim of this study was to conduct a systematic review of the literature of randomized controlled trials on the effect of testosterone (T) supplementation compared to the placebo group or lower dose on sarcopenic components (muscle mass, strength and physical performance) in middle-aged and elderly men. Major electronic databases were searched for articles published on or before December 2019. Studies including individuals with age ≥ 40 years and which described the effect of T supplementation on sarcopenic components were found eligible (11 studies). Outcomes were calculated as the difference in means between the experimental and control/placebo groups, and data were presented as effect size with 95% confidence limits (95%CI). The meta-analysis was performed using a random effects model. Regarding lean body mass (LBM), eight studies evaluated the effect of T supplementation on this outcome, of these, seven reported gains after the intervention period. Our meta-analysis showed a beneficial effect on LBM of 2.54kg (95% CI, 1.27 to 3.80) ($p < 0.001$). In muscle strength (MS), seven included studies evaluated the handgrip strength (HGS) and just one reported gain after the intervention period, but the meta-analysis showed an increase for HGS of 1.58 kgf (95% CI, 0.17 to 3.0) ($p = 0.03$). The second outcome for MS was leg strength (LS), where nine studies were included and five demonstrated gains in this parameter after the intervention period. In the meta-analysis, two out of three tests showed an effect on LS: T supplementation increase the leg press strength in 91.23 N (95%CI, 0.23 to 182.22) ($p = 0.05$) and leg extension in 144.10N (95%CI, 44.21 to 244.00) ($p < 0.01$). In physical performance, four studies evaluated this outcome, with three of them showing positive effects in this parameter. In the meta-analysis, only two studies that reported the same assessment test (Physical Performance Test) were included, but no effect of T supplementation on this parameter was found. It can be concluded that T supplementation influences sarcopenic components in middle-aged and older men, because is associated with increased in muscle mass and strength in addition to physical performance.

KEYWORDS

Testosterone; Muscle Strength; Body Composition; Physical Functional Performance; Middle-Age; Aged.

1. INTRODUCTION

The aging process naturally causes changes in the skeletal muscle system. These changes are, to some extent, related to the levels of certain hormones. Systemic concentrations of anabolic hormones gradually decrease over time, which may be associated with impairment of muscle strength and function (Guadalupe-Grau et al., 2017).

Among the hormones considered anabolic, testosterone (T), the main male sex hormone, has major metabolic and vascular actions, with multiple physiological effects in various tissues and organs, as well as an essential role in maintaining muscle mass and function (Saad et al., 2017; Harada, 2018; Oki et al., 2015). In muscle it is responsible for stimulating protein synthesis and inhibiting protein degradation, favoring muscle hypertrophy. The physiological effects of T are induced by its binding to the intracellular androgen receptor, which when moving to the nucleus induces the transcription of specific genes (Vingren et al., 2010).

In view of the complexity and the various implications of T, there is growing interest in investigating its clinical applications. T deficiency has already been proven to correlate with the onset of components of sarcopenia, a disease rooted in adverse muscle changes that accumulate throughout life. The presence of sarcopenia increases the risk of falls, fractures, and mobility disorders, and contributes to reductions in quality of life, loss of independence and need for long-term medical care (Cruz-Jentoft et al., 2018). In elderly men, the T replacement has already been shown to be capable of helping to reverse the deleterious effects of sarcopenia on muscle mass and function (Vingren et al., 2010), promoting changes in body composition, especially in lean body mass (LBM) gain and neuromuscular adaptation, mitigating the disability which results from senescence (Saad et al., 2017; Storer et al., 2016; Neto et al., 2015; Hsu et al., 2014; De Spiegeleer et al., 2018). However, the effects of such treatment are not fully understood; its objectives, dosages and duration vary widely (Tenover, 1992; Wittert et al., 2003; Bhasin et al., 2005; Skinner et al., 2018), which hinders comparisons and definitive conclusions (Neto et al., 2015).

Within this context, we conducted a systematic review and meta-analysis, where our research question was formulated according to the PICOS format: what is the effect of T supplementation (intervention) compared to the placebo group or lower dose (comparison) by randomized controlled trials (study) on sarcopenic components (outcome) in middle-aged and elderly men (population)? The protocol of this review was registered in PROSPERO on September 25, 2019, with accession number CRD42019126589.

2. MATERIAL AND METHODS

2.1. Data Sources and Search Strategy

The search strategy included randomized clinical trials published between January 1990 and December 2019 and indexed in the MEDLINE (via PubMed), Embase, and Cochrane Library databases, with no limitations on language. The following combinations of MeSH terms were used: ((“Sarcopenia”[Mesh] OR “Sarcopenia” OR “Sarcopenias” OR “Muscular Atrophy”[Mesh] OR “Muscular Atrophy” OR “Atrophies, Muscular” OR “Atrophy, Muscular” OR “Muscular Atrophies” OR “Atrophy, Muscle” OR “Atrophies, Muscle” OR “MuscleAtrophies” OR “MuscleAtrophy” OR “Neurogenic Muscular Atrophy” OR “Atrophies, Neurogenic Muscular” OR “Atrophy, Neurogenic Muscular” OR “Muscular Atrophies, Neurogenic” OR “Muscular Atrophy, Neurogenic” OR “Neurogenic Muscular Atrophies” OR “Neurotrophic Muscular Atrophy” OR “Atrophies, Neurotrophic Muscular” OR “Atrophy, Neurotrophic Muscular” OR “Muscular Atrophies, Neurotrophic” OR “Muscular Atrophy, Neurotrophic” OR “Neurotrophic Muscular Atrophies”) AND (“Testosterone”[Mesh] OR “Testosterone” OR “17-beta-Hydroxy-4-Androsten-3-one” OR “17 beta Hydroxy 4 Androsten 3 one” OR “Androtop” OR “Histerone” OR “Sterotate” OR “Sustanon” OR “Androderm” OR “Testoderma” OR “Testolin” OR “Testopel” OR “Testosterone Sulfate” OR “AndroGel” OR “8-Isotestosterone” OR “8 Isotestosterone” OR “17-beta-Hydroxy-8 alpha-4-Androsten-3-one” OR “17 beta Hydroxy 8 alpha 4 Androsten 3 one” OR “Andropatch” OR “Testin”)). Embase and the Cochrane Library were searched with a combination of the descriptors ‘muscle atrophy’, ‘sarcopenia’, and ‘testosterone’. First, retrieved entries/articles were screened according to their titles and abstracts. In a second step, the retrieved studies were read in full. In both steps, two independent reviewers (ÉCTR and SMP) evaluated the papers, following the eligibility criteria. Neither of the reviewers were blinded to article journals, institutions, and authors. A third reviewer (GCS) was consulted in case of disagreements. A hand search of the reference lists of selected articles was conducted to supplement the electronic search strategy. Studies that were part of the gray literature were not sought or included.

2.2. Eligibility Criteria

The inclusion criteria were randomized clinical trials of middle-aged men (age 40 to 60 years), elderly men (age >60 years), or both (age >40 years) that evaluated the effects of T supplementation on sarcopenic components (muscle mass, strength and physical performance). Articles were required to mention at least one of the variables established to evaluate the outcomes, based on the consensus definition of sarcopenia (Cruz-Jentoft et al.,

2019). The exclusion criteria were as follows: duplicates; study population with any active inflammatory disease or decompensated chronic disease; absence of a control or placebo group; studies that could not be located in full; and studies whose authors did not respond to attempts at contact (in case of incomplete data and/or questions about the study).

2.3. Data Extraction

Two independent reviewers (ÉCTR and SMP) performed data extraction and a third reviewer was consulted in case of disagreements (GCS). The outcomes of interest to the present study were sarcopenic components (muscle strength, muscle mass and physical performance). The established variables for muscle strength were handgrip strength (HGS) by manual dynamometer (kgf) and leg strength (LS) by 1-repetition-maximum strength test or measuring quadriceps peak torque (N); for muscle mass was LBM (by dual x-ray absorptiometry, hydrodensitometry and electrical bioimpedance analysis) (kg); and for physical performance, tests such as gait speed, the Short Physical Performance Battery (SPPB), and the Timed-Up and Go test (TUG). A structured data form was used to organize the collected information using the Microsoft Excel spreadsheet (Microsoft Office® 2016) and the EndNote X9® reference management software was used during the selection procedure. For each of the included studies, the following data were extracted: name of the authors, year of publication, baseline characteristics of the study population (location, age, total serum T, free T), intervention type (oral capsule, transdermal or intramuscular injection), dose, time and frequency of T supplementation, beyond the differences in means and/or medians values for the outcomes.

2.4. Methodological Quality of Studies

The methodological quality of the studies was assessed with the revised Cochrane risk-of-bias tool for randomized trials (RoB 2, version of 15 March 2019) (Sterne et al., 2019), that considers five domains for evaluation, and categorizes trials as having a “low risk of bias,” “high risk of bias,” or “some concerns”. The quality of the studies was performed by two independent reviewers (ÉCTR and SMP) and a third reviewer (GCS) was consulted in case of disagreements.

2.5. Data Synthesis and Analysis

All measurements were standardized and converted before analyzes, we reported mean difference. Articles with data that reported standard error were converted to standard deviation (SD), using the formula: $SD = SE * \sqrt{n}$. The Review Manager® Software, version 5.3, was used to conduct meta-analyses using a random-effects model in general and subgroups analysis, considering the high heterogeneity that was found in the analyses. Statistical

heterogeneity was calculated from I^2 statistic, with values of <25, 25-50 and >50% are considered indicative of small, medium, and large amounts of heterogeneity, respectively (Higgins et al., 2003). The χ^2 test for heterogeneity was also done and we judged a p value less than 0.05 significant. The inverse variance method and 95% total confidence intervals (95% CI) were used. Sensitivity analysis was performed in order to better investigate the heterogeneity. Subgroup analyses were also carried out, where studies were grouped according to the supplementation route (transdermal, intramuscular injection or oral capsule), intervention time (studies with an intervention time <12 months and studies with 12 months or more of intervention) and individual quality of articles (low risk of bias, some concerns about the risk of bias and high risk of bias). We proposed, but it was not possible to perform subgroups according to age (means adults 40 to 65 years age, and elderly > 65 years age), since all the articles included had an average of over 65 years age. It was also not possible to carry out analyses according to the nature of control group, only one study did not use placebo as a control group, thus, we detail it in the sensitivity analysis.

3. RESULTS

3.1 Search Results

The search strategies yielded 1668 records. The titles and abstracts of all were examined by two investigators to determine if they met the inclusion criteria. A total of 1629 articles were excluded after this procedure. The 39 remaining articles were read in full to determine their appropriateness for further analysis. After this step, 11 studies were selected for this review. Of these, eight were included in meta-analysis for LBM outcomes, seven in meta-analysis for LS, seven in meta-analysis for HGS, and two in meta-analysis for physical performance. This process is illustrated in Figure 1.

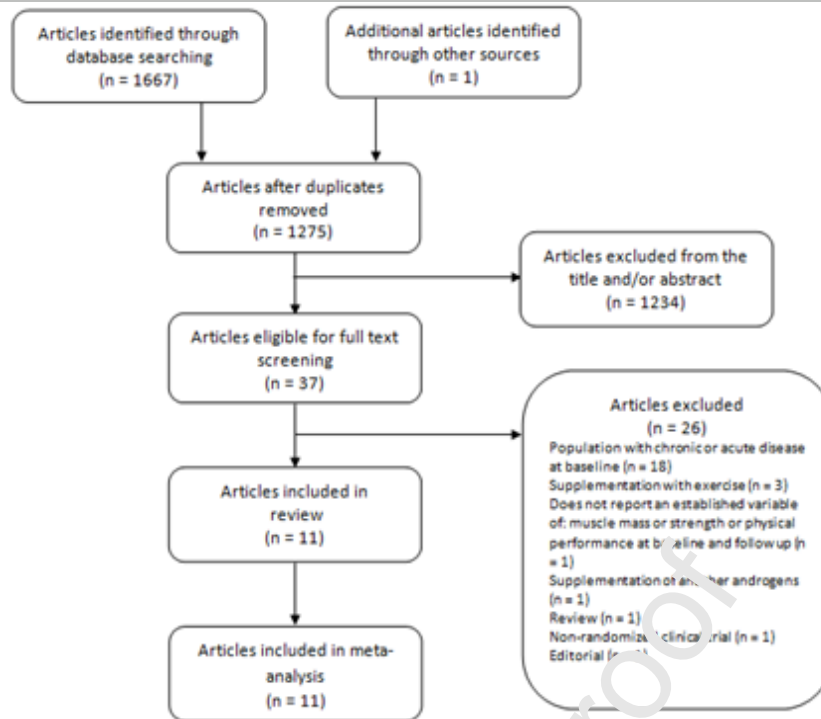


Figure 1. Flowchart of identification and selection of articles included in the systematic review and meta-analyses.

3.2 Characteristics of the Included Articles

The selected studies were published between 1992 and 2017, and carried out with men only, without restriction on the duration of the intervention. The number of participants in each study ranged from 12 to 262. A total of 773 participants were included in the systematic review and meta-analyses: 392 in intervention groups and 381 in placebo groups. The majority of the studies were conducted in community samples (Claggett et al., 1999; Fitts et al., 2015; Gagliano-Jucá et al., 2017; Page et al., 2005; Srinivas-Shankar et al., 2010; Tenover, 1992). The mean \pm SD age at baseline ranged from 66.0 \pm 7.93 to 77.9 \pm 7.3 years in the intervention groups and 65.0 \pm 3.0 to 76.3 \pm 8.0 years in the placebo groups.

The intervention method was different for each study. Testosterone enanthate was used in six studies (Bhasin et al., 2005; Claggett et al., 1999; Ferrando et al., 2002; Fitts et al., 2015; Page et al., 2005; Tenover, 1992), undecanoate in one study (Wittert, 2003), and Androgel in four studies (Gagliano-Jucá et al., 2017; Kenny et al., 2010; Snyder et al., 1999; Srinivas-Shankar et al., 2010). Three methods of administration were used: oral (Page et al., 2005; Wittert et al., 2003), intramuscular (Bhasin et al., 2005; Claggett et al., 1999; Ferrando et al.,

2002; Fitts et al., 2015; Tenover, 1992), and transdermal (Gagliano-Jucá et al., 2017; Kenny et al., 2010; Snyder et al., 1999; Srinivas-Shankar et al., 2010). The treatment duration ranged from 12 weeks to 36 months, and there was no standardization or pattern of dosage. Additional relevant characteristics are described in Table 1.

Table 1. Characteristics and main results of the articles included in the systematic review and meta-analysis.

Reference	Baseline total T per group or total (Mean±SD)	N per group	Age per group or total (Mean±SD)	Route	Dose	Testosterone formulation	Frequency	Time	Outcomes analyzed	Main results
Bhasin, 2005	T 600mg:12.6 ±3.7 ²	T 600mg: 10	66.0±4.0	IM	600mg	Enanthate (Delatestryl)	Weekly	21 weeks	Muscle strength	Changes in muscle strength (P <0.001) were correlated with the dose and concentration of T.
	T 300mg:10.8±3.8 ²	T 300mg:13			vs. 300mg					
	T 125mg:13.4±4.3 ²	T 125mg:12			vs. 125mg					
	T 50mg:11.4±2.7 ²	T 50mg:12			vs. 50mg					
	T 25mg:12.9±4.4 ²	T 25mg:13			vs. 25mg					
Claggue, 1999	T:11.3±1.7 ²	T: 7	T:68.1±6.6	IM	200mg	Enanthate	Biweekly	12 weeks	Muscle mass and muscle strength	No treatment effects were detected in any of the evaluated outcomes.
	PL:11.6±0.9 ²	PL:7	PL:65.3±1.8		vs. Placebo					
Ferrando, 2002	-	T: 7	T: 68.0±7.93	IM	Titulated to a target between 17 and 28 nmol/l	Enanthate	1st month: weekly 2nd – 6th month: Biweekly	6 weeks	Muscle mass and muscle strength	The intervention increased total muscle mass (p <0.001) and leg extension strength (p=0.015) after 6 months.
		PL: 5	PL: 67.0±6.70							
Fitts, 2015	-	PL:7	PL 63.5±3.0	IM	100mg	Enanthate	Weekly and monthly	5 weeks	Muscle strength	Both treatments (weekly and monthly) increased peak strength. The weekly treatment was five times more effective than the monthly one in increasing the peak strength of type I fibers. Strength was 1.5 times greater in slow fibers
		MO:7	MO: 72.0±8.0		vs. Placebo					
		WK:5	WK: 73.0±8.0							

										compared to fast fibers. In type II fibers, peak strength increased similarly between treatments.
Gagliano-Juca, 2017	T: 253.0±61.0 ³ PL:242.0±68.0 ³	T: 46 PL: 53	T: 73.0±5.0 PL: 73.0±5.0	TD	100mg vs. Placebo	Gel	Daily	6 months	Muscle strength and physical performance	The intervention increased leg muscle strength (p=0.027). Treatment significantly improved stair climbing power (effect size=32.3 W; 95% CI=1.8 to 62.9 W; p=0.041) compared to PL group.
Kenny, 2010	T: 380.4±179.5 ³ PL:417.8±192.5 ³	T: 69 PL: 62	T: 77.9±7.3 PL:76.3±8.0	TD	5mg vs. Placebo	AndroGel1%	Daily	12 months	Muscle mass, muscle strength, and physical performance	The intervention increased muscle mass (p=0.03). There were no differences in strength parameters or physical performance.
Page, 2005	T: 9.9±1.6 ² PL:10.5±1.7 ²	T: 24 PL: 24	T: 71.0±4.0 PL: 71.0±5.0	OC	200mg vs. Placebo	Enanthate	Biweekly	36 months	Muscle mass, muscle strength, and physical performance	The intervention increased HGS compared to baseline and placebo (p <0.0001 and p <0.01), as well as muscle mass (p <0.0001). After 36 months, the intervention significantly improved performance in a timed functional

										test when compared with baseline and placebo (4.3±1.6 mean±SEM)
Snyder, 1999	T: 367±79 ³ PL: 369±75 ³	T: 45 PL: 40	T: 73.1±5.8 PL: 73.0±5.9	TD	6 mg vs. Placebo	Scrotal patch Testoderm	Daily	36 months	Muscle mass and muscle strength	The intervention resulted in an increase in muscle mass (P <0.001). The knee extension and flexion strength did not differ between the two groups after treatment.
Srinivas-Shankar, 2010	T: 11.0±3.2 ² PL: 10.9±3.1 ²	T: 130 PL: 132	T: 73.7±5.7 PL: 73.9±6.4	TD	50 mg vs. Placebo	Testogel 1%;	Daily	6 months	Muscle mass, muscle strength and physical performance	The intervention increased the peak torque of isokinetic knee extension (p=0.02) and muscle mass (p<0.001). There was no difference in HGS values. Tinetti gait and balance, ALF, 6MWT, and PPT improved at 6-month assessment (vs. baseline) in the intervention group. However, adjusted differences between treatment groups did not reach statistical significance.
Tenover, 1992	11.6±1.44 ²	T: 13	66.0	IM	100mg vs.	100 mg/mL in sesame oil	Weekly	3 months	Muscle mass and muscle	After the intervention,

	PL: 13				Placebo				strength	muscle mass increased (P <0.001). There were no changes in HGS during treatment in either group.
Wittert, 2003	T:17.0±25.26 ²	T: 33	T:69.0±6.0	OC	160mg vs. Placebo	Undecanoate (Andriol)	Daily	12 months	Muscle mass and muscle strength	The intervention increased muscle mass (p=0.0001). No effects on muscle strength were detected.
	PL:15.6±22.4 ²	PL: 25	PL: 68.0±5.0							

Abbreviations: -: No information given in the article; ¹Values expressed in pmol/L; ²Values expressed in nmol/L; ³Values expressed in ng/dL; 6MWT: 6-min walk test; ALF: Aggregate Locomotor Function Test; CI: confidence interval; HGS: handgrip strength; IM: intramuscular; N: number of study participants; OC: oral capsule; PPT: Physical Performance Test; SD: standard deviation; SEM: standard error of the mean; SPPB: Short Physical Performance Battery; TD: transdermal.

3.3 Quality Assessment and Publication Bias

Evaluation of the quality of the included articles is described in Supplementary Table 1. Three of the eight studies that evaluated the muscle mass outcome were considered to be at high risk of bias. For muscle strength, five of the included studies (out of a total of 11) were considered to have risk of bias. For physical performance, most studies (3/4) showed some risk of bias. About the publication bias, no funnel plot were performed since both the visual assessment and the statistical hypothesis tests are not normally suggested when there are less than 10 studies in the meta-analysis given its low power to detection.

3.4. Outcomes

3.4.1 Lean Body Mass

Eight studies evaluated LBM (Clagge et al., 1999; Ferrando et al., 2002; Kenny et al., 2010; Page et al., 2005; Snyder et al., 1999; Srinivas-Shankar et al., 2010; Tenover, 1992; Wittert et al., 2003). Of these, seven showed a positive effect of T supplementation in the intervention group compared to the placebo group (Ferrando et al., 2002; Kenny et al., 2010; Page et al., 2005; Snyder et al., 1999; Srinivas-Shankar et al., 2010; Tenover, 1992; Wittert et al., 2003). All the eight studies were included in the meta-analysis. In total, 604 participants were included, 312 in intervention and 292 in placebo groups. T supplementation was associated with an increase in LBM of 2.54 kg [95% CI, 1.27 to 3.80, ($p < 0.001$)] (figure 2). Due to the high heterogeneity ($I^2 = 72\%$), we performed a sensitivity analysis, where we identified a high influence of the study Ferrando et al., 2002, because when it was removed, the positive effect of T supplementation was maintained with a gain of 1.12 kg (95% CI, 1.61 to 2.91), but with a significant reduction in heterogeneity ($I^2 = 41\%$). Also, another article that seemed to influence heterogeneity in a substantial way was Page et al., 2005, because where this and Ferrando et al., 2002 were removed from the analyzes together, we obtained a drastic reduction in heterogeneity ($I^2 = 0\%$), having the positive gains in LBM being maintained (effect size = 1.65kg [95% CI, 1.46 to 1.83]).

We performed a subgroup analysis by route of supplementation, time of intervention and individual quality of the articles. In the subgroup analysis by supplementation route, the positive effects of T supplementation gains in LBM continued to be observed in the subgroups that used the transdermal supplementation route and oral capsule, but lost its effect in the subgroup that used the intramuscular route (figure 2). Due to the high heterogeneity found in the subgroup of studies that used the intramuscular supplementation route ($I^2 = 63\%$), we performed a sensitivity analysis. Ferrando et al., 2002 seemed to substantially influence the heterogeneity of the analysis. When this was removed, the analysis still did not

indicate gains in LBM in this subgroup, but the heterogeneity was reduced [effect size 1.26 kg (95% CI, -2.38 to 4.90), $I^2 = 0\%$]. The subgroup of articles that used the oral capsule supplementation route also showed high heterogeneity ($I^2 = 89\%$), however due to this subgroup having only two articles, it was not possible to perform sensitivity analysis.

In the subgroup analysis by time of intervention, the positive effect of T supplementation on LBM was maintained in the group 12 months or more of intervention, but lost its positive effect in the group <12 months of intervention (figure 2). Due to the high heterogeneities found in both analyses ($I^2 = 75\%$ in the subgroup <12 months and $I^2 = 69\%$ in the subgroup 12 months or more) we performed a sensitivity analysis. When Ferrando et al., 2002 was removed from the subgroup of studies that had an intervention time <12 months, the analyzes continued not to indicate gains in LBM, but heterogeneity was reduced [effect size = 1.29kg (95% CI, -0.31 to 2.90), $I^2 = 0\%$]. When we removed the Page et al., 2005 study from the analysis of subgroup 12 months or more, the positive effects of T supplementation were maintained with a gain of 1.63 kg (95% CI, 1.47 to 1.83) and heterogeneity reduced ($I^2 = 0\%$).

In the subgroup analysis according to the individual quality of the articles, the positive effects of T supplementation continued to be observed in all subgroups (low risk of bias, some concerns about the risk of bias and high risk of bias) (figure 2). The subgroup some concerns about the risk of bias showed high heterogeneity ($I^2 = 60\%$), however due to this subgroup having only two articles, it was not possible to perform sensitivity analysis.

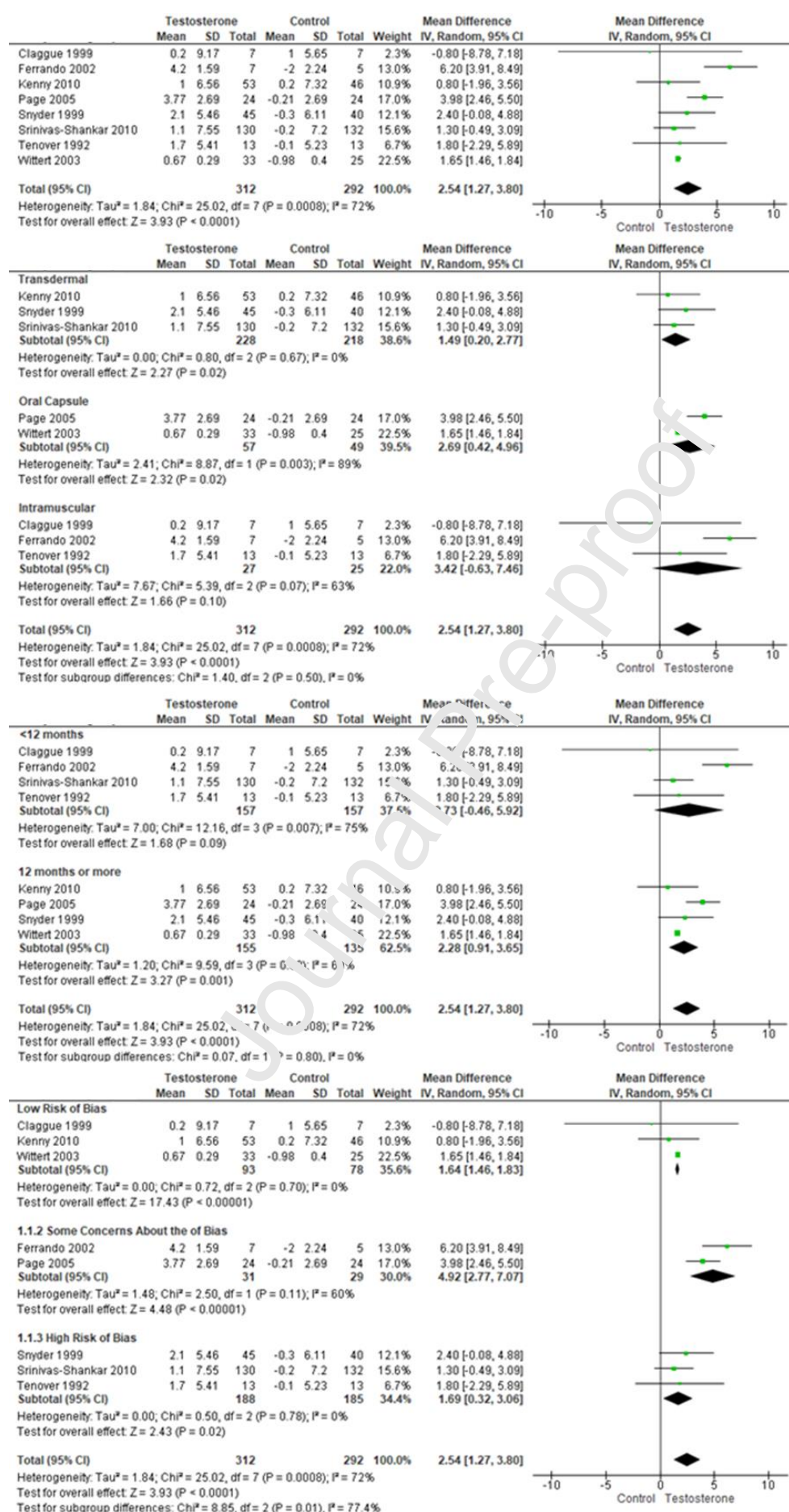


Figure 2. Effect of testosterone supplementation compared to control or placebo on lean body mass (kg).

3.5.2 Muscle Strength

In this systematic review and meta-analysis, we analyzed two indicators of MS: HGS and LS. Seven studies were evaluated for HGS, and all were included in the meta-analysis (Clagge et al., 1999; Kenny et al., 2010; Page et al., 2005; Snyder et al., 1999; Srinivas-Shankar et al., 2010; Tenover, 1992; Wittert et al., 2003). In total, 592 participants were included, 305 for intervention and 287 for placebo arms. T supplementation was associated with an increase in HGS of 1.58kg (95%CI, 0.17 to 3.0, (p=0.03), with moderate heterogeneity ($I^2 = 30\%$) (figure 3).

We also conducted the subgroup analysis and there was no effect of T supplementation for HGS (figure 3).

However, when we performed the subgroup analysis for the supplementation route, there was no effect of T supplementation for HGS, and due to the presence of only two studies, it was not possible to perform a sensitivity analysis in the subgroup oral capsule, which also has high heterogeneity.

Also, found no effect for HGS on the intervention time subgroup (figure 3), nor on the sensitivity analysis, but we observed a reduction in heterogeneity when Page et al., 2005 was removed from the subgroup 12 months or more [$I^2=0\%$, effect size 0.39kgf (95%CI, -1.14 to 1.92)].

As in the other analyses, we found no effect for HGS on the individual quality of articles subgroup, however there is a low heterogeneity in the sensitivity analysis when removing Page et al., 2005 or Kenny et al., 2010 from the subgroup some concerns about the of bias [$I^2=0\%$, effect size=0.72kgf (95%CI, -1.11 to 2.55) and $I^2=0\%$, effect size=2.69kgf (95%CI, -1.72 to 7.10) respectively].

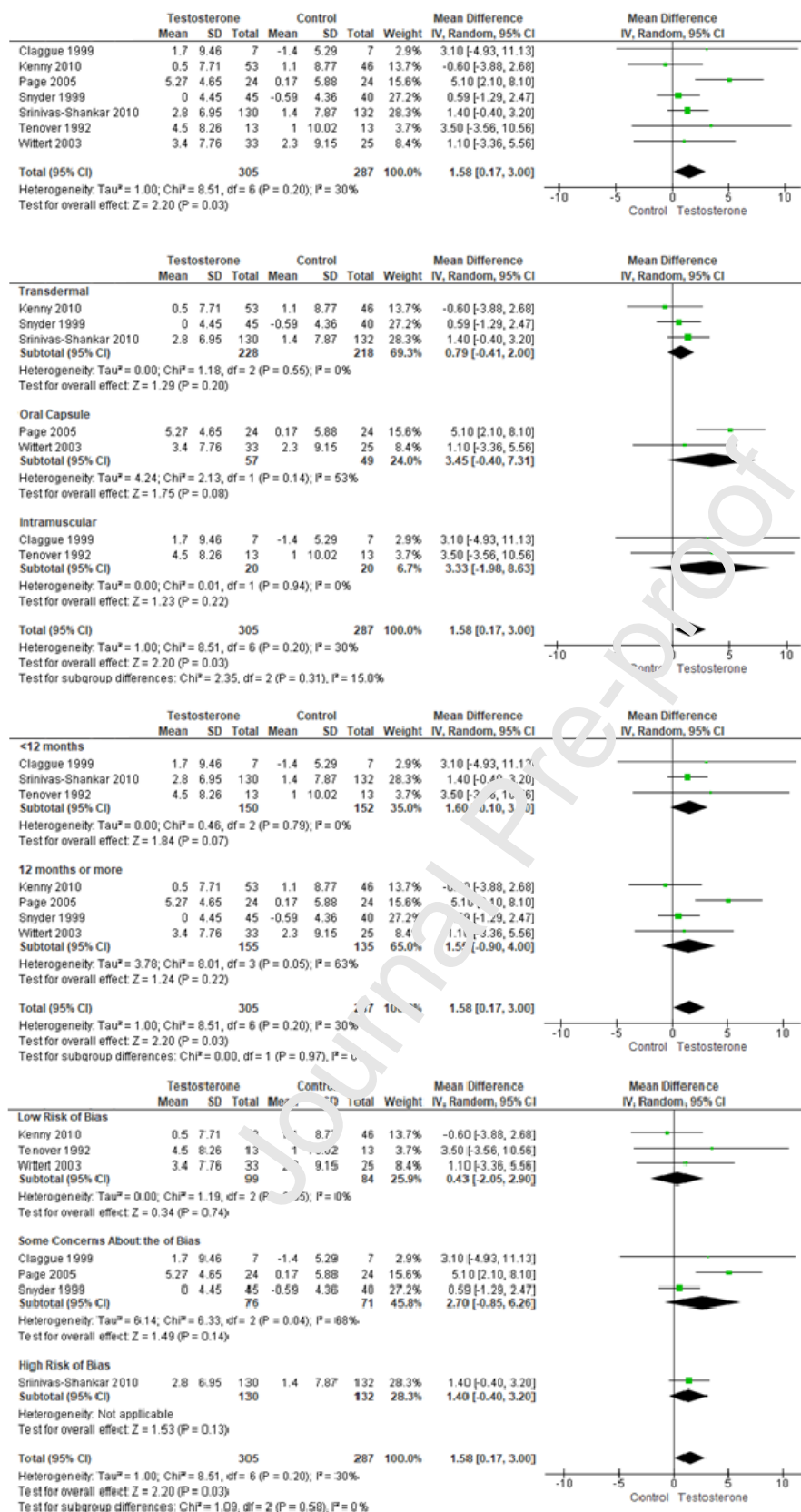


Figure 3. Effect of testosterone supplementation compared to control or placebo on handgrip strength (kgf).

The LS was evaluated in nine studies (Bhasin et al., 2005; Claggue et al., 1999; Ferrando et al., 2002; Fitts et al., 2015; Gagliano-Juca et al., 2017; Kenny et al., 2010; Snyder et al., 1999; Srinivas-Shankar et al., 2010; Wittert et al., 2003). The tests found to assess LS were the isometric and isokinetic strength of knee flexors and extensors, leg extension (LE) and leg press strength (LPS).

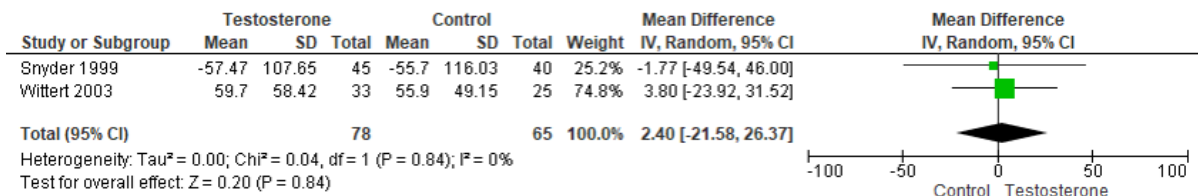
Two studies evaluated isometric strength of knee flexors and extensors (Claggue et al., 1999; Srinivas-Shankar et al., 2010), however only one of them (Claggue et al., 1999) provided the information of the angle used in the protocol for strength assessment (90°). Srinivas-Shankar et al., 2010 reported improvement in the isometric knee extension in the T group (vs. placebo at 6 months; adjusted difference 8.6N (95% CI, 1.3 to 16.0); (p=0.02). Claggue et al., 1999 did not report any significant difference for improvement in the strength of knee extensors and flexors between the groups in the follow up. Isokinetic strength of knee flexors and extensors were evaluated by four studies (Ferrando et al., 2002; Snyder et al., 1999; Srinivas-Shankar et al., 2010; Wittert et al., 2003), however, among the studies different speeds were used for strength assessment: 60°/s, 90°/s, 180°/s and 240°/s. Any of them reported a significant impact of T supplementation in LG. Only two studies reported the same assessment protocol for the isokinetic strength of knee extensors (IKE), enabling the performance of a meta-analysis of the results (Snyder et al., 1999; Wittert et al., 2003), where 143 participants were included, 78 from intervention and 65 from placebo arms, and no effect of T supplementation was observed for LS (figure 4).

For LE test (Ferrando et al., 2002; Fitts et al., 2015), the meta-analysis included a total of 24 participants, 12 from intervention group and 12 from placebo group. We observed an increase of 144.10N for LE in the T supplementation group (95%CI, 44.21 to 244.00); (p=0.005), and low heterogeneity ($I^2=0\%$) (figure 4).

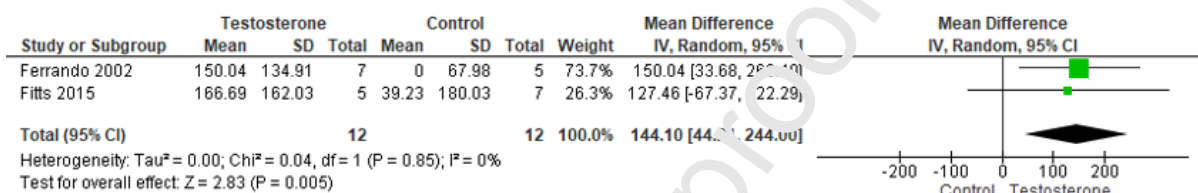
Finally, the LPS analyses was performed (Bhasin et al., 2005; Gagliano-Juca et al., 2017; Kenny et al., 2010) and included a total of 217 participants, 105 from intervention group and 112 from control or placebo group. There was an increase of 91.23N in LPS for the group that supplemented T (95%CI, 0.23 to 182.22); (p=0.05), with high heterogeneity ($I^2=62\%$) (figure 4). In sensibility analyzes, the effect is maintained just when removing Bhasin et al., 2005, with an increase of 81.01 N (95%CI, 2.17 to 159.86); (p=0.04). Both studies included in analyzes had transdermal gel T administration, using only placebo and were classified as having low risk of bias for the outcome, however, the high heterogeneity ($I^2=68\%$) was maintained. Also, when Gagliano-Juca et al., 2017 or Kenny et al., 2010 is removed the effect of T supplementation on LS is lost [effect size=179,10N (95%CI, -227.45

to 585.66), $I^2=67\%$; and effect size=202.36N (95%CI, -99.69 to 504.41), $I^2=51\%$, respectively]. We were unable to generate a subgroup analysis for LS, due to the low number of included studies.

A) Isokinetic knee extension 60°/s



B) Leg extension (1 repetition maximum)



C) Leg press strength (1 repetition maximum)

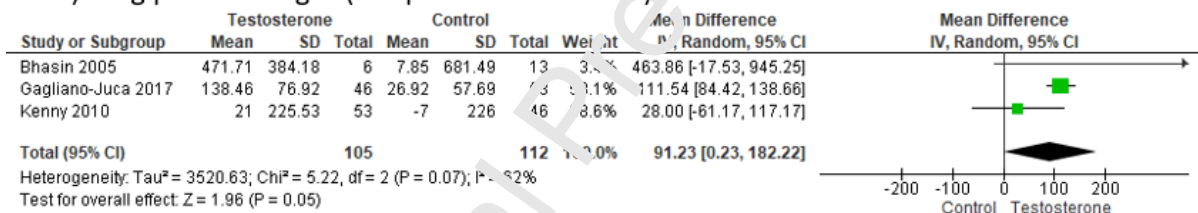


Figure 4. Effect of testosterone supplementation compared to control or placebo on leg strength (N).

3.5.3 Physical Performance

Four studies aimed to evaluate physical performance (Kenny et al., 2010; Page et al., 2005; Srinivas-Shankar et al., 2010; Gagliano-Juca et al., 2017). The validated tests found to assess physical performance were the aggregate locomotor function test (ALF) (McCarthy et al., 2004), 6-minute walk test (6MWT) (ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories, 2002), Tinetti gait and balance test (Tinetti et al., 1986), 12-step stair-climb test (LeBrasseur et al., 2008, 2009; Basaria et al., 2010), SPPB (Guralnik et al., 1989), supine-to-stand test (Alexander et al., 1997), Get up and Go test (Podsiadlo et al., 1991), and physical performance test (PPT) (Reuben et al., 1990).

The ALF, PPT, 6MWT, and Tinetti gait test were evaluated after 6 months of 50-mg transdermal Tsupplementation (Srinivas-Shankar et al., 2010). At the end of treatment, there was no difference between the intervention and placebo groups.

The 12-step stair-climb test was used by Gagliano-Jucá et al. (2017). They reported changes in the test associated with T supplementation [effect size=32.3W(95%CI, 1.8 to 62.9W), $p=0.041$] compared to the placebo group.

The SPPB, supine-to-stand test, and Get Up and Go test were employed by Kenny et al. (2010). Only the SPPB showed changes in the 12-month follow-up, being independent of intramuscular T treatment.

In the meta-analysis, only one test was evaluated, the PPT (Page et al., 2005; Srinivas-Shankar et al., 2010), where they were included a total of 310 subjects, 154 in the intervention group and 156 in the control group. There was no significant impact of t supplementation compared to the placebo group in physical performance in PPT (figure 5). The analysis showing high heterogeneity ($I^2=90\%$), but it was impossible to carry out a sensibility or subgroup analysis, once only two studies were included.

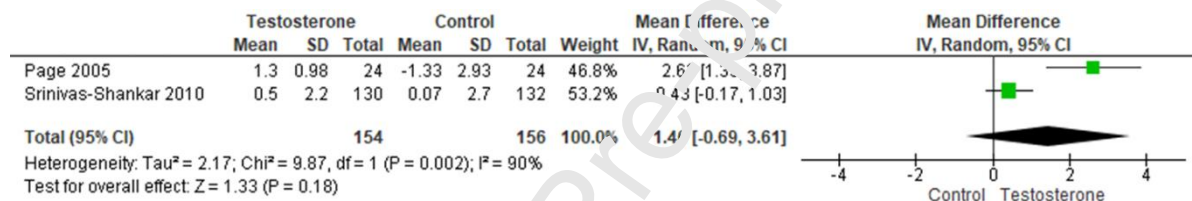


Figure 5. Effect of testosterone supplementation compared to control or placebo on the Physical Performance Test.

4. DISCUSSION

The results of this systematic review and meta-analysis indicate that T supplementation is associated with increases in muscle mass, strength and physical performance. Similar results have been reported elsewhere in the literature (Corona et al., 2016; Pasiakos et al., 2019; Gharahdaghi et al., 2019; Spiegeleer et al., 2018).

The exact mechanism of action by which T affects skeletal muscle is still unclear. The methodological differences between studies make it difficult to reach a clear conclusion on the subject. Some hypotheses suggest that supplementation may increase the size of muscle fibers, due to the high rate of reuse of intracellular amino acids by T, which would increase protein synthesis (Myung Jun Shin et al., 2018). Others suggest that the action of androgens would influence muscle hypertrophy via increased expression of insulin-like growth factor type 1 (Laurent et al., 2019; Neto et al., 2015). Furthermore, T has been speculated to play a role in activating a G-protein-linked receptor, which would increase the intracellular calcium

concentration in myoblasts, resulting in cell growth (Myung Jun Shin et al., 2018; Neto et al., 2015; Chiu et al., 2019).

Most studies available on the topic report positive results of T supplementation on lean mass gain. A systematic umbrella review and meta-analysis (Spiegeleer et al., 2018) that aimed to evaluate the available evidence on pharmacological interventions for sarcopenia demonstrated a strong effect of supplementation on muscle mass in men with low serum T levels and suggest that elderly patients with clinical muscle weakness and low serum T levels can benefit more from this intervention. In our analysis, positive gains in LBM were found in most analyzes. In the review, only one of the articles did not report gains in LBM after T supplementation (Clagge et al., 1999) and in our meta-analysis, gains in lean mass were not observed only in the subgroups of studies that used the intramuscular supplementation route and that had a intervention time <12 months. Corroborating the data obtained by the present study, results of an important clinical trial that studied the effects of T administration for 3 years in elderly men (Testosterone's Effects on Atherosclerosis Progression in Aging Men, TEAAM Trial) (Storer et al., 2017), also demonstrated a greater increase in LBM in the T group.

LS demonstrated gains after T supplementation in LPS and LE. Gharahdaghi et al. (2019) reported that short interventions are able to improve muscle mass/function in non-hypogonadal elderly men. They demonstrated that supplemental T for resistance training for 6 weeks increased the fat-free mass of the legs ($p=0.02$), the thickness of the *vastus lateralis* muscle, the length of the fascicle and the cross-sectional area of the quadriceps ($P < 0.05$), which may indicate that even short interventions are capable of promoting gains on this outcome. In our analysis both studies evaluated for LE strength (Ferrando et al., 2002; Fitts et al., 2015) used short interventions (5-6 weeks) which may corroborate the thesis that short interventions are capable of promoting gains in LS. The intramuscular route of administration has been reported to provide superior gains in relation to the others. Skinner et al. (2018), in a meta-analysis, reported that intramuscular supplementation was more effective than transdermal formulations in increasing muscle mass and improving muscle strength in middle-aged and elderly men. Corona et al. (2016) also reported this same association; when only randomized placebo-controlled clinical trials were considered, parenteral preparations were associated with higher circulating levels of T at follow-up when compared to those observed with oral and transdermal formulations. This suggests that the gains in muscle strength associated with T therapy may depend mainly on the route of administration (Skinner et al., 2018). However, in the present meta-analysis, in the analysis of subgroups by

route of administration, in the HGS outcome, no differences were found between the placebo/control and T groups. Due to the number of articles included, subgroup analyses could not be performed according to the supplementation route in the LS outcome.

The relationship between T and HGS has been explored in previous studies. One such study (Chiu et al., 2019) included more than 7000 individuals over age 20 from the NHANES database in order to verify the association between serum T levels and HGS, and found that HGS was correlated positively with T (beta coefficient: 6.244; 95% CI: 5.982, 6.506; $p < 0.001$) in the unadjusted model and persisting in the multivariate model, where individuals with the highest HGS tended to have higher levels of T, showing a prominent association between the two. Results from our meta-analysis indicate gains in HGS in the general analysis, but not in the analysis of subgroups by time of intervention, route of supplementation and individual quality of the articles, indicating that in this outcome, maybe the methodological difference of the studies does not have such a significant impact.

Of the four studies in this systematic review that assessed outcomes related to physical performance, only one (Srinivas-Shankar et al., 2010) did not indicate any association between T supplementation and better physical performance when compared to the placebo group. Again, the discrepant methodology of the studies and the different tests used to measure performance make it difficult to draw definitive conclusions. However, muscle strength may play a possible determining role in these results, considering that previous studies have already demonstrated the potential for an interaction (Bouchard et al., 2010; Iwamura et al., 2017). Two articles were included in the meta-analysis for the PPT variable; unlike Nam et al. (2015), who found a positive effect of T treatment on PPT results, in the present meta-analysis no differences were found after treatment (effect size=1.46 (95%CI, -0.69 to 3.61). This is probably due to the fact that the studies included in their analysis did not differ so markedly in methodology (all interventions consisted of transdermal T and the intervention period ranged from 6 months to 1 year), unlike in the present meta-analysis, where interventions varied between transdermal and oral-capsule T and the treatment period ranged from 6 to 36 months).

A central point when proposing to evaluate T supplementation is the fact that the methodology of the studies varies substantially. The duration of the treatment regimen, as well as the dose, route of administration, and even the ester of T and pharmaceutical formulation administered vary widely. In the present meta-analysis, most outcomes showed high or moderate heterogeneity ($I^2=72\%$ for muscle mass, $I^2=30\%$ for HGS and $I^2=62\%$, $I^2=0\%$, $I^2=0\%$, for LS (LPS, LE and IKE, respectively), and $I^2=90\%$ for physical

performance, in general analysis). In the analysis of subgroups, heterogeneity had a slight drop in most outcomes, emphasizing that the methodological difference of the studies can impact the analyzes. However, high heterogeneity is not uncommon in studies of this topic. A meta-analysis carried out by Neto et al. (2015), who proposed to evaluate the effect of T supplementation on muscle mass in the elderly, also found very high heterogeneity among the included clinical trials ($I^2=98\%$), even after a sensibility analysis by time and route of administration.

Therefore, T supplementation can be a beneficial strategy to mitigate the age-related decline in parameters of muscle mass, strength, and physical performance in men. Additional studies, conducted with appropriate methodological rigor, are needed to elucidate the testosterone-muscle interaction.

5. CONCLUSIONS

The results of this systematic review and meta-analysis indicate that T supplementation is associated with increases in muscle mass, strength and physical performance in middle-aged and older men. Our meta-analysis showed that, compared with placebo or control group T supplementation had a beneficial effect on LBM, HGS and LS (LPS and LE). Future studies should focus on standardizing T supplementation protocols, particularly with regard to dosage.

AUTHOR CONTRIBUTION

CC and PB conceived the presented idea and designed the study. ISP and GCS conceived the presented idea, designed the study, and edited and reviewed the manuscript. ÉCTR and SMP collected and analyzed data and wrote the manuscript. All authors discussed the results and contributed to the final manuscript. All authors approved the final version of the manuscript and agree to be accountable for the study. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

SOURCES OF SUPPORT

Fundo de Incentivo à Pesquisa, Hospital de Clínicas de Porto Alegre (FIPE/HCPA).

DECLARATION OF INTEREST

None.

REFERENCES

- Alexander, N. B., Ulbrich, J., Raheja, A., & Channer, D., 1997. Rising from the floor in older adults. *J Am Geriatr Soc.* 45(5), 564-569.
- ATS statement: guidelines for the six-minute walk test, 2002. ATS committee on proficiency standards for clinical pulmonary function laboratories. *Am J Respir Crit Care Med.* 166(1), 111-117.
- Basaria, S., Coviello, A.D., Travison, T.G., Storer, T.W., Farwell, W.R., Jette, A.M., ... & Bhasin, S., 2010. Adverse events associated with testosterone administration. *N Engl J Med.* 363, 109–122.
- Bhasin, S., Woodhouse, L., Casaburi, R., Singh, A. B., Mac, R. P., Lee, M., ... & Magliano, L., 2005. Older men are as responsive as young men to the anabolic effects of graded doses of testosterone on the skeletal muscle. *The J Clin Endocrinol Metab.* 90(2), 678-688.
- Bouchard, D. R., & Janssen, I., 2010. Dynapenic-obesity and physical function in older adults. *J GerontolA Biol Sci Med Sci.* 65(1), 71-77.
- Chiu, H. T., Shih, M. T., & Chen, W. L., 2019. Examining the association between grip strength and testosterone. *Aging Male.* 1-8.
- Clague, J. E., Wu, F. C., & Horan, M. A., 1999. Difficulties in measuring the effect of testosterone replacement therapy on muscle function in older men. *Int J Androl.* 22(4), 261-265.
- Corona, G., Giagulli, V. A., Mascioni, E., Vignozzi, L., Aversa, A., Zitzmann, M., ... & Maggi, M., 2016. Testosterone supplementation and body composition: results from a meta-analysis study. *Eur J Endocrinol.* 174(3), R99-R116.
- Cruz-Jentoft, A. J., Bahat, G., Bauer, J., Boirie, Y., Bruyère, O., Cederholm, T., ... & Schneider, S. M., 2013. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing.* 48(1), 16-31.
- De Spiegeleer, A., Beckwée, D., Bautmans, I., & Petrovic, M., 2018. Pharmacological interventions to improve muscle mass, muscle strength and physical performance in older people: an umbrella review of systematic reviews and meta-analyses. *Drugs Aging.* 35(8), 719-734.
- Ferrando, A. A., Sheffield-Moore, M., Yeckel, C. W., Gilkison, C., Jiang, J., Achacosa, A., ... & Urban, R. J., 2002. Testosterone administration to older men improves muscle function: molecular and physiological mechanisms. *Am J Physiol Endocrinol Metab.* 282(3), E601-E607.

- Fitts, R. H., Peters, J. R., Dillon, E. L., Durham, W. J., Sheffield-Moore, M., & Urban, R. J., 2015. Weekly versus monthly testosterone administration on fast and slow skeletal muscle fibers in older adult males. *The J Clin Endocrinol Metab* 100(2), E223-E231.
- Gagliano- Jucá, T., Storer, T. W., Pencina, K. M., Travison, T. G., Li, Z., Huang, G., ... & Basaria, S. , 2018. Testosterone does not affect agrin cleavage in mobility- limited older men despite improvement in physical function. *Andrology*. 6(1), 29-36.
- Gharahdaghi, N., Rudrappa, S., Brook, M. S., Idris, I., Crossland, H., Hamrock, C., ... & Constantin- Teodosiu, D., 2019. Testosterone therapy induces molecular programming augmenting physiological adaptations to resistance exercise in older men. *J Cachexia Sarcopenia Muscle*. 10(6), 1276-1294.
- Guadalupe-Grau, A., Carnicero, J. A., Losa-Reyna, J., Tresguerras, J., del Carmen Gómez-Cabrera, M., Castillo, C., ... & García-García, F. J., 2017. Endocrinology of aging from a muscle function point of view: results from the Toledo study for healthy aging. *J Am Med Dir Assoc*. 18(3), 234-239.
- Guralnik, J.M., Branch, L.G., Cummings, S.R., & Curb, J.D., 1989. Physical performance measures in aging research. *J Gerontol*. 44(5), 141–146.
- Harada, N., 2018. Role of androgens in energy metabolism affecting on body composition, metabolic syndrome, type 2 diabetes, cardiovascular disease, and longevity: lessons from a meta-analysis and rodent studies. *Biosci Biotechnol Biochem*. 82(10), 1667-1682.
- Higgins, J. P., Thompson, S. G., Deeks, J. J., & Altman, D. G. 2003. Measuring inconsistency in meta-analyses. *Bmj*. 327(7414), 557-560.
- Hsu, B., Cumming, R. G., Nagathan, V., Blyth, F. M., Le Couteur, D. G., Seibel, M. J., ... & Handelsman, D. J. 2014. Longitudinal relationships of circulating reproductive hormone with functional disability muscle mass, and strength in community-dwelling older men: the Concord Health and Ageing in Men project. *The J Clin Endocrinol Metab*. 99(9), 3310-3318.
- Iwamura, M., & Kanauchi, M., 2017. A cross-sectional study of the association between dynapenia and higher-level functional capacity in daily living in community-dwelling older adults in Japan. *BMC geriatrics*. 17(1), 1.
- Kenny, A. M., Kleppinger, A., Annis, K., Rathier, M., Browner, B., Judge, J. O., & McGee, D., 2010. Effects of transdermal testosterone on bone and muscle in older men with low bioavailable testosterone levels, low bone mass, and physical frailty. *J Am Geriatr Soc*. 58(6), 1134-1143.
- Laurent, M. R., Dedeyne, L., Dupont, J., Mellaerts, B., Dejaeger, M., & Gielen, E., 2019. Age-related bone loss and sarcopenia in men. *Maturitas*. 122, 51-56.

- LeBrasseur, N.K., Bhasin, S., Miciek, R., & Storer, T.W., 2008. Tests of muscle strength and physical function: reliability and discrimination of performance in younger and older men and older men with mobility limitations. *J Am Geriatr Soc.* 56, 2118–2123.
- LeBrasseur, N.K., Lajevardi, N., Miciek, R., Mazer, N., Storer, T.W., & Bhasin, S., 2009. Effects of testosterone therapy on muscle performance and physical function in older men with mobility limitations (The TOM Trial): design and methods. *Contemp Clin Trials.* 30, 133–140.
- McCarthy, C. J., & Oldham, J. A., 2004. The reliability, validity and responsiveness of an aggregated locomotor function (ALF) score in patients with osteoarthritis of the knee. *Rheumatology.* 43(4), 514-517.
- Moher D., Liberati A., Tetzlaff J., Altman D. G., PRISMA Group Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Annals of Internal Medicine.* 2009;151(4):264–269.
- Nam, Y. S., Lee, G., Yun, J. M., & Cho, B., 2018. Testosterone replacement, muscle strength, and physical function. *World J Mens Health.* 36(2), 110-122.
- Neto, W. K., Gama, E. F., Rocha, L. Y., Ramos, C. C., Taets, W., Scapini, K. B., ... & Caperuto, É., 2015. Effects of testosterone on lean mass gain in elderly men: systematic review with meta-analysis of controlled and randomized studies. *Age (Dordr).* 37(1), 5.
- Oki, K., Law, T. D., Loucks, A. B., & Clark, B. C., 2015. The effects of testosterone and insulin-like growth factor 1 on motor system form and function. *Exp Gerontol.* 64, 81-86.
- Page, S. T., Amory, J. K., Bowman, F. D., Anawalt, B. D., Matsumoto, A. M., Bremner, W. J., & Tenover, J. L. (2005). Exogenous testosterone (T) alone or with finasteride increases physical performance, grip strength, and lean body mass in older men with low serum T. *The J Clin Endocrinol Metab.* 90(3), 1502-1510.
- Pasiakos, S. M., Berryman, C. E., Karl, J. P., Lieberman, H. R., Orr, J. S., Margolis, L. M., ... & Vartanian, O., 2019. Effects of testosterone supplementation on body composition and lower-body muscle function during severe exercise-and diet-induced energy deficit: A proof-of-concept, single centre, randomised, double-blind, controlled trial. *EBioMedicine.* 46, 411-422.
- Podsiadlo, D., & Richardson, S., 1991. The timed “Up & Go”: a test of basic functional mobility for frail elderly persons. *J Am Geriatr Soc.* 39(2), 142-148.
- Reuben, D. B., & Siu, A. L., 1990. An objective measure of physical function of elderly outpatients: the Physical Performance Test. *J Am Geriatr Soc.* 38(10), 1105-1112.

- Saad, F., Roehrig, G., von Haehling, S., & Traish, A., 2017. Testosterone deficiency and testosterone treatment in older men. *Gerontology*. 63(2), 144-156.
- Shin, M. J., Jeon, Y. K., & Kim, I. J., 2018. Testosterone and sarcopenia. *World J Mens Health*. 36(3), 192-198.
- Skinner, J. W., Otzel, D. M., Bowser, A., Nargi, D., Agarwal, S., Peterson, M. D., ... & Yarrow, J. F., 2018. Muscular responses to testosterone replacement vary by administration route: a systematic review and meta-analysis. *J Cachexia Sarcopenia Muscle*. 9(3), 465-481.
- Skinner, J. W., Otzel, D. M., Bowser, A., Nargi, D., Agarwal, S., Peterson, M. D., ... & Yarrow, J. F., 2018. Muscular responses to testosterone replacement vary by administration route: a systematic review and meta-analysis. *J Cachexia Sarcopenia Muscle*. 9(3), 465-481.
- Snyder, P. J., Peachey, H., Hannoush, P., Berlin, J. A., Loh, L., Lenrow, D. A., ... & Strom, B. L., 1999. Effect of testosterone treatment on body composition and muscle strength in men over 65 years of age. *The J Clin Endocrinol Metab*. 84(8), 2647-2653.
- Srinivas-Shankar, U., Roberts, S. A., Connolly, M. L., O'Connell, M. D., Adams, J. E., Oldham, J. A., & Wu, F. C., 2010. Effects of testosterone on muscle strength, physical function, body composition, and quality of life in intermediate-frail and frail elderly men: a randomized, double-blind, placebo-controlled study. *The J Clin Endocrinol Metab*. 95(2), 639-650.
- Sterne, J. A., Savović, J., Page, M. J., Elbers, R. G., Blencowe, N. S., Boutron, I., ... & Emberson, J. R., 2019. RoB 2: a revised tool for assessing risk of bias in randomised trials. *Bmj*. 366.
- Storer, T. W., Basaria, S., Traustadottir, T., Harman, S. M., Pencina, K., Li, Z., ... & Huang, G., 2017. Effects of testosterone supplementation for 3 years on muscle performance and physical function in older men. *The J Clin Endocrinol Metab*. 102(2), 583-593.
- Tenover, J. S., 1992. Effects of testosterone supplementation in the aging male. *The J Clin Endocrinol Metab*. 75(4), 1092-1098.
- Tinetti, M. E., 1986. Performance-oriented assessment of mobility problems in elderly patients. *J Am Geriatr Soc*. 34(2), 119-126.
- Vingren, J. L., Kraemer, W. J., Ratamess, N. A., Anderson, J. M., Volek, J. S., & Maresh, C. M., 2010. Testosterone physiology in resistance exercise and training. *Sports Med*. 40(12), 1037-1053.
- Wittert, G. A., Chapman, I. M., Haren, M. T., Mackintosh, S., Coates, P., & Morley, J. E., 2003. Oral testosterone supplementation increases muscle and decreases fat mass in healthy

elderly males with low-normal gonadal status. J GerontolA Biol Sci Med Sci. 58(7), M618-M625.

Journal Pre-proof

HIGHLIGHTS

- Testosterone supplementation is associated with positive gains in lean body mass;
- Testosterone it is also associated with gains in strength in mens;
- The testosterone supplementation it may be a strategy to prevent sarcopenia in men;
- A better standardization of the testosterone supplementation method is necessary.