Cognitive response to testosterone replacement added to intensive lifestyle intervention in older men with obesity and hypogonadism: prespecified secondary analyses of a randomized clinical trial

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ABSTRACT

Background: Both obesity and hypogonadism are common in older men which could additively exacerbate age-related declines in cognitive function. However, little is known about the effects of lifestyle intervention plus testosterone replacement therapy in this population.

Objectives: In this secondary analysis of the LITROS (Lifestyle Intervention and Testosterone Replacement in Obese Seniors) trial, we examined whether testosterone replacement therapy would improve cognitive function when added to intensive lifestyle intervention in older men with obesity and hypogonadism.

Methods: Eighty-three older, obese hypogonadal men with frailty were randomly assigned to lifestyle therapy (weight management and exercise training) plus testosterone (LT + Test) or lifestyle therapy plus placebo (LT + Pbo) for 6 mo. For this report, the primary outcome was change in the global cognition composite z score. Secondary outcomes included changes in z score subcomponents: attention/information processing, memory, executive function, and language. Changes between groups were analyzed using mixed-model repeated-measures ANCOVAs following the intention-to-treat principle.

Results: Global cognition z score increased more in the LT + Test than in the LT + Pbo group (mean change: 0.49 compared with 0.21; between-group difference: −0.28; 95% CI: −0.45, −0.11; Cohen's $d = 0.74$). Moreover, attention/information z score and memory z score increased more in the LT + Test than in the LT + Pbo group (mean change: 0.55 compared with 0.23; between-group difference: −0.32; 95% CI: −0.55, −0.09; Cohen's $d = 0.49$ and mean change: 0.90 compared with 0.37; between-group difference: −0.53; 95% CI: −0.93, −0.13; Cohen's $d = 1.43$, respectively). Multiple regression analyses showed that changes in peak oxygen consumption, strength, total testosterone, and luteinizing hormone were independent predictors of the improvement in global cognition ($R^2 = 0.38$; $P < 0.001$).

Conclusions: These findings suggest that in the high-risk population of older men with obesity and hypogonadism, testosterone replacement may improve cognitive function with lifestyle behaviors controlled via lifestyle intervention therapy. This trial was registered at clinicaltrials.gov as NCT02367105. *Am J Clin Nutr* 2021;114:1590–1599.

Keywords: adiposity, obesity, aging, frailty, cognition, diet, exercise, androgens

Introduction

In the older adult population, the prevalence of obesity is anticipated to grow markedly in the coming years because of an increase in the aging population and in the prevalence of obesity itself. In fact, more than one-third of persons 65 y or older in the United States are now classified as having obesity (1). This expanding population is particularly vulnerable, because

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Abbreviations used: AD, Alzheimer disease; FSH, follicle-stimulating hormone; hs-CRP, high-sensitivity C-reactive protein; IGF-I, insulin-like growth factor I; LH, luteinizing hormone; LITROS, Lifestyle Intervention and Testosterone Replacement in Obese Seniors; LT + Pbo, lifestyle therapy plus placebo; LT + Test, lifestyle therapy plus testosterone; MEDVAMC, Michael E DeBakey VA Medical Center; RAVLT, Rey Auditory Verbal Learning Test; RCT, randomized clinical trial; SCWT, Stroop Color and Word Test; SDMT, Symbol Digital Modalities Test; Trail A and B, Trail-Making Test Parts A and B; VAT, visceral adipose tissue; $\dot{V}O_{2peak}$, peak oxygen consumption; 1-RM, 1 repetition maximum; 3MS, Modified Mini-Mental State.

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obesity not only exacerbates the age-related decline in physical function but also the age-related decline in cognitive function that increases the risk of dementia. Obesity further predisposes to dementia in older adults because of convergent mechanisms such as insulin resistance and chronic inflammation (2). However, little is known about the effect of weight-loss intervention on cognitive function (3), especially in the at-risk population of older adults with obesity. Previously, we showed that weight loss and exercise each improved cognition compared with control (no weight loss or exercise) but their combination may provide benefits similar to exercise alone (4), a finding corroborated by 2 other reports suggesting that calorie restriction may not enhance or diminish the cognitive benefits of regular exercise (5, 6).

Obesity is also associated with hypogonadism, which could exacerbate the age-related decline in testosterone concentrations. In fact, some studies have shown that obesity is the single most important factor associated with low testosterone, outweighing the effects of age and comorbidities (7, 8). A high prevalence of hypogonadism (∼50%) was reported in middle-aged and older adults with obesity in a large community-based study (9), whereas BMI and age were reported as the dominant drivers of low testosterone in another study (10). In a testosterone trial that recruited older men with evidence of clinical androgen deficiency and low testosterone concentrations, 63% of participants were obese (11). In our prior study, we also found that ∼78% of older obese men with frailty had hypogonadism which persisted despite intensive lifestyle intervention (12). Accordingly, the increasing population of older adults with obesity and clinical hypogonadism (9, 10) is a public health challenge and an important target for more effective interventions.

Because low testosterone concentrations may contribute to increased risk of cognitive impairment and dementia (13), hypogonadism could additively exacerbate the obesity- and age-related cognitive declines. Indeed, dementia is multifactorial in nature (14); therefore, an approach that concurrently targets coexisting factors such as sex hormone deficiency and obesity could have additive effects in improving cognition. Epidemiologic studies have shown that higher testosterone concentrations are associated with improved cognitive function (15, 16) but results from randomized clinical trials (RCTs) of testosterone therapy in older men have been inconsistent, with some reporting improvements (17) and others showing no benefit (18). However, to the best of our knowledge, none of the previous RCTs of testosterone has focused on the prevalent, high-risk, and understudied population of older men with obesity and clinical hypogonadism. Moreover, no previous RCT has directly compared the effects of lifestyle intervention plus testosterone and lifestyle intervention without testosterone on cognition in this population. Therefore, the purpose of this RCT was to test the hypothesis that testosterone replacement therapy would improve cognitive function when added to an intensive lifestyle intervention in frail, older men with obesity and hypogonadism. The data reported in this article are the result of secondary analyses of the LITROS (Lifestyle Intervention and Testosterone Replacement in Obese Seniors) trial.

Methods

Overview

The LITROS trial (NCT02367105) was a parallel, double-blind RCT to determine whether testosterone replacement therapy would improve physical function in combination with a lifestyle intervention in older men with obesity and hypogonadism. The principal results of the parent RCT showed that in older obese hypogonadal men, adding testosterone to a lifestyle intervention does not further improve overall physical function but may prevent or attenuate the weight loss–induced reduction in muscle and bone mass and further improve aerobic capacity (19). The present study reports secondary analyses of the trial examining changes in cognitive function, as prespecified in the protocol.

The LITROS trial was conducted from 2 February, 2015 through 31 December, 2019 at the Michael E DeBakey VA Medical Center (MEDVAMC), Houston, TX. The study was approved by the Institutional Review Board of Baylor College of Medicine and the Research and Development Committee of the MEDVAMC. Study oversight was provided by an independent Data and Safety Monitoring Board.

After baseline assessments, participants were randomly assigned, with stratification for BMI (in kg/m^2) (<35 compared with ≥35), into 2 groups: *1*) lifestyle therapy plus testosterone replacement therapy (LT + Test) or *2*) lifestyle therapy plus placebo (LT + Pbo) for 26 wk. Participants and all study personnel were masked to treatment allocation. The randomization algorithm was generated and maintained by the research pharmacy of the MEDVAMC. All baseline assessments were repeated at 6 mo.

Study population

Volunteers were recruited through advertisements and review of medical records at the MEDVAMC. Persons were eligible for inclusion if they were veterans, older (age ≥ 65 y), obese (BMI ≥ 30), hypogonadal (fasting testosterone concentrations < 10.4 nmol/L on 2 separate mornings) (20), sedentary (regular exercise < 1 h/wk), and with stable body weight (±2 kg) and receiving stable medications for 6 mo before enrolment. In addition, all participants had to have evidence of mild-to-moderate physical frailty, as defined by a score of 18–31 on the modified Physical Performance Test (total scores range from 0 to 36 points, with higher scores indicating better performance) (21). Persons with severe cardiopulmonary disease (e.g., recent myocardial infarction, unstable angina), musculoskeletal or neuromuscular impairments that precluded exercise training, history of prostate cancer, venous thromboembolism, untreated sleep apnea, hematocrit > 50%, prostate findings of palpable nodule, prostate-specific antigen ≥4 ng/mL, an International Prostate Symptom score > 19 (22), a Mini Mental Status Exam score < 24, and untreated major depression or schizophrenia were excluded.

Procedures

Intensive lifestyle intervention and testosterone replacement therapy.

The lifestyle therapy consisted of a weight management program and exercise training. Participants were prescribed a balanced diet that provided an energy deficit of 500–750 kcal/d and contained ∼1 g · kg^{-1} · d^{-1} of high-quality protein (23). Participants met weekly with a dietician for dietary adjustments and behavioral therapy. They were instructed to set weekly behavioral goals and attend weekly weigh-in sessions. Food

diaries were reviewed, and new goals were set based on diary reports. The goal was to achieve weight loss of ∼10% at 6 mo. The exercise training involved combined aerobic and resistance training sessions thrice weekly. The sessions lasted 90 min and began with 15 min of warm-up flexibility exercises followed by 30 min of aerobic exercises, 30 min of resistance exercises, and 15 min of balance exercises. The aerobic exercises consisted of treadmill walking, stationary cycling, and stair climbing. Participants exercised at ∼65% of their peak heart rate, which was gradually increased to 70%–85%. The resistance exercises consisted of 9 upper-body and lower-body exercises using weight-lifting machines. The initial sessions were 1–2 sets of 8–12 repetitions at 65% of 1 repetition maximum (1-RM), which were increased progressively to 2–3 sets at ∼85% of 1-RM. Exercise sessions were supervised by exercise trainers at our exercise center.

The testosterone preparation was testosterone gel 1.62% (Androgel, purchased from AbbVie) applied topically once daily in the morning. The placebo gel was formulated to have an identical appearance to the active gel. The initial dosage was 40.5 mg/d, which was expected to increase the testosterone concentration to within the normal range for young men (19–40 y of age). To verify that the testosterone concentration was in the target range, the testosterone concentration was measured 2 wk after starting the intervention. If the testosterone concentration was not in the target range, the dosage was adjusted by the unblinded physician, and the measurement was repeated after another 2 wk. To maintain blinding when the dosage was adjusted in a participant receiving testosterone, the apparent dosage was changed simultaneously in a participant receiving placebo (i.e., a corresponding participant in the LT + Pbo group was treated similarly to the participant in the LT + Test group to preserve blinding to treatment).

Additional details about the interventions including compliance data, exercise adaptations, and adverse events have been reported in the primary article (19).

Outcome assessments.

The primary outcome for this secondary analysis of the trial was change in the global cognition composite *z* score. Secondary outcomes included changes in the subcomponent *z* scores: attention/information processing, memory, executive function, and language. Tertiary outcomes included changes in the raw cognitive scores.

Cognitive testing.

The following validated tests of cognitive functions, which have been demonstrated to be related to aging, obesity, and sex steroids, were used (3, 24, 25): Trail-Making Test Parts A and B (Trail A and B), Stroop Color and Word Test (SCWT), Rey Auditory Verbal Learning Test (RAVLT), Symbol Digital Modalities Test (SDMT), Word List Fluency Test, and Modified Mini-Mental State (3MS) test.

The following cognitive domains were assessed.

Attention/information processing was assessed by Trail A, the SDMT, and the SCWT. Trail A provides information on visuospatial scanning, speed of processing, and mental flexibility (26). It involves connecting 25 consecutively numbered circles. Shorter time to completion indicates better performance. The SDMT assesses divided attention, visual scanning, tracking, and motor speed. Using a reference key, participants are given 90 s to pair specific numbers with given geometric figures, which is relatively culture free (27). The SCWT provides additional information on selective attention and information processing by analyzing cognitive processes (28). Accordingly, the mean speed of cards 1 and 2 in the SCWT (described below) was also included as a measure of attention/information processing.

Memory was assessed by the RAVLT. The RAVLT is a word list memory task. Participants are given a list of 15 unrelated words and asked to repeat them over 5 different trials. Another list of 15 unrelated words is given and they are asked to again repeat the original list of 15 words and again after 30 min (29). The RAVLT total is the total number of words recalled over the 5 different trials; it reflects the individual's ability to accumulate words across repeated learning trials. The RAVLT delayed recall is the total number of words recalled after the 30-min delay interval; it reflects the individual's long-term retention and forgetting rate.

Executive function was assessed by Trail B and the SCWT. Trail B involves connecting numbers and letters in an alternating progressive sequence (26). In order to measure central executive functioning, the difference in the time taken to complete Trail B, which stresses the central executive process of task set inhibition, cognitive flexibility, and the ability to maintain a response set, and the time to complete Trail A, which has little executive input, was calculated (i.e., Trail B-A) (30). The SCWT assesses the ability to inhibit the cognitive interference that occurs when the processing of a specific stimulus feature affects the simultaneous processing of a second stimulus attribute, well-known as the Stroop Effect (28). Participants are given 45 s each to complete the following: *1*) reading the words on a page as fast as possible (card 1: word reading), *2*) naming the colors on a page as fast as possible (card 2; color naming), and *3*) naming the colors of the ink of printed words as fast as possible (card 3; color-word). The Stroop Interference score was calculated by subtracting the predicted color-word score from the color-word score.

Language was assessed by the Word List Fluency Test. The Word List Fluency Test measures verbal production, semantic memory, and language. Participants are asked to name as many animals as possible in a 1-min period. Higher scores indicate better performance (31).

Overall cognitive function was assessed by the 3MS test. The 3MS test has components for orientation, registration, attention, language, praxis, and immediate and delayed memory (32). Scores range from 0 to 100, with higher scores indicating better performance. The 3MS test is more sensitive for mild cognitive impairment than the traditional 30-point Mini-Mental State Examination (32). Participants' raw test scores were standardized to *z* scores based on the baseline mean and SD. Composite *z* scores of cognitive functions were then calculated for each participant: attention *z* score with Trail A and the SDMT, and the SCWT (mean speed of cards 1 and 2), memory *z* score with total recall and delayed recall in the RAVLT, executive function *z* score with Trail B-A and the SCWT, and language *z* score with the Word List Fluency Test. A global cognition composite *z* score was generated by computing the mean standardized changes of all cognitive tests, including the 3MS (33, 34).

All cognitive testing was administered in a single session in a controlled environment. All participants were evaluated in the morning (from 08:00 to 11:00) by the same blinded trained examiner. The same time of day was scheduled for evaluation and re-evaluation to minimize circadian variations.

Potential mediators of intensive lifestyle intervention and testosterone replacement therapy on cognitive outcomes grouped in blocks.

Body weight and visceral adipose tissue. Body weight was measured in the morning after a 12-h fast. Visceral adipose tissue (VAT) was evaluated by DXA (using Hologic Horizon APEX Software 5.5.2) (35).

Insulin sensitivity, chronic inflammation, and insulin-like growth factor I. Blood samples were obtained in the fasting state for measurements of glucose and insulin using the glucose oxidase method (YSI Inc.) and RIA. HOMA-IR was calculated as fasting glucose (mg/dL) multiplied by fasting insulin (μU/mL) divided by 22.5 (36). As a marker of chronic inflammation, high-sensitivity C-reactive protein (hs-CRP) was measured by immunoturbidimetric assay (Hitachi 917; Roche). Insulin-like growth factor I (IGF-I) was measured by RIA (Diagnostic Products). We queried participants for any current or recent (within 5 d) symptoms of a systemic infection (e.g., fever, swollen glands, persistent cough) before proceeding with the blood sampling.

Muscle strength and peak oxygen consumption. The 1-RMs (the maximal weight a person can lift at 1 repetition) for biceps curl, bench press, seated row, knee extension, knee flexion, and knee extension were summed to calculate total 1-RM strength (37). Peak oxygen consumption ($\dot{V}O_{2peak}$) was assessed during graded treadmill walking by indirect calorimetry (True Max 2400; ParvoMedics), as previously described (38). Briefly, the incremental test started at a speed determined during a warm-period to elicit ∼70% of age-predicted maximum heart rate and remained constant throughout the test, and the grade was increased by 3% every 2 min. The test continued until the participant could no longer exercise because of exhaustion.

Sex hormones and gonadotrophins. Blood samples were also obtained in the fasting state for the measurements of gonadal hormones. Total testosterone, estradiol, and estrone were measured by LC–tandem MS; free testosterone was measured by equilibrium dialyses (Mayo Clinic Laboratories). Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were measured by chemiluminescent immunoassay (UnicCel Exl 600, Beckman Coulter). The CVs for these measurements ranged from 5.6% to 10.2%.

Statistical analysis

The main outcome for this report was the prespecified change in the global cognition composite z score. Accordingly, the sample size of the parent RCT (19) was sufficient to detect a clinically important mean ± SD difference between groups of 0.28 ± 0.39 (Cohen's $d = 0.74$) in global cognition z score at an α level of 5%. An effect size of Cohen's $d = 0.74$ is larger than Cohen's $d = 0.25$ generated from significant practice effects (39) and is clinically important (40). Intention-to-treat analyses were performed with SAS software, version 9.4 (SAS Institute), by analyzing data from all participants originally randomly assigned. Baseline characteristics were compared by using ANOVA or Fisher's exact test. Longitudinal changes between groups were tested with the use of mixed-model repeated-measures ANCOVAs. Change from baseline was used as the dependent variable with group, visit, and group × time as independent effects and baseline values, age, and years of education as covariates. The primary focus of analyses was the significance of the interaction between group and time point. Within the framework of the mixed model, when the P value for an interaction was <0.05, the specific contrasts were used to test the null hypothesis that changes between the 2 time points in 1 group were equal to corresponding changes in the other group. P values for secondary outcomes were controlled for multiple testing using the Holm–Bonferroni procedure for maintaining family-wise error rate. Analyses testing for within-group changes were also performed using mixed-model repeated-measures ANOVA when the group × time interaction was significant; otherwise, the main effects were tested. Cohen's d was calculated to quantify the magnitude of differences in outcome variables between the intervention groups. These statistics were included because they are, unlike P values, independent of sample size and provide information on the size of the observed effect.

Pearson's correlation was used to examine relations among changes in selected variables and changes in global cognition; stepwise multiple linear regression was used to identify which among the variables were the important independent predictors for the changes in global cognition.

Sensitivity analyses that supported the statistical results obtained included multiple imputation for missing fitness data (which showed the same pattern of results). Additional analyses included logistic regression that verified that the data were consistent with an assumption that data were missing at random. Data are presented as least-square means ± SEs unless otherwise specified. P values < 0.05 were considered to indicate statistical significance.

Results

The Consolidated Standards of Reporting Trials (CONSORT) diagram (**Supplemental Figure 1**) has been reported previously (19). Briefly, 83 participants were randomly assigned and 70 (84%) completed the study. Thirteen participants (8 in the LT + Test and 5 in the LT + Pbo group) discontinued the intervention and were included in the intention-to-treat analyses. Baseline characteristics were not different between the groups (**Table 1**). Almost half of the participants were of black race. Participants had an average of 4 chronic diseases, which is consistent with an at-risk older population. Attendance at the behavioral-diet sessions was 84.9% ± 4.0% for the LT + Test group and 89% ± 3.1% for the LT + Pbo group. Attendance at the supervised-exercise sessions was 86.7% ± 4.1% for the LT + Test group and 92.8% ± 3.0% for the LT + Pbo group.

The scores for Trail A and Stroop speed improved more in the LT + Test than in the LT + Pbo group (between-group difference: 6.2; 95% CI: 2.1, 10.3; Cohen's $d = 0.64$ and between-group

TABLE 1 Baseline characteristics of participants by intervention group[1]

	LT + Test ($n = 42$)	LT + Pbo ($n = 41$)	*P* value
Age, y	72.6 ± 0.6	72.2 ± 0.5	0.57
BMI, kg/m^2	37.1 ± 0.9	36.9 ± 0.8	0.86
Weight, kg	115.5 ± 3.2	114.2 ± 2.4	0.87
Visceral adipose tissue, cm^3	1395 ± 65	1311 ± 51	0.31
Years of education	12.1 ± 0.5	12.7 ± 0.4	0.36
Race[2]			0.38
White	19 (45)	22 (54)	
Black	20 (48)	16 (39)	
Other	3 (7)	3 (7)	
Ethnic group[2]			0.36
Hispanic or Latino	5 (11)	8 (20)	
Not Hispanic or Latino	37 (88)	32 (78)	
Unknown	0 (0)	1 (2)	
Chronic diseases (no)[3]	4.0 ± 0.4	4.6 ± 0.4	0.45
Physical Performance Test score	28.4 ± 0.4	28.3 ± 0.5	0.77
Total 1-RM strength, kg	314 ± 11	311 ± 11	0.81
$\dot{V}O_{2peak}$, mL · kg^{-1} · min^{-1}	17.1 ± 0.6	16.8 ± 0.5	0.73
HOMA-IR	12.2 ± 4.2	14.2 ± 3.6	0.27
hs-CRP, mg/dL	0.61 ± 0.13	0.55 ± 0.08	0.70
Insulin-like growth factor 1, ng/mL	80.3 ± 5.5	83.1 ± 8.7	0.79
Total testosterone, nmol/L	7.6 ± 0.2	7.3 ± 0.3	0.33
Free testosterone, nmol/L	0.18 ± 0.01	0.19 ± 0.01	0.27
Estradiol, pmol/L	100.6 ± 8.1	102.4 ± 7.3	0.88
Follicle-stimulating hormone, mIU/mL	11.0 ± 1.9	10.9 ± 1.2	0.95
Luteinizing hormone, mIU/mL	6.3 ± 0.9	6.7 ± 0.6	0.69
Use of CNS-affecting drugs			
Antidepressant	2	3	0.67
Anticholinergic	2	1	1.00
Sedative-hypnotic	0	2	0.21

[1] Values are means ± SEMs, *n*, or *n* (%) unless otherwise indicated. CNS, central nervous system; hs-CRP, high-sensitivity C-reactive protein; LT + Pbo, lifestyle therapy plus placebo; LT + Test, lifestyle therapy (weight management and exercise training) plus testosterone replacement; $\dot{V}O_{2peak}$, peak oxygen consumption; 1-RM, 1-repetition maximum.

[2] Race and ethnic group were reported by the participants.

[3] Chronic diseases included hypertension, diabetes, coronary artery disease, congestive heart failure, arthritis, and chronic lung disease.

difference: −3.2; 95% CI: −5.0, −1.4 ; Cohen's $d = 0.70$, respectively) (**Table 2**). Likewise, the scores in RAVLT total recall and delayed recall improved more in the LT + Test than in the LT + Pbo group (between-group difference: −3.9; 95% CI: −6.6, −1.2; Cohen's $d = 0.60$ and between-group difference: −1.2; 95% CI: −2.1, −0.4; Cohen's $d = 0.62$, respectively). There was a trend for the score in the SDMT to improve more in the LT + Test than in the LT + Pbo group (Table 2). The scores in the Trail B-A, Stroop interference, and 3MS also improved from baseline in the LT + Test and LT + Pbo groups (significant main effect of time), although there were no significant differences between groups (Table 2).

The global cognition *z* score increased more in the LT + Test than in the LT + Pbo group (between-group difference: −0.28; 95% CI: −0.45, −0.11; Cohen's $d = 0.74$) (**Table 3**, **Supplemental Figure 2**). Moreover, attention/information *z* score and memory *z* score increased more in the LT + Test than in the LT + Pbo group (between-group difference: −0.32; 95% CI: −0.50, −0.09; Cohen's $d = 0.49$ and between-group difference: −0.53; 95% CI: −0.93, −0.13, Cohen's $d = 1.43$, respectively) (Table 3). Bivariate analyses showed that changes in each of the subcomponent *z* scores correlated with the change in the global cognition *z* score (**Supplemental Table 1**).

VAT, HOMA-IR, and hs-CRP decreased to the same extent in the LT + Test and LT + Pbo groups (between-group difference: 19; 95% CI: −65, 103; Cohen's $d = 0.02$; between-group difference: 0; 95% CI: −0.63, 0.63; Cohen's $d = 0.00$; and between-group difference: 0.01; 95% CI: −0.01, 0.03; Cohen's $d = 0.03$, respectively). IGF-I also increased similarly in the LT + Test and LT + Pbo groups (between-group difference: 5.1; 95% CI −3.1, 13.3; Cohen's $d = 0.04$). The between-group differences in changes in body weight, 1-RM strength, $\dot{V}O_{2peak}$, testosterones, estrogens, FSH, and LH have been reported elsewhere (19).

Bivariate analyses showed that changes in several clinical variables correlated with changes in the global cognition composite *z* score (**Supplemental Table 2**). Using these as candidate variables, stepwise multiple regression revealed that changes in $\dot{V}O_{2peak}$ ($\beta = 0.34$), 1-RM strength ($\beta = 0.28$), testosterone ($\beta = 0.24$), and LH ($\beta = -0.22$) were independent predictors, explaining 38% of the variance, for the changes in global cognition ($R^2 = 0.38$; $P < 0.001$) (**Table 4**).

Discussion

Our secondary analyses of the randomized, double-blind, placebo-controlled LITROS trial involving frail adults 65 y of age or older with obesity and hypogonadism suggest that testosterone replacement therapy added to intensive lifestyle

TABLE 2 Baseline raw cognitive test scores and changes by intervention group[1]

	LT + Test ($n = 42$)	LT + Pbo ($n = 41$)	Difference (95% CI)	P value[2]	Cohen's d
Attention/information processing					
Trail A[3]					
Baseline	54.0 ± 2.7	52.1 ± 1.7			
Change at 6 mo	−13.9 ± 1.5***	−7.7 ± 1.4***	6.2 (2.1, 10.3)	0.03	0.64
Symbol Digit Modalities Test					
Baseline	38.7 ± 0.9	39.1 ± 1.2			
Change at 6 mo	2.8 ± 0.5	1.1 ± 0.5	−1.7 (−3.1, 0.3)	0.09	0.52
Stroop speed[4]					
Baseline	71.3 ± 1.8	70.1 ± 1.5			
Change at 6 mo	2.3 ± 0.7**	−0.9 ± 0.6	−3.2 (−5.0, −1.4)	0.01	0.70
Memory					
RAVLT total recall					
Baseline	35.9 ± 1.7	36.6 ± 1.3			
Change at 6 mo	7.0 ± 1.0***	3.1 ± 0.9*	−3.9 (−6.6, −1.2)	0.03	0.60
RAVLT delayed recall					
Baseline	6.0 ± 0.4	6.4 ± 0.5			
Change at 6 mo	2.5 ± 0.3***	1.3 ± 0.3*	−1.2 (−2.1, −0.4)	0.04	0.62
Executive function					
Trail B-A[3]					
Baseline	59.8 ± 5.8	72.4 ± 7.3			
Change at 6 mo	−19.5 ± 4.0†	−9.1 ± 4.1†	10.4 (−0.9, 21.7)	0.44	0.41
Stroop interference					
Baseline	−7.4 ± 1.8	−5.9 ± 0.9			
Change at 6 mo	4.6 ± 0.7††	3.1 ± 0.7††	−1.5 (−3.3, 0.3)	0.21	0.33
Language					
Word list fluency test					
Baseline	18.2 ± 0.8	18.8 ± 0.8			
Change at 6 mo	0.9 ± 0.5	0.2 ± 0.5	−0.8 (−2.6, 1.1)	0.41	0.25
3MS					
Baseline	94.1 ± 0.6	94.2 ± 0.6			
Change at 6 mo	2.1 ± 0.4††	1.2 ± 0.3††	−0.9 (−1.9, 0.1)	0.16	0.35

[1]Baseline values are observed means ± SEs; change score values are least-squares adjusted means ± SEs from the repeated-measures ANOVAs. *,**,***Significant difference for the comparison of the value at the follow-up time with the baseline value within the group, as calculated with the use of mixed-model repeated-measures ANOVAs: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. †,††Significant main effect of time: †$P < 0.01$; ††$P < 0.001$. LT + Pbo, lifestyle therapy plus placebo; LT + Test, lifestyle therapy (weight management and exercise training) plus testosterone; RAVLT, Rey Auditory Verbal Learning Test; Trail A and B, Trail-Making Test Parts A and B; 3MS, Modified Mini-Mental State Examination.

[2]P values for between-group comparisons of changes from baseline to 6 mo were calculated with the use of mixed-model repeated-measures ANOVAs (with baseline values, age, and years of education as covariates).

[3]Lower scores indicate better performance.

[4]Mean of cards 1 and 2 in the Stroop Color and Word Test.

intervention (weight management and exercise training) may further improve cognitive function. Specifically, in this high-risk population, testosterone replacement therapy in combination with lifestyle therapy resulted in the largest improvement in the global cognition composite z score obtained by averaging the z scores from all cognitive tests. This positive finding was associated with significant improvement in the subcomponent attention/information processing and memory z scores. To our knowledge, the current study is the first RCT to examine whether testosterone replacement therapy may improve cognitive function with lifestyle behaviors controlled via lifestyle intervention therapy in this prevalent but understudied population of older men with coexisting obesity and hypogonadism.

Several features of our RCT design set our study apart from prior studies of testosterone therapy in older adults. We specifically enrolled older men who were obese and had evidence suggestive of biochemical and clinical hypogonadism (i.e., unequivocally low early-morning testosterone associated with evidence of physical frailty) (20, 41). We provided testosterone replacement therapy, adjusting the dosage to keep the serum testosterone within the mid-young target range while participants underwent ongoing behavioral weight loss and exercise training to improve obesity- and age-related metabolic and physical complications. Accordingly, the positive effect of testosterone therapy above and beyond lifestyle therapy on cognition that we observed in this specific population is distinct from that reported in the most recent systematic review and meta-analysis of 23 independent RCTs of testosterone to date, none of which specifically treated clinically hypogonadal men or involved intensive diet and exercise interventions combined with testosterone (25).

Given the multiple risk factors for cognitive decline in our study participants, we used a multifactorial intervention consisting of intensive lifestyle intervention with or without testosterone replacement therapy. The matched weight loss and exercise training between our study groups facilitated the

TABLE 3 Baseline cognitive function measured with composite *z* scores and changes by intervention group[1]

	LT + Test ($n = 42$)	LT + Pbo ($n = 41$)	Difference (95% CI)	*P* value[2]	Cohen's *d*
Primary outcome					
Global cognition					
Baseline	−0.03 ± 0.09	0.03 ± 0.09			
Change at 6 mo	0.49 ± 0.06***	0.21 ± 0.06***	−0.28 (−0.45, −0.11)	0.001	0.74
Secondary outcomes					
Attention/information processing					
Baseline	−0.02 ± 0.10	0.02 ± 0.09			
Change at 6 mo	0.55 ± 0.08***	0.23 ± 0.08**	−0.32 (−0.55, −0.09)	0.005	0.49
Memory					
Baseline	−0.05 ± 0.15	0.04 ± 0.14			
Change at 6 mo	0.90 ± 0.14***	0.37 ± 0.14*	−0.53 (−0.93, −0.13)	0.009	1.43
Executive function					
Baseline	−0.07 ± 0.09	0.08 ± 0.10			
Change at 6 mo	0.31 ± 0.09†	0.18 ± 0.08†	−0.13 (−0.37, 0.11)	0.28	0.22
Language					
Baseline	−0.05 ± 0.15	0.07 ± 0.16			
Change at 6 mo	0.34 ± 0.13	0.08 ± 0.13	−0.26 (−0.62, −0.10)	0.17	0.31

[1] Baseline values are observed means ± SEs; change score values are least-squares adjusted means ± SEs from the repeated-measures ANOVAs. *,**,***Significant difference for the comparison of the value at the follow-up time with the baseline value within the group, as calculated with the use of mixed-model repeated-measures ANOVAs: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. †$P < 0.001$ for main effect of time. LT + Pbo, lifestyle therapy plus placebo; LT + Test, lifestyle therapy (weight management and exercise training) plus testosterone.

[2] *P* values for between-group comparisons of changes from baseline to 6 mo were calculated with the use of mixed-model repeated-measures ANOVAs (with baseline values, age, and years of education as covariates). In a Holm–Bonferroni correction to adjust for multiple comparisons in the secondary outcomes, the corrected *P* values were 0.02 for attention/information processing, 0.03 for memory, 0.34 for executive function, and 0.34 for language.

assessment of the effect of testosterone above and beyond that of lifestyle therapy alone. Indeed, we found that LT + Test improved overall cognitive function more than LT + Pbo, a finding corroborated by multiple regression analyses showing that change in testosterone independently predicted improvement in global cognition. Testosterone has been found to delay neuronal apoptosis (42) and accelerate the rate of nerve regeneration (43), effects that are likely mediated through the androgen receptor (44). Testosterone can also be aromatized to estrogen, which has additional neuroprotective and neurotrophic effects (45). However, change in estradiol was not an independent predictor in the final model probably because our participants were not estrogen deficient owing to obesity (46). On the other hand, a potential novel finding is that change in LH was also an independent predictor of improvement in global cognition. LH receptors are expressed in the brain and the loss of sex steroids which leads to increased LH has been found to correlate with cognitive deficits in men (47). Our finding is in agreement with emerging literature showing that decreasing peripheral LH reduces cognitive deficits in Alzheimer disease (AD) models (48).

TABLE 4 Final model in the stepwise multiple regression analyses identifying predictors of changes in the global cognition composite[1]

	β	*P* value
Change in $\dot{V}O_{2peak}$	0.34 ± 0.10	0.001
Change in total 1-RM strength	0.28 ± 0.14	0.006
Change in total testosterone	0.24 ± 0.01	0.02
Change in LH	−0.22 ± 1.02	0.03

[1] Values entered in the model were change in body weight, change in visceral adipose tissue, change in HOMA-IR, change in high-sensitivity C-reactive protein, change in $\dot{V}O_{2peak}$, change in 1-RM strength, change in total testosterone, change in free testosterone, change in estradiol, change in follicle-stimulating hormone, change in LH, age, and years of education. The sample size for analysis was 70. Multiple $R = 0.62$; $R^2 = 0.38$; $P < 0.001$. LH, luteinizing hormone; $\dot{V}O_{2peak}$, peak oxygen consumption; 1-RM, 1-repetition maximum.

As in our prior RCT in this population (4), cognitive scores in the lifestyle intervention alone (LT + Pbo) group also increased from baseline. However, because there was no control group without lifestyle intervention in the current study, we cannot exclude that the increase in scores in the LT + Pbo group could be due in part to a learning effect (39). Given that our lifestyle intervention was multimodal, involving a combination of diet-induced weight loss, aerobic training, and resistance training, the results of multiple regression also helped to sort out their relative contributions to the changes in cognition. Although indeed dietary calorie restriction induced significant weight loss associated with improvement in insulin sensitivity and reduction in chronic inflammation in our participants, these variables were not independent predictors of the improvement in global cognition. On the other hand, aerobic training–induced improvement in $\dot{V}O_{2peak}$ and resistance training–induced improvement in 1-RM strength were among the independent predictors in the final model. These findings suggest that lifestyle intervention improved cognitive function primarily through aerobic- and resistance-training-specific physiologic adaptations to exercise rather than through dietary weight loss metabolic adaptations. These results are consistent with those of our previous report (4) and of Hugenschmidt et al. (5), both conducted in a population similar to the current study, which demonstrated that among the lifestyle approaches, exercise is the more potent intervention, such that adding diet-induced weight

loss provides little additional benefit over exercise. Moreover, the long-term Look AHEAD (Action for Health in Diabetes) study showed that a behavioral diet-induced weight-loss intervention did not alter cognitive function in adults with obesity and type 2 diabetes (6, 49).

Because most prior studies showing cognitive benefits of exercise involved aerobic training alone (24), our RCT extends the literature by adding novel findings on the effects of combined aerobic and resistance training in this high-risk older population. The few studies involving resistance training alone have been equivocal with positive results limited to those using high-intensity protocols (50, 51). In the current RCT, we used moderate- to high-intensity aerobic training to improve $\dot{V}O_{2peak}$ and moderate- to high-intensity resistance training to improve 1-RM strength in older men with obesity and hypogonadism. It has been suggested that the increase in IGF-I in response to these exercise adaptations may mediate some of the cognitive benefits (52). However, we did not find the change in IGF-I to be an independent predictor of improvement in global cognition, probably because of the modest increase in IGF-I that we observed, consistent with diminished growth hormone/IGF-I response with aging (53). Future studies might include measurements of other candidate cellular and molecular mediators such as the novel myokines, apelin and osteocalcin, which are released during muscle contraction and have been found to have neurotrophic effects (54, 55). Taken together, the results of our RCT suggest that most effective in improving the cognitive function of older obese hypogonadal men is a multifactorial intervention that corrects both their unhealthy lifestyles and sex steroid deficiencies.

Strengths of our trial included the concealed randomization, placebo-controlled, parallel design, the comprehensive lifestyle programs and assessments of outcomes, the degree of adherence resulting in matched weight loss across groups, and the well-characterized, unique, but increasing population. We assessed cognitive function with a comprehensive set of standardized well-validated neuropsychological tests, including measures sensitive to obesity and gonadal steroids. We measured testosterone concentrations with the gold-standard assay of LC–tandem MS. Moreover, we included exploratory analyses of the potential mediators of cognitive responses to our intensive interventions, such as changes in insulin sensitivity, inflammation, sex and gonadotrophic hormones, and physiologic adaptations to aerobic and resistance training.

Our study also had limitations. Importantly, we did not include a group that had testosterone therapy alone or a group that had no lifestyle therapy. However, we have shown that weight loss plus exercise is most effective in improving the functional status of older adults with obesity (37, 56), so for ethical purposes, all participants in this study received lifestyle therapy. Accordingly, our study design did not address any synergistic effects of lifestyle therapy and testosterone. Participants who volunteered may be different from the general population so our results may not necessarily generalize to the broader population of older men with obesity and hypogonadism. Despite multiple risk factors for cognitive decline in our participants, our study was not powered to detect differences in the incidence of the spectrum of mild cognitive impairment and AD, which would require a very large sample size and prolonged duration of follow-up. Because our results are from secondary analyses of a parent RCT trial, further carefully conducted RCTs are needed to confirm and extend our results in different settings.

In conclusion, findings from our secondary analyses of prespecified outcomes in the LITROS trial suggest that in the high-risk population of older men with obesity and hypogonadism, testosterone replacement therapy may improve cognitive function when combined with intensive lifestyle intervention. Our results should stimulate further clinical trials examining the cognitive response to intensive lifestyle intervention combined with testosterone replacement in this vulnerable population. At present, the decision to add testosterone replacement to lifestyle intervention in this expanding older population with obesity should be decided on an individual basis after carefully weighing the risks and benefits of testosterone in combination with lifestyle therapy (19). Although eating specific foods (e.g., herbs, spices) may be an alternative option for increasing testosterone concentrations, the current evidence is largely based on young nonclinical populations (57) and, as such, their efficacy in correcting clinical hypogonadism in older men is largely undocumented.

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Data availability

Data described in the article, code book, and analytic code will be made available upon reasonable request pending a specific Data User Agreement (DUA) or other written agreements (aggregated data that can be released without privacy and confidentiality risks).

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