Metabolism 136 (2022) 155290

Contents lists available at ScienceDirect

Metabolism

journal homepage: www.journals.elsevier.com/metabolism

Metformin, testosterone, or both in men with obesity and low testosterone: A double-blind, parallel-group, randomized controlled trial

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ARTICLE INFO

Keywords:
Obesity
Metformin
Testosterone
Erectile dysfunction
Insulin resistance

ABSTRACT

Background: Men with obesity tend to be insulin resistant and often have low-normal testosterone concentrations. We conducted a clinical trial aimed to evaluate potential therapeutic strategies for low testosterone in men with obesity.

Methods: We did a 1-year, parallel, randomized, double-blind, placebo-controlled trial, where we evaluated the independent and combined effects of metformin and testosterone in 106 men with obesity, aged 18–50 years, who had low levels of testosterone and no diabetes mellitus. The primary outcome was change in insulin resistance, measured as Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) index. Secondary outcomes included changes in total and free serum testosterone, body composition, metabolic variables, erectile function, and health-related quality of life (HRQoL).

Results: In the intention-to-treat analysis, the HOMA-IR index decreased significantly in all active groups compared to placebo (metformin −2.4, 95 % CI −4.1 to −0.8, $p = 0.004$; testosterone −2.7, 95 % CI −4.3 to −1.1, $p = 0.001$; combination −3.4, 95 % CI −5.0 to −1.8, $p < 0.001$). Combination therapy was not superior to testosterone alone in decreasing insulin resistance (−0.7, 95 % CI −2.3 to 0.9, $p = 0.383$). Only the combination of metformin plus testosterone significantly increased total and free testosterone concentrations, compared to placebo. No significant changes in body composition (except for a higher decrease in fat mass in the metformin and combination group), metabolic variables, erectile function, or HRQoL were found with any treatment.

Conclusions: Among men with obesity and low testosterone concentrations, the combination of metformin plus testosterone, metformin only, and testosterone only, compared to placebo, reduced insulin resistance with no evidence of additive benefit.

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https://doi.org/10.1016/j.metabol.2022.155290
Received 16 February 2022; Accepted 11 August 2022
Available online 18 August 2022

1. Introduction

Obesity is a multifactorial chronic disease that has become a serious global public health problem. Its prevalence has more than doubled since 1980, and currently 12 % of adults aged 18 years and over have obesity, totaling >700 million adults with obesity worldwide [1,2].

Excess body weight is associated with a number of metabolic complications such as hypertension, type 2 diabetes, some types of cancer, and cardiovascular disease [3]. Moreover, obesity is the leading cause of low testosterone concentrations in men, affecting 20 % to 40 % of men with obesity according to several epidemiological studies [4–6], which contrasts with the prevalence of 4 % to 5 % in the general male population [7].

Low testosterone concentrations in men have profound health implications, including visceral obesity, reduced lean body mass, type 2 diabetes, erectile dysfunction, and decreased quality of life [8]. On the other hand, the influence of testosterone on risk for cardiovascular events in men is uncertain; while some studies report that testosterone deficiency may increase cardiovascular morbidity and all-cause mortality risk [9,10], other studies indicate that serum testosterone concentrations are not associated with incident cardiovascular events [11].

Several approaches have been used to address low concentrations of testosterone in men with obesity, including weight reduction (either through lifestyle modifications or bariatric surgery), treatment with aromatase inhibitors, selective estrogen receptor modulators, or IL-1 receptor antagonists [12–14]. Despite loss of weight excess is the best approach for reduced testosterone levels in men with obesity, testosterone replacement therapy has increased substantially in recent years, despite the highly controversial risk/benefit ratio and the lack of robust data on its long-term clinical efficacy [15,16].

Insulin resistance and compensatory hyperinsulinemia are pathophysiological hallmark features of low testosterone in obesity, resulting in impaired gonadotropin secretion and subsequent decreased secretion of testosterone from the testes [17]. Therefore, a potential treatment for low testosterone concentrations in men with obesity could be a therapy aimed at reducing insulin resistance. In addition, a simultaneous approach both targeting insulin resistance and reduced testosterone concentrations could also constitute another effective treatment for this condition.

We conducted a randomized, double-blind, placebo-controlled trial of testosterone and metformin, testosterone only, metformin only and dual placebo with the endpoint of insulin resistance in young men with obesity and low testosterone. We also evaluated changes in testosterone concentrations, body composition, metabolic variables, erectile function, and health-related quality of life (HRQoL).

2. Methods

2.1. Trial design and oversight

The TESEO (**T**estosterone, m**E**tformin, or both, for low te**S**tosterone in m**E**n with **O**besity) trial was a 1-year, single-center, double-blind, parallel-group, randomized, placebo-controlled independent trial, performed in the Department of Endocrinology and Nutrition at the Virgen de la Victoria University Hospital (Malaga, Spain).

The study protocol was approved by the institutional review boards and the local ethics committee. The trial was performed in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. All patients provided written informed consent before study entry. The trial was monitored by an independent data and safety monitoring board (Delos Clinical, Seville, Spain). The protocol was registered with ClinicalTrials.gov (NCT02514629).

2.2. Patients

We included young adult men (18–50 years) with obesity (defined by a body-mass index [BMI] ≥30 kg/m^2) who had reduced serum concentrations of testosterone, defined by either total testosterone levels <230 ng/dl (8 nmol/l) or free testosterone levels <70 pg/ml (240 pmol/l) [18,19].

The main exclusion criteria included diabetes mellitus (diagnosed by fasting plasma glucose ≥126 mg per deciliter [7 mmol per liter], glycated hemoglobin ≥6.5 %, as confirmed by repeated testing, or by the use of any anti-diabetic medication); evidence of intercurrent pituitary disease; hyperprolactinemia; increased luteinizing hormone (LH) levels; additional pituitary hormone deficiencies; hemochromatosis, malignancy; prostate-specific antigen (PSA) level >4 ng/ml; hematocrit >50 %; severe lower urinary tract symptoms; hepatic, renal, or cardiovascular disease; and uncontrolled hypertension. In addition, the use of androgens, phosphodiesterase 5 inhibitors, clomiphene, human chorionic gonadotropin, or alprostadil was not permitted. All subjects had normal pubertal development and reported an intact sense of smell. Patients with extremely low total testosterone levels (<150 ng/dl [5.2 nmol/l]) underwent magnetic resonance imaging; no brain disorders were found in any patient.

2.3. Randomization, masking, and intervention

Eligible patients were randomly assigned in a 1:1:1:1 ratio (via a computer-generated block randomization procedure) to receive twice-daily placebo tablets and placebo injections in weeks 0, 6, 18, 30, and 42 (placebo group), twice-daily 850 mg metformin tablets plus placebo injections in weeks 0, 6, 18, 30, and 42 (metformin group), twice-daily oral placebo tablets plus 1000 mg testosterone undecanoate injections in weeks 0, 6, 18, 30, and 42 (testosterone group), or twice-daily 850 mg metformin tablets plus 1000 mg testosterone undecanoate injections in weeks 0, 6, 18, 30, and 42 (metformin-testosterone group).

Metformin hydrochloride 850 mg was acquired by the investigators from Merck (Darmstadt, Germany), and matching placebo tablets were manufactured by Toll Manufacturing Services (Madrid, Spain). Testosterone undecanoate 1000 mg/4 ml (Reandron) and matching placebo ampoules were donated by Bayer Pharma AG (Berlin, Germany). None of the manufacturers had any other role in the study. Packaging and labeling of both the active drugs and matching placebos were performed by the Clinical Trials Unit of the Pharmacy Service of Virgen de la Victoria University Hospital (Malaga, Spain).

2.4. Clinical assessments and measurements

Serum insulin levels were measured by immunoassay using an ADVIA Centaur autoanalyzer (Siemens Healthineers, Erlangen, Germany). Analytical sensitivity was 0.3 mU/l, and inter- and intra-assay coefficients of variability were <10 %. Total testosterone was determined by 2 different techniques: 1) for recruitment, a competitive immunoassay using electrochemiluminescence (ECLIA) (ADVIA Centaur System, Siemens) was used; 2) At the study end, the concentrations of all study samples (batched during the clinical trial) were determined by high-performance liquid chromatography-mass spectrometry (HPLC-MS) using a triple quadrupole mass spectrometry system (Model 6460; Agilent Technologies, Santa Clara, California).

Sex hormone–binding globulin (SHBG) was determined by electrochemiluminescence immunoassay (Elecsys SHBG; Roche, Basel, Switzerland) (reference range: 15–50 nmol/l). Free testosterone was estimated from total testosterone and SHBG using Vermeulen's formula [20]. LH was determined by direct chemiluminometric assay (ADVIA Centaur; Siemens Healthineers, Erlangen, Germany) (reference values: 1.5–7.7 mIU/ml).

2.4.1. Body composition analysis

Weight and body composition was assessed with a Tanita multifrequency body composition analyzer MC-180MA (Tanita Corporation, Tokyo, Japan). This instrument has been validated against other

weighing methods and is repeatedly checked in relation to the reference standards of dual-energy X-ray absorptiometry (DEXA) [21].

2.4.2. Erectile function and health-related quality of life (HRQoL)

We used the simplified International Index of Erectile Function (IIEF-5) questionnaire to assess erectile function. This questionnaire comprises 5 questions and each IIEF-5 item is scored on a 5-point ordinal scale. The possible scores for the IIEF-5 range from 5 to 25, with lower values representing poorer sexual function. Erectile dysfunction is determined as an IIEF-5 score ≤21 points [22].

HRQoL was assessed using the Aging Males' Symptoms (AMS) scale. Scores on this scale range from 17 to 85, with higher scores indicating worse health status. The intensity of complaints is rated as no/little (≤26 points), mild (27–36 points), moderate (37–49 points), or severe (≥50 points) [23].

2.5. Follow-up assessments

All baseline assessments were repeated at 26 and 52 weeks. Additional study visits at weeks 6, 18, 30, and 42 were undertaken for the administration of injections.

2.6. Study outcomes

The primary endpoint was change in insulin resistance, measured as Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index, from baseline to 1 year. HOMA-IR was calculated as fasting glucose (measured in millimoles per liter) multiplied by fasting insulin (measured in microunits per milliliter) divided by 22.5 [24]. The HOMA-IR index has been validated against the gold-standard hyperinsulinemic-euglycemic clamp (an invasive, intensive, and technically difficult procedure), and is considered a reliable index to assess insulin sensitivity in clinical studies [25].

Pre-specified secondary outcomes were changes in serum levels of total and free testosterone, BMI, fat mass, fat-free mass, parameters related to glucose and lipid metabolism, erectile function (measured by the IIEF-5 questionnaire), and HRQoL (measured by the AMS scale).

2.7. Statistical analyses

Given that a previous study [26] reported that the treatment with testosterone undecanoate reduced the HOMA-IR index from 8.4 to 3.3 after 1 year of treatment in patients with obesity (standard deviations of 5.7 and 1.7, respectively), assuming a statistical power of 80 %, a significance level of 5 %, and an estimated 15 % of follow-up dropouts, we estimated that 25 patients were required for each study group.

Analyses were done by intention to treat, which included all randomized patients who received at least one dose of the study drugs, and the last observation carried forward approach was used. In addition, we performed a per-protocol analysis, including only those patients who completed the treatment originally allocated. Continuous variables are reported as mean (± SD), whereas categorical variables are reported as numbers and percentages. Baseline characteristics were compared using analysis of variance or Fisher's exact test.

As primary analysis we used generalized estimating equations (GEE), assuming an unstructured correlation matrix for all compared variables with the exception of anthropometric indexes which met the criteria for an exchangeable matrix. The primary focus of the analyses was the change in the HOMA-IR index (primary end-point) in the 4 groups, considering the time scale of the repeated measurements as a continuous variable. Each of the three active treatments was compared with the reference group (placebo). In addition, we tested the contrast between the combination treatment group (metformin plus testosterone) versus the testosterone group alone. Also, we tested for possible interactions (on the additive scale) of the effects of the active treatments. All these procedures also were applied to each secondary end-point. Finally, we added a sensitivity analysis by rerunning all these comparisons using mixed linear models (with the subject as a random variable) instead of GEE. The analyses were performed using Stata (15.0, StataCorp LP, Tx. USA). *p*-Values <0.05 were considered to indicate statistical significance.

3. Results

3.1. Trial population

Between July 18, 2013 and July 17, 2015, a total of 334 participants were screened for eligibility, and 106 were randomly assigned to receive placebo (n = 26), metformin (n = 27), testosterone (n = 26), or metformin-testosterone (n = 27). Of these patients, 85 (80.2 %) completed a 1-year follow-up. In addition, 8 patients who had discontinued the intervention were included in the intention-to-treat analyses, since all provided follow-up data at 6 months. Therefore, 93 patients were available for the primary analysis (Fig. 1).

There were no significant between-group differences in baseline characteristics (Table 1). The mean age of the participants was 37.9 years, mean BMI was 42.5 kg/m^2, and mean HOMA-IR index values were 6.0. The mean total testosterone and free testosterone levels by ECLIA were 239 ng/dl and 57.1 pg/ml, and the mean total testosterone and free testosterone levels by HPLC-MS were 313 ng/dl and 77.5 pg/ml, respectively.

3.2. Insulin resistance

After 1 year of treatment, the mean (± SD) HOMA-IR index values increased in the placebo group (4.6 ± 13.3), while they decreased in the metformin group (−0.3 ± 4.0), in the testosterone group (−0.7 ± 3.3), and in the combination group (−2.2 ± 7.8) (Table 2) (Fig. 2).

In the between-group comparison, all active treatments significantly decreased insulin resistance over 1 year compared with placebo: mean differences, metformin −2.4 (95 % CI −4.1 to −0.8; p = 0.004); testosterone −2.7 (95 % CI −4.3 to −1.1; p = 0.001); combination −3.4 (95 % CI −5.0 to −1.8; p < 0.001). Additionally, we tested if the combination of metformin-testosterone was more effective than testosterone alone in decreasing insulin resistance, but no significant differences were found (mean differences −0.7, 95 % CI −2.3 to 0.9, p = 0.383) (Table 2).

Similar results were found in the per-protocol analysis; in comparison with placebo, treatment with metformin, testosterone or the combination of metformin plus testosterone were more effective in reducing insulin resistance over 1 year: metformin −2.7 (95 % CI −4.6 to −0.9, p = 0.003), testosterone −3.0 (95 % CI −4.7 to −1.2, p = 0.001), metformin plus testosterone −3.8 (95 % CI −5.6 to −2.0, p < 0.001). Again, the combination of metformin plus testosterone was not more effective than testosterone alone in reducing insulin resistance (mean differences −0.9, 95 % CI −2.6 to 0.9, p = 0.335) (Supplementary Table 1).

Lastly, we tested if the effects of metformin plus testosterone were additive in terms of changes in HOMA-IR index; the effect of the combination group on HOMA-IR index was not greater than the sum of the other two intervention groups (metformin-only and testosterone-only) separately (p = 0.336 in the simple comparison of final-basal, p = 0.286 in the mixed linear model).

3.3. Testosterone concentrations

At 1 year, total testosterone concentrations increased by 108 ± 261 ng/dl in the combination group, by 49 ± 124 ng/dl in the testosterone group, and by 15 ± 96 points in the metformin group.

Despite we did not find a global effect in the between-group comparison (p = 0.118, intention-to-treat analysis; p = 0.093 per-protocol analysis), the combination of metformin plus testosterone significantly increased total testosterone concentrations compared to placebo (mean

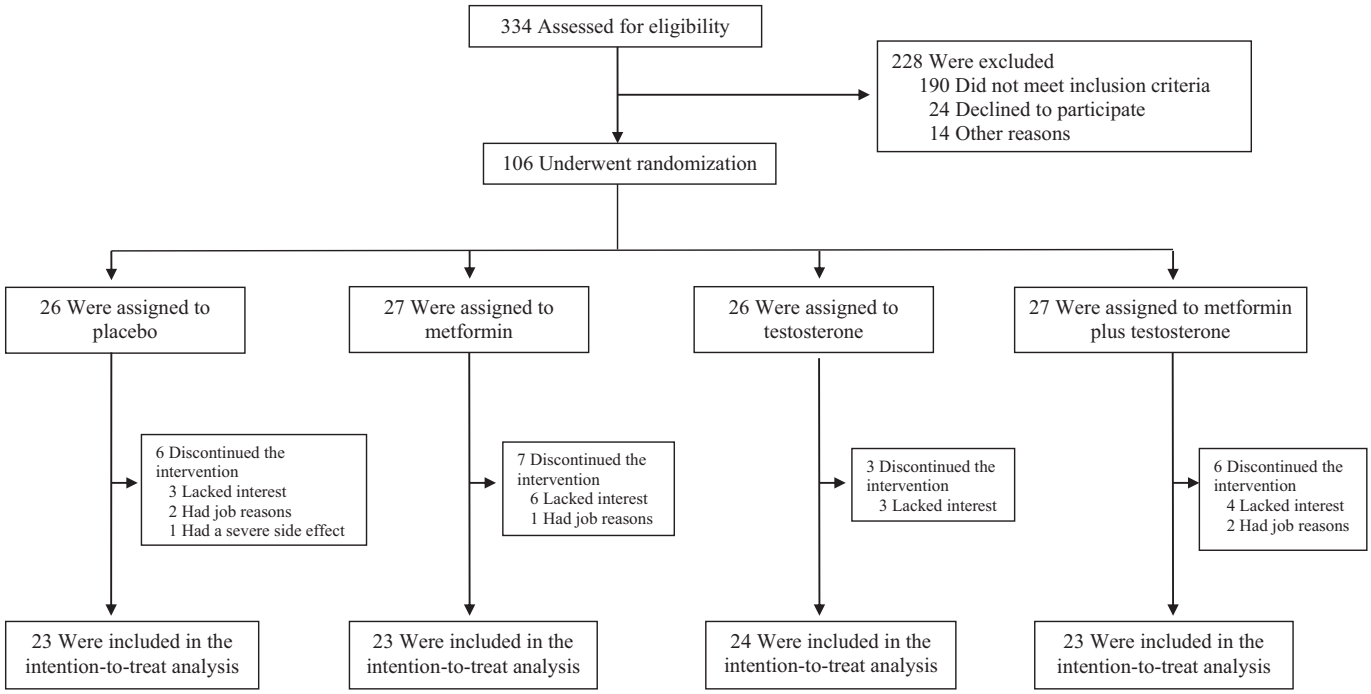

Fig. 1. Screening, randomization, and follow-up.
The study groups included a placebo group, a metformin group, a testosterone group, and a metformin-testosterone combination treatment group.

differences 42.9 ng/dl, 95 % CI 2.1 to 83.7, $p = 0.040$) (Table 2 and Supplementary Table 1).

Similarly, free testosterone was increased by 31.5 ± 82.2 pg/ml in the combination group, by 15.6 ± 32.2 pg/ml in the testosterone group, and by 5.5 ± 28.6 pg/ml in the metformin group. Again, despite the lack of a global effect in the between-group comparison ($p = 0.095$, intention-to-treat analysis; $p = 0.052$ per-protocol analysis), the combination treatment was superior to placebo increasing free testosterone concentrations (mean differences 14.9 pg/ml, 95 % CI 2.7 to 27.4; $p = 0.020$) (Table 2 and Supplementary Table 1).

Finally, we tested a potential additive effect of metformin on free testosterone concentrations; the effect of the combination group on free testosterone was not greater than the sum of the other two intervention groups (metformin-only and testosterone-only) separately ($p = 0.571$ in the simple comparison of final-basal; $p = 0.518$ in the mixed linear model).

3.4. Body weight and composition

Table 2 shows the changes in BMI and body composition of the participants. Despite we found no significant differences compared to placebo between study groups in BMI reduction ($p = 0.357$), or in fat-free mass ($p = 0.068$), metformin and the combination of metformin plus testosterone significantly decreased fat mass compared to placebo (mean differences, metformin −1.7 kg [95 % CI −3.0 to −0.4; $p = 0.011$]; testosterone −1.0 kg [95 % CI −2.6 to 0.3; $p = 0.124$]; combination −1.8 kg [95 % CI −3.1 to −0.5; $p = 0.007$]).

3.5. Erectile function

Erectile function, measured using the IIEF-5 questionnaire, increased by 2.3 ± 2.9 points in the combination group, by 1.0 ± 2.7 points in the metformin group, and by 0.9 ± 3.1 points in the testosterone group. However, in the between-group comparison, none of the treatments significantly improved erectile function compared to placebo ($p = 0.247$) (Table 2).

3.6. Health-related quality of life

The AMS score did not differ between the study groups ($p = 0.192$), indicating no substantial clinical improvement in the quality of life with any of the assigned treatments (Table 3). In addition, compared with baseline, there were no significant between-group changes in the AMS severity category at 1 year.

3.7. Other variables

No significant differences were found in glycated hemoglobin, HDL, LDL, or triglyceride levels between the study groups. Likewise, there were no significant changes in PSA levels in any active group compared to placebo (Table 3). Moreover, the effect of the combination group (metformin + testosterone) on HbA1c or PSA was not greater than the sum of the other two intervention groups (metformin-only and testosterone-only) separately ($p = 0.832$ for HbA1c and $p = 0.685$ for PSA, in mixed linear models).

On the other hand, the combination treatment was associated with a significant increase in hematocrit concentrations compared to placebo (mean differences 0.9 %, CI 95 % 0.1 to 1.8; $p = 0.033$). In addition, LH concentrations decreased significantly in the testosterone and combination group compared to placebo: mean differences, testosterone −1.2 mUI/ml (95 % CI −1.7 to −0.7; $p < 0.001$); combination −1.2 mUI/ml (95 % CI −1.7 to −0.7; $p < 0.001$), with no significant differences between both groups ($p = 0.829$) (Table 3).

Overall, results from sensitivity analysis and per-protocol analysis were similar to those obtained in the intention-to-treat analysis (Supplementary Table 1 and Supplementary Table 2).

3.8. Adverse events

Overall, 33.3 % of patients had side effects. Adverse events of any cause were reported in 16.1 % of the patients in the placebo group, in 38.7 % of patients in the metformin group, in 12.9 % of patients in the testosterone group, and in 32.3 % of patients in the metformin-testosterone group. Most side effects in the metformin and the

Table 1
Baseline characteristics of the participants according to study group.[a]

	Placebo (*N* = 23)	Metformin (N = 23)	Testosterone (*N* = 24)	Combination (N = 23)
Age - yr	37.5 ± 7.9	36.1 ± 6.6	40.0 ± 7.6	37.6 ± 7.5
Education - no. (%)				
Primary studies	11 (47.8)	10 (43.5)	11 (45.8)	10 (43.5)
Secondary studies	8 (34.8)	10 (43.5)	11 (45.8)	10 (43.5)
College degree	4 (17.4)	3 (13.0)	2 (8.4)	3 (13.0)
Smoking status – no. (%)				
Never smoked	12 (52.3)	15 (65.2)	7 (29.2)	15 (65.2)
Former smoker	6 (26.1)	7 (30.4)	11 (45.8)	6 (26.1)
Current smoker	5 (21.7)	1 (4.4)	6 (25.0)	2 (8.7)
Race or ethnic group - no. (%)				
White, from Europe	23 (100)	22 (95.6)	22 (91.6)	23 (100)
Other	0 (0)	1 (4.4)	2 (8.4)	0 (0)
Body mass index[b]				
Mean – kg/m^2	43.1 ± 7.5	44.4 ± 8.9	42.4 ± 6.9	40.0 ± 7.1
30–34.9 - no. (%)	5 (21.7)	3 (13.0)	3 (12.5)	5 (21.8)
35–39.9 - no. (%)	4 (17.4)	6 (26.1)	6 (25)	7 (30.4)
≥40 - no. (%)	14 (60.9)	14 (60.9)	15 (62.5)	11 (47.8)
Blood pressure - mm Hg				
Systolic	131.5 ± 13.2	130.0 ± 10.0	132.1 ± 12.9	131.1 ± 11.0
Diastolic	85.4 ± 7.8	83.0 ± 7.4	87.4 ± 8.7	83.3 ± 12.9
Waist circumference - cm	132.7 ± 16.6	134.7 ± 17.1	133.0 ± 12.2	126.0 ± 17.3
Hematocrit - %	45.4 ± 3.0	45.5 ± 2.7	45.9 ± 2.8	45.0 ± 3.0
LH - mUI/ml	3.8 ± 1.6	3.6 ± 1.6	3.1 ± 1.5	3.1 ± 1.4
Total testosterone - ng/dl (ECLIA)	233 ± 53	240 ± 58	238 ± 48	252 ± 49
Total testosterone -ng/dl (HPLC-MS)	306 ± 67	305 ± 83	312 ± 125	327 ± 84
SHBG -nmol/l	24.9 ± 11.5	23.3 ± 7.9	23.2 ± 10.0	22.5 ± 9.0
Free testosterone - pg/ml (ECLIA)	55.2 ± 11.6	57.0 ± 11.5	54.9 ± 7.6	61.3 ± 7.9
Free testosterone -pg/ml (HPLC-MS)	74.5 ± 17.8	75.8 ± 20.1	75.6 ± 25.4	84.2 ± 22.1
Glycated hemoglobin - %	5.6 ± 0.3	5.4 ± 0.4	5.5 ± 0.3	5.4 ± 0.4
Glucose - mg/dl	93.1 ± 8.6	93.4 ± 11.0	95.6 ± 10.6	93.3 ± 10.6
Insulin - mUI/ml	29.1 ± 20.9	23.4 ± 16.7	24.7 ± 11.4	23.3 ± 31.4
HOMA-IR index - points	6.8 ± 5.0	5.6 ± 4.6	5.8 ± 2.7	5.9 ± 9.6
Cholesterol – mg/dl				
LDL	111.9 ± 24.2	111.3 ± 27.1	113.1 ± 29.1	111.9 ± 31.6
HDL	40.6 ± 5.0	43.8 ± 8.8	40.5 ± 12.0	42.3 ± 7.8
Triglycerides - mg/dl	161.6 ± 77.7	146.1 ± 77.2	187.1 ± 112.4	146.0 ± 64.2
PSA - ng/ml	0.7 ± 0.5	0.7 ± 0.4	0.7 ± 0.4	0.7 ± 0.4
		21.4 ± 2.9	21.0 ± 3.4	19.0 ± 5.1
IIEF-5 total score - points	21.2 ± 3.4			
Erectile dysfunction no. (%)	10 (43.5 %)	9 (40.9 %)	12 (50 %)	15 (65.2 %)
AMS total score - points	41.2 ± 12.7	37.4 ± 15.1	39.5 ± 14.5	38.9 ± 11.9
Pathological AMS score - no. (%)	19 (82.6 %)	17 (73.9 %)	18 (75.0 %)	19 (82.6 %)

[a] Plus–minus values are means ± SD. There were no significant differences between the groups at baseline. To convert total testosterone from ng/dl to nmol/l, multiply by 0.0346. To convert free testosterone from pg/ml to nmol/l, multiply by 3.46. To convert the values for glucose to millimoles per liter, multiply by 0.05551. To convert the values for cholesterol to millimoles per liter, multiply by 0.02586. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129. AMS denotes Aging Males' Symptoms, ECLIA electrochemiluminescence immunoassay, HDL high-density lipoprotein, HOMA-IR homeostasis model assessment of insulin resistance, HPLC-MS high-performance liquid chromatography-mass spectrometry, IIEF-5 simplified International Index of Erectile Function, LDL low-density lipoprotein, LH luteinizing hormone, PSA prostate-specific antigen, and SHBG sex hormone–binding globulin. Scores on the IIEF-5 range from 5 to 25, with a lower value denoting more severe symptoms. Scores on the AMS scale range from 17 to 85, with a higher value denoting more severe symptoms.

[b] Body mass index is the weight in kilograms divided by the square of the height in meters.

metformin plus testosterone group consisted of mild gastrointestinal symptoms. No significant rise in PSA by >1.0 ng/ml was found, independent of the assigned treatment. Polycythemia (hematocrit level ≥52 %) was more commonly found in the testosterone and the metformin plus testosterone group (week 26: 16.6 % testosterone, 21.7 % metformin-testosterone; week 52, 12.5 % testosterone, 15 % metformin-testosterone).

4. Discussion

Although the finding of low testosterone concentrations in men with obesity is a frequent occurrence, the therapeutic approach for this situation remains an unresolved issue. Current mainstay treatment for decreased testosterone levels in obesity is based on weight loss interventions, mainly through the implementation of lifestyle modifications such as energy-restricted diets and/or physical exercise [13]. Moreover, considering that bariatric surgery is associated with the restoration of testosterone concentrations in a large percentage of patients with extreme obesity, low testosterone concentrations have been advocated as a potential indication for bariatric procedures [27]. In addition, given the diminished secretion of testosterone in men with this condition, pharmacological treatment has focused on the administration of different formulations of testosterone, with the goal of increasing serum testosterone concentrations and reducing insulin resistance [4].

Here we report the results of the TESEO study, a 1-year, randomized, placebo-controlled, pathophysiology-based, independent clinical trial involving young men with obesity and low testosterone. In this study, we evaluated potential therapeutic strategies to lower insulin resistance in men with obesity, which could have a potential impact on testosterone levels and other outcomes. Our main results indicate that metformin, testosterone, or the combination of both, were effective in reducing insulin resistance, in comparison with placebo.

Our trial tested several pharmacological approaches for the treatment of low testosterone concentrations in men with obesity. The pathophysiological findings of this condition can be summarized as increased resistance and compensatory hyperinsulinemia (rationale for using an insulin-sensitizer such as metformin), resulting in impaired gonadotropin secretion and the subsequent decreased secretion of

Table 2
Effect of metformin, testosterone, or both on primary and secondary outcome variables.[a]

Outcome variable	Placebo (n−23)	Metformin (n–23)	Testosterone (n-24)	Combination (n−23)	Generalized estimating equations (time as a continuous variable) ***[Mixed linear models]***				
					Interaction group*time	Metformin vs placebo	Testosterone vs placebo	Combination vs placebo	Combination vs testosterone
Primary outcome									
HOMA-IR									
Baseline	6.8 ± 5.0	5.6 ± 4.6	5.8 ± 2.7	5.9 ± 9.6					
Change at 6 mo	0.2 ± 4.7	−1.1 ± 2.9	−0.1 ± 2.8	−3.0 ± 8.8					
Change at 1 yr	4.6 ± 13.3	−0.3 ± 4.0	−0.7 ± 3.3	−2.2 ± 7.8	<0.001 ***[0.013]***	−2.4 (−4.1 to −0.8) p = 0.004 ***[−2.4 (−4.5 to −0.3) p = 0.036]***	−2.7 (−4.3 to −1.1) p = 0.001 ***[−2.6 (−4.7 to −0.5) p = 0.015]***	−3.4 (−5.0 to −1.8) p < 0.001 ***[−3.4 (−5.5 to −1.3) p = 0.002]***	−0.7 (−2.3 to 0.9) p = 0.383 ***[−0.8 (−2.9 to 1.3) p = 0.465]***
Secondary outcomes									
Total testosterone - ng/dl									
Baseline	306 ± 67	305 ± 83	312 ± 125	327 ± 83					
Change at 6 mo	8 ± 91	18 ± 89	45 ± 111	75 ± 132					
Change at 1 yr	14 ± 62	15 ± 96	49 ± 124	108 ± 261	0.118 ***[0.054]***	−1.6 (−42.5 to 39.2) p = 0.937 ***[0.3 (−38.1 to 38.6) p = 0.990]***	13.1 (−27.3 to 53.5) p = 0.524 ***[17.2 (−20.8 to 55.1) p = 0.375]***	42.9 (2.1 to 83.7) p = 0.040 ***[46.9 (8.6 to 85.3) p = 0.016]***	−29.7 (−10.7 to 70.1) p = 0.149 ***[29.8 (−8.2 to 67.7) p = 0.124]***
Free testosterone - pg/ml									
Baseline	74.5 ± 17.8	75.8 ± 20.1	75.6 ± 25.4	84.2 ± 22.1					
Change at 6 mo	−0.6 ± 18.9	4.8 ± 22.4	16.0 ± 33.3	21.6 ± 41.9					
Change at 1 yr	0.6 ± 12.1	5.5 ± 28.6	15.6 ± 32.2	31.5 ± 82.2	0.095 ***[0.054]***	2.2 (−10.3 to 14.7) p = 0.727 ***[2.5 (−9.4 to 14.3) p = 0.683]***	6.7 (−5.7 to 19.1) p = 0.288 ***[7.5 (−4.2 to 19.2) p = 0.211]***	14.9 (2.7 to 27.4); p = 0.020 ***[15.5 (3.6 to 27.3) p = 0.011]***	8.2 (−4.2 to 20.5) p = 0.195 ***[8.0 (−3.8 to 19.7) p = 0.183***]
Body mass index[b] - kg/m^2									
Baseline	43.1 ± 7.5	44.4 ± 8.9	42.4 ± 6.9	40.0 ± 7.1					
Change at 6 mo	−0.7 ± 2.1	−2.0 ± 3.1	−1.4 ± 2.3	−1.6 ± 2.3					
Change at 1 yr	−0.6 ± 0.7	−1.9 ± 3.4	−1.3 ± 3.2	−1.5 ± 2.8	0.357 ***[0.367]***				
Fat-free mass - kg									
Baseline	79.6 ± 8.0	79.7 ± 9.5	79.6 ± 6.6	77.2 ± 9.1					
Change at 6 mo	−1.1 ± 2.3	−1.6 ± 2.7	−0.4 ± 1.9	−0.4 ± 2.1					
Change at 1 yr	−0.1 ± 2.0	−1.5 ± 3.1	−0.7 ± 2.4	−0.4 ± 2.5	0.068 ***[0.359]***				
Fat mass - kg									
Baseline	50.7 ± 16.9	53.0 ± 19.1	49.0 ± 13.6	44.5 ± 15.3					
Change at 6 mo	−0.5 ± 4.6	−3.7 ± 6.9	−2.5 ± 4.8	−3.9 ± 5.5					
Change at 1 yr	−0.6 ± 6.0	−3.4 ± 7.5	−2.2 ± 6.8	−3.6 ± 6.2	0.025	−1.7 (−3.0 to −0.4) p = 0.011[c]	−1.0 (−2.6 to 0.3) p = 0.124[c]	−1.8 (−3.1 to −0.5) p = 0.007[c]	−0.8 (−2.1 to 0.4) p = 0.206[c]
IIEF-5 total score - points									
Baseline	21.3 ± 3.4	21.4 ± 2.9	21.0 ± 3.7	19.0 ± 5.1					
Change at 6 mo	1.4 ± 3.2	0.7 ± 2.8	1.0 ± 3.1	2.2 ± 3.1					
Change at 1 yr	1.5 ± 3.3	1.0 ± 2.7	0.9 ± 3.1	2.3 ± 2.9	0.247 ***[0.298]***				

(*continued on next page*)

Table 2 (*continued*)

Outcome variable	Placebo (n−23)	Metformin (n–23)	Testosterone (n-24)	Combination (n−23)	Generalized estimating equations (time as a continuous variable) ***[Mixed linear models]***				
					Interaction group*time	Metformin vs placebo	Testosterone vs placebo	Combination vs placebo	Combination vs testosterone
AMS total score - points									
Baseline	41.2 ± 12.7	37.5 ± 15.1	39.5 ± 14.5	38.9 ± 11.9					
Change at 6 mo	−7.7 ± 8.1	−6.0 ± 11.7	−10.0 ± 9.2	−8.5 ± 9.8					
Change at 1 yr	−9.0 ± 9.6	−6.8 ± 11.6	−11.5 ± 12.6	−7.6 ± 9.8	0.192 ***[0.350]***				

To convert total testosterone from ng/dl to nmol/l, multiply by 0.0346. To convert free testosterone from pg/ml to nmol/l, multiply by 3.46.
HOMA-IR denotes homeostasis model assessment of insulin resistance, IIEF-5 simplified International Index of Erectile Function, AMS Aging Males' Symptoms. Scores on the IIEF-5 range from 5 to 25, with a lower value denoting more severe symptoms. Scores on the AMS scale range from 17 to 85, with a higher value denoting more severe symptoms.

[a] Plus–minus values are means ± SD. *P* values for the comparison among the groups of changes from baseline to 1 year were calculated with the use of generalized estimation equations, with adjustment for baseline values, and are reported when the overall P value was <0.05 for the interaction among the four groups over time.

[b] Body mass index is the weight in kilograms divided by the square of the height in meters.

[c] An exchangeable correlation matrix was used in the GEE models and the results were basically identical to those obtained with linear mixed models.

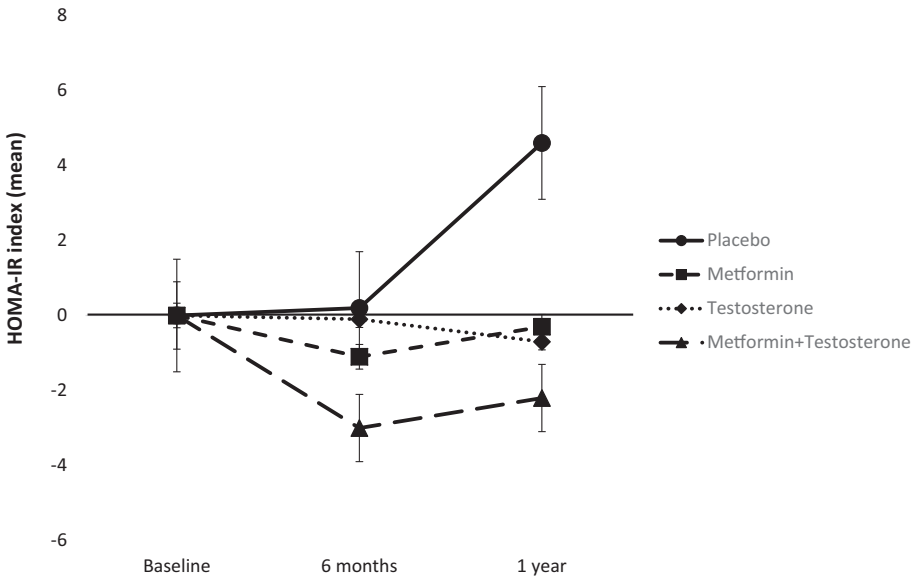

Fig. 2. Change in insulin resistance during the 1-year intervention
The mean (± SEM) change in the Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) index in the four study groups is shown from baseline to 1 year. Patients who received at least one dose of a study drug and had a post-baseline measurement were included in the analysis. HOMA-IR index was calculated as the level of fasting glucose (measured in millimoles per liter) multiplied by the level of fasting insulin (measured in microunits per milliliter) divided by 22.5. The change in the HOMA-IR index was the primary outcome of the trial.

testosterone from the testes (rationale for using testosterone therapy) [17].

Testosterone replacement therapy has been commonly used in men with obesity and low testosterone concentrations, even though much of the evidence available is derived from small, short-term, non-randomized, uncontrolled studies. Nevertheless, a handful of well-designed trials have reported that testosterone replacement therapy improves insulin sensitivity and other metabolic outcomes in obese men with low testosterone [28]. Indeed, in our trial, testosterone treatment significantly decreased insulin resistance compared to placebo. Moreover, in a recent large placebo-controlled trial that included patients with low-normal testosterone concentrations and impaired glucose tolerance or newly diagnosed type 2 diabetes, treatment with testosterone undecanoate for 2 years reduced the proportion of participants with type 2 diabetes. Compared with the placebo group, the testosterone group had greater decreases in fasting glucose, waist circumference, and improvements in body composition [29]. Although not reported in the article, it is feasible that testosterone also exerted beneficial effects on insulin resistance. Conversely, a study conducted in men with obesity and low testosterone, assigned to either testosterone or placebo (and background intervention with a calorie-restricted diet) found no significant between-group changes in insulin resistance [30]. The different baseline characteristics of the patients included in these trials may explain these divergent results.

Metformin, a biguanide widely-used for type 2 diabetes, could hypothetically constitute a plausible treatment for low testosterone in men with obesity, given its effects as an insulin sensitizer. Thus, in one non-randomized uncontrolled study conducted in subjects with low and normal testosterone concentrations, treatment with metformin (along with healthy dietary modifications and mild physical activity) resulted in decreased insulin resistance and increased serum testosterone levels [31]. Conversely, in another non-randomized uncontrolled study, testosterone levels were lower in metformin-treated men with obesity when compared with a control group [32]. In our randomized, double-blind, placebo-controlled trial, we found that metformin exerted beneficial effects on insulin resistance when compared to placebo.

Finally, a novel combination therapy with metformin plus testosterone was tested in this trial, with the aim of simultaneously targeting insulin resistance and decreased testosterone concentrations. Our results show that the concomitant use of metformin plus testosterone decreased insulin resistance, and it was the only therapy that significantly increased total and free testosterone levels.

Table 3
Effect of metformin, testosterone, or both on other outcomes.

Outcome variable	Placebo (n-23)	Metformin (n-23)	Testosterone (n-24)	Combination (n-23)	Generalized estimating equations (time as continuous variable) ***[Mixed linear models]***				
					Interaction group*time	Metformin vs placebo	Testosterone vs placebo	Combination vs placebo	Combination vs testosterone
Glycated hemoglobin - %									
Baseline	5.6 ± 0.3	5.4 ± 0.4	5.5 ± 0.3	5.4 ± 0.4					
Change at 6 mo	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.1					
Change at 1 yr	0.0 ± 0.3	0.1 ± 0.2	−0.1 ± 0.3	0.0 ± 0.2	0.092 ***[0.657]***	0.0 (−0.1 to 0.1) $p = 0.914$ [***0.0*** (***−0.1 to 0.1***) $\boldsymbol{p = 0.694}$]	0.1 (0.0 to 0.1) $p = 0.025$ [***0.0*** (***0.0 to 0.1***) $\boldsymbol{p = 0.407}$]	0.0 (0.0 to 0.1) $p = 0.394$ [***0.0*** (***−0.1 to 0.1***) $\boldsymbol{p = 0.896}$]	0.0 (−0.1 to 0.0) $p = 0.168$ [***0.0*** (***−0.1 to 0.0***) $\boldsymbol{p = 0.486}$]
SHBG -nmol/l									
Baseline	24.9 ± 11.5	23.3 ± 7.9	23.2 ± 10.0	22.5 ± 9.0					
Change at 6 mo	0.8 ± 4.7	−0.6 ± 6.5	0.0 ± 5.3	−0.1 ± 4.1					
Change at 1 yr	2.0 ± 5.1	0.2 ± 4.5	0.6 ± 4.9	0.5 ± 5.0	0.591 ***[0.591]***				
LDL - mg/dl									
Baseline	111.9 ± 24.2	111.3 ± 27.1	113.1 ± 29.1	111.9 ± 31.6					
Change at 6 month	−3.2 ± 19.7	−2.8 ± 25.6	−2.9 ± 19.7	−4.6 ± 16.7					
Change at 1 yr	−4.1 ± 15.4	−0.8 ± 24.6	3.7 ± 27.2	1.5 ± 21.0	0.388 ***[0.676]***				
HDL - mg/dl									
Baseline	40.6 ± 5.0	43.8 ± 8.8	40.5 ± 12.0	42.3 ± 7.8					
Change at 6 mo	0.4 ± 6.7	1.8 ± 8.5	2.1 ± 7.0	0.7 ± 5.7					
Change at 1 yr	−1.8 ± 5.3	2.0 ± 7.6	0.6 ± 8.6	0.4 ± 6.0	0.241 ***[0.284]***				
Triglycerides - mg/dl									
Baseline	161.6 ± 77.7	146.1 ± 77.2	187.1 ± 112.4	146.0 ± 64.2					
Change at 6 mo	−4.0 ± 69.4	15.4 ± 72.0	−26.2 ± 89.8	−14.2 ± 43.7					
Change at 1 yr	15.3 ± 77.1	−5.8 ± 41.6	−33.8 ± 82.9	−4.1 ± 61.3	0.088 ***[0.079]***	−14.3 (−35.4 to 6.7) $p = 0.182$ [***−11.8*** (***−32.3 to 8.8***) $\boldsymbol{p = 0.261}$]	−26.2 (−46.4 to −6.0) $p = 0.011$ [***−26.0*** (***−45.7 to −6.3***) $\boldsymbol{p = 0.010}$	−11.5 (−32.3 to 9.2) $p = 0.275$ [***−11.7*** (***−31.9 to 8.5***) $\boldsymbol{p = 0.257}$]	14.6 (−5.4 to 34.7) $p = 0.152$ [***14.3*** (***−5.2 to 33.8***) $\boldsymbol{p = 0.154}$]
Hematocrit - %									
Baseline	45.4 ± 3.0	45.5 ± 2.7	45.9 ± 2.8	45.0 ± 3.0					
Change at 6 mo	0.7 ± 1.8	−0.7 ± 2.5	1.9 ± 2.4	2.1 ± 2.3					
Change at 1 yr	0.0 ± 2.6	−0.6 ± 3.1	1.8 ± 3.1	2.2 ± 2.7	***0.017*** ***[0.003]***	−0.2 (−1.1 to 0.3) $p = 0.587$ [***−0.4*** (***−1.2 to 0.4***) $\boldsymbol{p = 0.302}$]	0.8 (−0.1 to 1.6) $p = 0.075$ [***0.9*** (***0.1 to 1.6***) $\boldsymbol{p = 0.026}$]	0.9 (0.1 to 1.8) $p = 0.035$ [***1.0*** (***0.3 to 1.8***) $\boldsymbol{p = 0.009}$]	0.2 (−0.7 to 1.0) $p = 0.694$ [***0.2*** (***−0.6 to 0.9***) $\boldsymbol{p = 0.633}$]
LH - mUI/ml									
Baseline	3.8 ± 1.6	3.6 ± 1.6	3.1 ± 1.5	3.1 ± 1.4					
Change at 6 mo	0.0 ± 1.4	−0.2 ± 1.2	−2.5 ± 1.6	−2.4 ± 1.6					
Change at 1 yr	−0.1 ± 1.2	−0.2 ± 1.4	−2.4 ± 1.8	−2.4 ± 1.8	<0.001 ***[<0.001]***	−0.1 (−0.6 to 0.4) $p = 0.819$ [***−0.1*** (***−0.5 to 0.4***) $\boldsymbol{p = 0.837}$]	−1.2 (−1.7 to −0.7) $p < 0.001$ [***−1.1*** (***−1.6 to −0.7***) $\boldsymbol{p < 0.001}$]	−1.2 (−1.7 to −0.7) $p < 0.001$ [***−1.2*** (***−1.7 to −0.7***) $\boldsymbol{p < 0.001}$]	−0.1 (−0.1 to 0.4); $p = 0.831$ [***−0.1*** (***−0.1 to 0.4***) $\boldsymbol{p = 0.814}$]
PSA - ng/ml									
Baseline	0.7 ± 0.5	0.7 ± 0.4	0.7 ± 0.4	0.7 ± 0.4					
Change at 6 mo	0.0 ± 0.2	0.0 ± 0.3	0.1 ± 0.5	0.1 ± 0.3					
Change at 1 yr	0.0 ± 0.3	0.1 ± 0.4	0.1 ± 0.3	0.1 ± 0.3	0.789 ***[0.753]***				

[a] Plus–minus values are means ±SD. *p*-Values for the comparison among the groups of changes from baseline to 1 year were calculated with the use of generalized estimation equations, with adjustment for baseline values, and are reported when the overall *p* value was <0.05 for the interaction among the four groups over time. To

convert the values for cholesterol to millimoles per liter, multiply by 0.02586. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129. SHBG denotes sex hormone–binding globulin, LDL low-density lipoprotein, HDL high-density lipoprotein, LH luteinizing hormone, and PSA prostate-specific antigen.

As secondary outcomes in our trial, we evaluated changes in erectile function, HRQoL, body composition, and several metabolic variables. Our trial did not detect significant differences in any of these outcomes compared to placebo (with the exception of decreased fat mass in those patients assigned to metformin or combination therapy), but it is important to highlight that the study was not powered for these outcomes and that the inclusion criteria did not require included patients to have baseline erectile dysfunction or impaired HRQoL.

Some of our results merit further consideration. First, in those patients assigned to testosterone alone or to the combination of testosterone plus metformin, serum total and free testosterone levels were only modestly elevated in the within-group comparisons, and were not increased in the between-group comparisons (except for combination therapy). In this regard, it has been previously reported that markedly obese patients (who, indeed, constitute the study population of our trial) probably need shorter testosterone dosing intervals to achieve ideal therapeutic ranges [33]. It is also important to highlight that baseline total testosterone concentrations obtained during the recruitment process (which were determined by immunoassay), were significantly lower than those obtained after study end by HPLC-MS (a more reliable and accurate method to measure testosterone in obesity). Therefore, with higher than expected total testosterone concentrations, the efficacy of the interventions tested may have been reduced. This underscores the importance of accurately diagnosing low testosterone levels in men with obesity, as an inaccurate diagnosis can lead to unnecessary treatment and potential undesirable side effects [34]. Second, although insulin resistance was reduced in those patients randomized to metformin or testosterone, compared to placebo, it did not exert a remarkable impact on the increase in testosterone concentrations. In this regard, it is important to note that within-group reductions in insulin resistance in both study groups were very modest and, therefore, could have precluded the improvement in testosterone concentrations.

A distinctive feature of our trial was the intentional exclusion of patients with type 2 diabetes, a condition that is also frequently associated with low testosterone [35]. Several trials have tested whether treatment with testosterone improves various outcomes in patients with type 2 diabetes and low testosterone, reporting no benefit for insulin resistance, glycemic control, constitutional symptoms, or sexual symptoms [36,37]. Therefore, by conducting a trial in patients with obesity and low testosterone, but without type 2 diabetes, we were able to evaluate this novel therapeutic approach with metformin, which might be challenging to conduct in patients with type 2 diabetes, as metformin is the background therapy in the majority of these patients. Another characteristic feature of the trial was the exclusion of subjects >50 years, to limit the well-known deleterious effect of age on testosterone levels [38].

The safety profile of each drug alone and of the combination of metformin and testosterone was consistent with the known side-effects of both drugs. Thus, patients under treatment with metformin (alone or in combination) presented more gastrointestinal symptoms and patients assigned to testosterone (alone or in combination) had a higher incidence of polycythemia.

Our study has certain limitations but also some important strengths. The strengths of our study include the randomized, double-blind, placebo-controlled design, the selection of patients (young patients without type 2 diabetes and no additional pituitary dysfunction), the use of erectile function and HRQoL scales, and the determination of serum testosterone using HPLC-MS. Also, the 1-year duration of the trial was adequate to assess the long-term efficacy of the tested therapies. An important, albeit intentional, limitation is that the results apply only to men aged 18–50 years without diabetes mellitus. In addition, despite we used a modern body composition analyzer, the utilization of a DEXA scan would have been preferable to track between-group changes in body composition, as it is considered a more accurate technique. Finally, another limitation of our trial is the small sample size, which was calculated for the primary outcome and, therefore, our secondary outcomes should be regarded as exploratory and hypothesis-generating.

5. Conclusions

Metformin, testosterone, or the combination of metformin plus testosterone over 1 year improved insulin resistance compared to placebo in young nondiabetic men with obesity and low testosterone, with no evidence of additive benefit.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metabol.2022.155290.

CRediT authorship contribution statement

Conception and design: JCFG and FJT. Acquisition of data: JCFG, MAP, LML, and MMV. Statistical analysis and interpretation of data: JCFG, RBR, FRD, MAM and JJJM. Obtaining funding: JCFG and FJT. Administrative, technical, or material support: AGG. Supervision: JCFG. Drafting of the manuscript: JCFG, RBR, BRM, JJJM, EMYS, SV, MAM, and FJT. Critical revision of the manuscript for important intellectual content: All authors.

Trial registration

ClinicalTrials.gov Identifier: NCT02514629.

Declaration of competing interest

None of the authors report a conflict of interest.

Acknowledgments

The authors wish to thank all the study participants for their collaboration and Maria Repice for English language editing of this article. Also, the authors thank Bayer Pharma AG for the donation of testosterone undecanoate and matching placebo. JCFG was supported by a research contract from the *Servicio Andaluz de Salud*, Spain (SAS; B-0003-2017) and by an intensification research program (INT21/00078, *Instituto de Salud Carlos III*, Spain; co-funded by the Fondo Europeo de Desarrollo Regional-FEDER); BRM was supported by the Miguel Servet Type I program (CP19/00098, *Instituto de Salud Carlos III*, Spain; co-funded by the *Fondo Europeo de Desarrollo Regional-FEDER*); MMV was supported by a *Rio Hortega* grant from the *Instituto de Salud Carlos III*, Spain (CM18/00120); EMYS was the recipient of the Nicolas Monardes Programme from the *Servicio Andaluz de Salud*, *Junta de Andalucia*, Spain (C1-0005-2019). This work was supported by a grant from *Servicio Andaluz de Salud* (PI-0173-2013). The funding organization played no role in the design and performance of the study, choice of enrolled subjects, review and interpretation of the data, or final approval of the manuscript. No other disclosures were reported. The graphical abstract was created with BioRender.com.

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