Send Orders for Reprints to reprints@benthamscience.net

Endocrine, Metabolic & Immune Disorders-Drug Targets, **XXXX,** ***XX*****, 1-7**

CLINICAL TRIAL STUDY

Overall Sexual Function in Dysmetabolic Obese Men with Low Testosterone Levels Treated with Clomiphene Citrate

Carla Pelusi[1], Nicola Bartolomeo[2], Giuseppe Lisco[2], Margherita Baccini[1], Flaminia Fanelli[1], Giovanni De Pergola[3,4], Vincenzo Triggiani[2], Uberto Pagotto[1] and Vito Angelo Giagulli[3,5,*]

[1]Division of Endocrinology and Diabetes Prevention and Care, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Department of Medical and Surgical Sciences (DIMEC), Alma Mater Studiorum University of Bologna, Bologna, Italy;; [2]Interdisciplinary Department of Medicine-Section of Internal Medicine, Geriatrics, Endocrinology, and Rare Diseases, University of Bari "Aldo Moro", School of Medicine, Policlinico, Bari, Italy; [3]National Institute of Gastroenterology "Saverio de Bellis," Research Hospital, Castellana Grotte, Bari, Italy; [4]Clinical Nutrition Unit, Medical Oncology, Department of Biomedical Science and Human Oncology, University of Bari, School of Medicine, Policlinico, Piazza Giulio Cesare 11, 70124 Bari, Italy; [5]Outpatients Clinic of Endocrinology and Metabolic Disease, Conversano Hospital, Conversano, Bari, Italy

ARTICLE HISTORY

Received: August 04, 2021
Revised: October 20, 2021
Accepted: January 4, 2022

DOI:
10.2174/1871530322666220304110958

Abstract: ***Background*****:** Sexual disorders are the most common clinical manifestations of hypogonadism. Functional hypogonadism is the most frequent form, and clomiphene citrate (CC) has been recently introduced as a possible off-label therapeutic option for these patients.

Objectives**:** This study aimed to evaluate the effects of CC on the overall sexual function in dysmetabolic obese men with low testosterone (T) levels.

Methods**:** This was a sub-study of a randomized, double-blind, cross-over, placebo-controlled trial that included twenty-four obese or overweight subjects with impaired glucose tolerance or type 2 diabetes and confirmed low total T (≤10.4 nmol/l) levels. Subjects were treated with CC or placebo (Plac) for 12 weeks, with an interval wash-out period of 6 weeks between treatments. All subjects were on metformin 2gr/day and a low-calorie diet. The between-treatment difference in the overall sexual function was assessed by IIEF-15 and a qADAM questionnaire.

Results**:** IIEF-15 and qADAM questionnaire data were available for 18 individuals. In unadjusted analyses, CC was associated with lower IIEF-15 total, erectile function, and intercourse satisfaction domain scores than Plac. After adjustments for multiple variables, CC was associated with a higher IIEF-15 sexual desire domain score (+0.9 ± 0.8; $p<.001$) despite a lower qADAM score (-2.1 ± 0.9; p=.008) with respect to Plac. No differences were found for the other domains between groups.

Discussion**:** The clinical significance of the absolute changes in IIEF-15 and qADAM scores during CC versus Plac is limited. However, CC has a reliable effect on sexual desire and is also as safe as Plac. According to the sample size, duration of follow-up, and inclusion criteria defined for the main study, further studies are therefore needed to assess the long-term efficacy of CC.

Conclusion**:** Compared to Plac, CC was found to be associated with a neutral effect on overall sexual function.

Keywords: Clomiphene citrate, international index of erectile function, IIEF-15, hypogonadism, metabolism, randomized controlled trial, sexual functions, Dysmetabolic Obese Men, testosterone.

1. INTRODUCTION

Reliable evidence has proved that testosterone (T) replacement therapy (TRT) enhances erectile function (EF) in hypogonadal men and, in particular, in those with the severe form (T < 8 nmol/L) [1]. Similar results have been obtained in trials involving dysmetabolic men, such as those with obesity and type 2 diabetes mellitus (T2DM), with low T levels, although to a lesser extent than in men with overt hypogonadism [2, 3]. TRT has been shown to improve both body composition and fasting glycaemic and insulin resistance in men with metabolic disturbances in observational and controlled studies [4] and limit the progression from prediabetes to overt T2DM [5, 6]. T levels are often lower in men with dysmetabolic disorders; however,

*Address correspondence to this author at the Outpatients Clinic of Endocrinology and Metabolic Disease, Conversano Hospital, via Edmondo de Amicis 36, 70014 Conversano, Bari, Italy; Tel: +39 (0) 80 409 13 68;
E-mail: vitogiagulli58@gmail.com;

1871-5303/22 $65.00+.00

this condition is usually reversible [7, 8], as demonstrated by T normalization after body-weight reduction or after the administration of drugs improving glycemic control [9, 10]. This form of hypogonadism, mainly present in adult/elderly men, has in the last few years been defined as "functional hypogonadism" [11, 12], and due to its characteristics, alternative treatment options to hormonal substitution have been suggested.

In recent years, selective estrogen receptor modulators (SERMs) have been assessed off-label as an option for managing function hypogonadism with promising results. They were shown to restore T and eugonadal levels while promoting or preserving spermatogenesis in men with T deficiency and ameliorating glucose metabolism [12]. In addition, SERMs are well-tolerated, usually with short-lasting and mild symptoms [13-16].

However, sexual disorders are the most prevalent clinical presentation of adult hypogonadism, including erectile dysfunction, a reduction in both libido and morning erections [17]. A few studies have been carried out on the effects of CC in men with erectile dysfunction with inconclusive results [16]. In the present sub-study, we, therefore, analyzed the overall sexual function in dysmetabolic obese men with low testosterone levels, comparing groups treated with CC *vs*. Plac using IIEF-15 and qADAM questionnaires.

2. MATERIALS AND METHODS

2.1. Study Design and Participants

As extensively reported in our previous works, we carried out a randomized, double-blind, cross-over, placebo-controlled trial at two Italian centers, the Endocrinology Unit of the S. Orsola-Malpighi Hospital (Bologna) and the Endocrinology and Metabolic Outpatient Clinic of Conversano Hospital (Bari). Twenty-four obese male subjects aged between 35 and 55 years old, newly diagnosed with impaired glucose tolerance (IGT) or type 2 diabetes and confirmed total testosterone (TT) ≤3 ng/ml (10.4 nmol/l) were recruited [13,18,19]. Subjects with organic hypogonadism and on previous therapy with glucose-lowering or lipid-lowering drugs were excluded. Given the strict criteria above, a limited number of screened subjects were found to be eligible. We, therefore, decided to also include three overweight subjects with a BMI of 28.8, 29.6, and 29.7 kg/m^2, respectively.

This study was approved by the Ethics Committees of the S. Orsola-Malpighi Hospital of Bologna (protocol number UOE/012011) and the Local Health District of ASL Bari (protocol number 778), registered with EudraCT (number 2011-000439-10) and in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants [13, 19].

2.2. Randomization and Masking

We randomly allocated eligible participants (1:1) to either clomiphene citrate plus metformin (CC/Met) group or a placebo plus metformin (Plac/Met) group according to a prespecified, computer-generated randomization list, using block sizes of six. A pharmacy-controlled randomization method was used to conceal allocation. Sequentially numbered containers with clomiphene citrate or placebo capsules of identical appearance, size, weight, and taste were issued to the investigator by the pharmacy and then to the eligible patients by the investigator. Metformin was supplied as 1000 mg tablets in the form of commercially available packs. Participants, pharmacists, and investigators were masked to the treatment allocation [13, 19].

2.3. Procedures

Metformin tablets were administered twice a day orally. Clomiphene citrate (25 mg; Serophene, Merck Serono, Rome, Italy) or placebo capsules were administered orally once a day. The study drugs were administered for 12 weeks; after a six-week wash-out period, subjects were assigned to the other drug group. All subjects were prescribed a hypocaloric diet (1600 kcal/day) and moderate physical activity (30 min/day brisk walk) [20]. Visits were performed at baseline, every six weeks, and study completion. The following examinations were performed: 1) clinical history and ongoing therapy; 2) physical examination; 3) questionnaires; 4) laboratory assessment, including metabolic parameters, such as hormones and proteins (as previously described) [13, 19].

The overall sexual function was assessed by a self-administered IIEF-15 and qADAM questionnaires before each treatment allocation and upon each treatment completion. The IIEF-15 comprises 15 items classified into five domains: 1) erectile function, 2) orgasmic function, 3) sexual desire, 4) intercourse satisfaction, and 5) overall satisfaction. A score of 0-5 was awarded to each of the 15 questions, with lower values representing a poorer sexual function. The maximum score for each domain was 30, 10, 10, 15, and 10, respectively. In the IIEF-15, erectile dysfunction is defined as severe for a score of 0-10, moderate for 11-16, mild or mild to moderate for 17-25, and absent for 26-30; the minimal clinically important difference is 4 [21]. There is also an abridged 5-item version of the IIEF-15, also known as IIEF-5 or the Sexual Health Inventory for Men (SHIM). It comprises four items of the erectile function domain and one from the intercourse satisfaction domain [22].

The qADAM questionnaire comprises ten items rated on a scale of 1-5, in which 5 represents the absence of a given symptom and one represents maximal symptoms. Sexual desire, energy, strength/endurance, enjoyment of life, happiness, erectile function, level of sleepiness, work performance, sports ability, and height loss are assessed. The overall score ranges from 10 to 50 [23].

2.4. Outcomes

The primary outcome of this sub-study was the between-treatment difference in the overall sexual function assessed by IIEF-15 and qADAM questionnaires among dysmetabolic men with low T levels during CC/Met versus Plac/Met. Both the total IIEF-15 score and the scores of each of the five domains were evaluated.

2.5. Statistical Analysis

The analyses reported here were performed following the per-protocol approach; participants were included if they

received the intended intervention in accordance with the protocol. Firstly, for continuous variables, Shapiro-Wilk's statistics were applied to test normality, and all variables showed a normal distribution. Secondly, we assessed the treatment effect on questionnaire scores using a mixed-effects model that analyses cross-over studies that included repeated measures [24]. The effect of each treatment was estimated by comparing pre-treatment and post-treatment values, while the efficacy of clomiphene citrate was assessed by calculating the between-treatment difference during CC/Met versus Plac/Met. The characteristics of subjects allocated to CC/Met followed by Plac/Met versus Plac/Met followed by CC/Met were compared to assess differences between time points; a carry-over effect was ruled out. Thirdly, a bivariate linear mixed model with interaction terms was used to evaluate whether questionnaire scores could have been influenced by clinical, hormonal, or metabolic parameters as well as by the AR-CAG polymorphism [25]. Age and parameters showing a p-value <0.25 in the bivariate analysis were included in a multivariable mixed model. Akaike's information criterion (AIC) and the significance level of the F-statistic were used in the stepwise procedure to estimate the final model. Multivariable analysis was used to adjust the between-treatment differences during CC/Met versus Plac/Met for these pre-treatment variables. Waist circumference was not included in the models because of multicollinearity with BMI. DHT was also not included as its increase on the CC/Met arm was due to increased serum TT and SHBG levels [13]. Data were expressed as mean and standard deviation (SD) or standard error (SE). All analyses were two-sided and carried out using Statistical Application Software (SAS) software (version 9.4, SAS Institute Inc., Cary, North Carolina, USA); significance was set at $p<0.05$.

3. RESULTS

Between December 2011 and February 2016, a total of 24 patients were recruited for the study. Two patients were excluded because of the use of some medications (*e.g*., steroids and human chorionic gonadotropin), while in one, the decision to withdraw was based on a personal choice. Three other patients did not fill out either the IIEF-15 or qADAM questionnaires at all four-time points.

As a result, data from the first two questionnaires were completed by 18 patients and were thus available for analysis. The characteristics of these participants are reported in Table **1**. Ten patients were diagnosed with type 2 diabetes, and eight with IGT. Sixteen subjects were obese. The mean TT and FT levels were 2.8 ± 0.4ng/ml (9.7 ± 1.4 nmol/l) and 68.1 ± 15 pg/ml (220 ± 50 pmol/l), respectively. The IIEF-15 total score was 45.8 ± 20.2, with the erectile function domain score ranging from 10 to 29 (from severe form to no erectile dysfunction) [26] and the qADAM score was 32.9 ± 5.3.

After assessing the effects of treatment on IIEF-15 scores, no changes in IIEF-15 or qADAM scores were found after CC/Met. The scores for IIEF-15, erectile function, and intercourse satisfaction increased during Plac/Met treatment (+8.7, +3.8, and +2.4, respectively). This was confirmed when the between-treatment difference was also evaluated (Table **2**). Similar results were found for those subjects allocated to CC/Met followed by Plac/Met versus Plac/Met followed by CC/Met (data not shown). No differences were found for the other outcomes. No carry-over effect was found for any score.

Table 1. Baseline characteristics of included subjects.

Characteristic	All Subjects (n=18)
Age (years)	47.6 ± 6.7
Clinical features	
Body mass index (kg/m^2)	35.0 ± 5.4
Waist circumference (cm)	115.3 ± 10.2
Hormones	
Total testosterone (ng/ml)	2.8 ± 0.4
Free testosterone (pg/ml)	68.1 ± 15
Total estradiol (pg/ml)	24.4 ± 7.3
Free estradiol (pg/ml)	0.3 ± 0.1
SHBG (nM/L)	21.5 ± 8.0
LH	3.8 ± 1.6
FSH	4.6 ± 2.1
Leptin (ng/ml)	17.7 ± 15.2
Metabolic Features	
Fasting plasma glucose (mg/dl)	113.1 ± 22.9
HbA1c (%)	6.0 ± 0.7
HOMA-IR	5.0 ± 3.1
Total cholesterol (mg/dl)	187.8 ± 36.4
HDL cholesterol (mg/dl)	40.9 ± 17.0
LDL cholesterol (mg/dl)	114.6 ± 38.2
Triglycerides (mg/dl)	183.6 ± 76.7
Genetic Features	
Androgen Receptor CAG repeats (n)	20.1 ± 3.4
Questionnaire Scores	
IIEF-15 total	45.8 ± 20.2
IIEF-15 erectile function domain	18.7 ± 9.7
IIEF-15 orgasmic function domain	7.1 ± 4.2
IIEF-15 sexual desire domain	6.2 ± 2.3
IIEF-15 intercourse satisfaction domain	7.8 ± 4.8
IIEF-15 overall satisfaction domain	6.1 ± 2.7
qADAM	32.9 ± 5.3

Note: Legend - Data are reported as mean ± SD.

We then evaluated whether other variables influenced questionnaires scores by calculating adjusted estimates for other parameters in a multivariable analysis (Table **3**, Supplementary Tables **S1** and **S2**). During CC/Met, a statistically significant reduction was found in IIEF-15

Table 2. Unadjusted IIEF-15 total and subscale and qadam scores before and after each treatment period in all participants.

-	CC/Met				Plac/Met				Between-Treatment Difference (CC/Met vs Plac/Met)	
-	Pre-Treatment	Post-Treatment	Change from Baseline	p	Pre-Treatment	Post-Treatment	Change from Baseline	p	Estimate	p
IIEF-15 Total	50.8±4.4	46.9±4.4	-3.9 ± 2.9	.188	42.5±4.4	51.2±4.4	8.7 ± 2.9	.005	-12.7 ± 4.2	.004
IIEF-15 Erectile function	21.2±2.1	18.7±2.1	-2.5 ±1.5	.097	17.0±2.1	20.8±2.1	3.8 ± 1.5	.014	-6.3 ± 2.1	.005
IIEF-15 Orgasmic function	7.6±0.8	7.1±0.8	-0.6 ± 0.7	.459	6.8±0.8	7.8±0.8	1.0 ± 0.7	.164	-1.6 ± 1.1	.134
IIEF-15 Sexual desire	6.6±0.5	6.5±0.5	-0.1 ± 0.4	.901	5.9±0.5	6.3±0.5	0.4 ± 0.4	.384	-0.4 ± 0.6	.481
IIEF-15 Intercourse satisfaction	8.7±1.0	8.5±1.0	-0.2 ± 0.8	.769	7.1±1.0	9.6±1.0	2.4 ± 0.8	.003	-2.7 ± 1.1	.017
IIEF-15 Overall satisfaction	6.7±0.6	6.1±0.6	-0.6 ± 0.7	.372	5.7±0.6	6.7±0.6	1.1 ± 0.7	.127	-1.7 ± 1.0	.090
qADAM	33.8 ± 1.2	32.8 ± 1.2	-1.0 ± 0.7	.146	32.7 ± 1.2	33.6 ± 1.2	0.9 ± 0.7	.214	-1.9 ± 1.0	.061

Note: Legend - Data are reported as mean ± SE.

Table 3. Adjusted between-treatment differences in iief-15 total and subscale and qadam scores in all participants.

-	Change from Baseline to end of Treatment During CC/Met		Change from Baseline to end of Treatment During Plac/Met		Between-Treatment Difference	
-	Estimate	p	Estimate	P	Estimate	p
IIEF-15 Total	-2.1 ± 3.4	.521	2.7± 2.0	.183	-4.9 ± 3.8	.171
IIEF-15 Erectile function	-2.8±1.0	.010	3.0± 1.0	.007	-5.8 ± 2.4	.457
IIEF-15 Orgasmic function	-1.0 ± 0.8	.234	1.0 ± 0.7	.154	-1.9 ± 1.0	.180
IIEF-15 Sexual desire	0.4 ± 0.7	.574	-0.5 ± 0.5	.363	0.9 ± 0.8	<.001
IIEF-15 Intercourse satisfaction	1.7± 0.6	.012	1.4± 0.3	.003	0.3 ± 0.6	.951
IIEF-15 Overall satisfaction	-0.5 ± 0.6	.351	1.1 ± 0.6	.069	-1.6 ± 0.8	.068
qADAM	-0.8 ± 0.7	.224	1.3 ± 0.7	.059	-2.1 ± 0.9	.008

Note: Legend - Data are reported as mean ± SE.

erectile function, and an increase was found in intercourse satisfaction (-2.8 and +1.7, respectively). The significant association between Plac/Met treatment and changes in erectile function and intercourse satisfaction were unchanged (+3.0 and +1.4, respectively), while results of the IIEF-15 total score were not confirmed. Concerning the between-treatment difference, compared to Plac/Met, CC/Met treatment was associated with a statistically significant increase in IIEF-15 sexual desire score despite a lower qADAM score. No other differences were found (Table **3**).

4. DISCUSSION

This sub-study aimed to assess the effect of CC on overall sexual function in dysmetabolic obese men with low testosterone levels. Eighteen subjects were included, either with type 2 diabetes or IGT. Subjects were treated with CC/Met or Plac/Met for 12 weeks in a randomized, double-blind, cross-over, placebo-controlled setting. Compared to placebo, the overall results of our study showed that CC was associated with an increase in the IIEF-15 sexual desire score, despite a lower qADAM score. No other differences were found. CC was administered at the dose of 25 mg since it could increase both T and estradiol levels by stimulating gonadotropins secretion [13, 27]. Due to these effects, CC is expected to exert positive actions on overall sexual function, as both T and E2 play a role in maintaining sex function [28]. CC could therefore reduce the risk of an inappropriate increase in estrogen levels with a consequent imbalance of T-to-E2 ratio, possibly responsible for an unnecessary increase in thromboembolic risk and gynecomastia, especially in our population in which several dysmetabolic conditions coexisted [29]. Aromatase inhibitors and SERMs could negatively affect the male sexual *function* as they affect the

balance between T and E2 [30, 31]. As an example, tamoxifen was found to decrease testicular expression of aromatase enzyme with a consequent decline in intra-testicular estradiol concentration, affecting both steroidogenesis and spermatogenesis [31, 32] and deteriorating erectile function [33].

Erectile function (EF) involves complex and multifactorial processes, and multiple chronic comorbidities may affect it, including metabolic, hormonal, vascular, neuronal, and psychological disturbances [12]. EF is the most common clinical presentation in subjects with hypogonadism [11, 12, 18, 34] and the disorder for which the most significant benefits are expected [18]. It can be assessed. Moreover, other sexual functions were evaluated using questionnaires, both during the first evaluation and during follow-up. These instruments support the physician in evaluating if the subject is affected by a disorder, stratifying its severity, and measuring changes during treatments [18]. The present study adopted two validated and widely used questionnaires (i.e., IIEF-15 and qADAM) that were completed before the beginning of each treatment and following 12 weeks of either CC/Met or Plac/Met**.** Response rates were generally adequate.

Included subjects were characterized by dysmetabolic conditions and low T levels, with no reference to signs or symptoms of hypogonadism. A wide range of scores was reported, and subjects with normal erectile function or severe erectile dysfunction were both included. Although a more homogeneous group of subjects (*e.g*., a mild-to-moderate form of erectile dysfunction) could have been enrolled, the abovementioned criteria were defined according to the primary outcome of the main study, in which changes in total testosterone following CC were assessed [13]. To the same end, the duration of this sub-study could be deemed as being sufficient for some questionnaire endpoints while inadequate for others. In hypogonadal men, TRT is associated with changes in IIEF-15 sexual desire, intercourse satisfaction, and overall satisfaction after only six weeks of treatment, while several months may be required before observing an improvement in erectile and ejaculatory function [3, 35, 36]. However, in our study, during CC/Met therapy, there was a significant improvement in serum T, FT, and estradiol (E2) levels as well as the T/E2 ratio. All steroids remained unchanged from baseline in the CC/Met group. Moreover, the results of sexual questionnaires obtained from CC/Met compared to the Plac/Met group were not different even if the participants were subdivided into subjects with the prediabetic condition or overt diabetes, considering different metabolic results obtained during CC/Met treatment compared to Plac/Met therapy (data not shown) [13].

Despite these limitations, few differences were found when CC/Met and Plac/Met groups were compared. Statistically significant results were found only for a higher IIEF-15 sexual desire score and a lower qADAM score. Given the absolute values (+0.9 and -2.1, respectively), the statistical significance of these results may not also be clinically relevant. Also, compared to IIEF-15, the qADAM questionnaire evaluates and pools a wider range of items, including ones that are insufficiently specific for our study purposes (e.g., height loss) [37].

From a clinical perspective, CC/Met should thus be regarded as at least as safe as Plac/Met. On the other hand, further studies, including subjects with hypogonadism and erectile dysfunction treated for at least 6-12 months, maybe needed for the adequate assessment of the other items included in the questionnaires.

A randomized controlled study evaluating the effects of CC on the qADAM questionnaire was published in 2018. Habous *et al.* allocated 282 hypogonadal subjects to CC 50 mg/day, human chorionic gonadotropin, or both for three months and found CC to be associated with an increased qADAM score [38]. Other studies, both randomized and observational, have assessed the effects of CC on the ADAM questionnaire, and an increase in the number of positive questions was found [39, 40]. One retrospective study found improvements in qADAM despite finding no changes in the IIEF-5 among 77 hypogonadal or infertile subjects treated with CC after a mean follow-up of 1 year [41]. However, the lack of a placebo arm in the Habous *et al.*, the use of a poor specific questionnaire in Kats *et al.* and Soares *et al.*, and the retrospective design of Chandrapal *et al.* make it very difficult to compare the findings and draw a definitive conclusion on the topic.

Our study has three main limitations. First, this was a sub-study of a randomized controlled trial aimed at assessing the effect of clomiphene on T levels. The sample size calculation was not based on this secondary outcome, resulting in a limited statistical power to assess differences. In fact, despite the non-significant carry-over effect, the difference before CC/Met and Plac/Met in the unadjusted analysis could be considered clinically significant [21]. Second, data on IIEF-15 and qADAM were not available for all the treated patients. Third, the use of two questionnaires may have been perceived as being unnecessary by patients, leading to a low response rate, whereas in fact, the aspects assessed by each questionnaire differ, as none of them are characterized by a greater accuracy in detecting low T levels [42].

CONCLUSION

CC has been assessed for the management of functional hypogonadism. Positive results have been found in terms of androgens levels and fertility, while limited evidence is currently available in terms of the effects on overall sexual function. Compared to Plac/Met and using two validated questionnaires, the present sub-study found that CC/Met was associated with no differences or marginally clinically significant ones in dysmetabolic subjects with low T levels. CC/Met should therefore be regarded to be at least as safe as Plac/Met. However, further randomized placebo-controlled studies, including a more significant number of subjects with hypogonadism and treated for a longer period, are needed to assess the efficacy of CC on overall sexual function.

AUTHORS' CONTRIBUTION

VAG and CP contributed to the conceptualization. The methodology utilized was selected by VAG, CP, GL, MB, FF, VT, UB, and GDP. An investigation was done by VAG, CP, MB, and GDP and formal analysis was performed by NB. Writing an original draft, reviewing, and editing were done by VAG and CP. VAG, CP, and GL supervised the study. VAG

and CP contributed to the project administration. VAG, CP, and UB participated in the funding acquisition.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethics Committees of the S. Orsola-Malpighi Hospital of Bologna (protocol number UOE/012011) and the Local Health District of ASL Bari (protocol number 778), registered with EudraCT (number 2011-000439-10).

HUMAN AND ANIMAL RIGHTS

No animal are used. All human procedures were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

Written informed consent was obtained from all participants.

STANDARD OF REPORTING

CONSORT guidelines were followed in this study.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

FUNDING

This study was funded by the Italian Research Project of Relevant Interest (Grant No. PRIN 2010-2011).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGMENTS

The authors are grateful to Prof. Renato Pasquali for his mentorship and useful advice in this field.

SUPPLEMENTARY MATERIALS

Supplementary material is available on the publisher's website along with the published article.

REFERENCES

[1] Corona, G.; Torres, L.O.; Maggi, M. Testosterone therapy: What we have learned from trials. *J. Sex. Med.*, **2020**, *17*(3), 447-460. http://dx.doi.org/10.1016/j.jsxm.2019.11.270 PMID: 31928918

[2] Giagulli, V.A.; Carbone, M.D.; Ramunni, M.I.; Licchelli, B.; De Pergola, G.; Sabbà, C.; Guastamacchia, E.; Triggiani, V. Adding liraglutide to lifestyle changes, metformin and testosterone therapy boosts erectile function in diabetic obese men with overt hypogonadism. *Andrology*, **2015**, *3*(6), 1094-1103. http://dx.doi.org/10.1111/andr.12099 PMID: 26447645

[3] Corona, G.; Rastrelli, G.; Morgentaler, A.; Sforza, A.; Mannucci, E.; Maggi, M. Meta-analysis of results of testosterone therapy on sexual function based on international index of erectile function scores. *Eur. Urol.*, **2017**, *72*(6), 1000-1011. http://dx.doi.org/10.1016/j.eururo.2017.03.032 PMID: 28434676

[4] Corona, G.; Giagulli, V.A.; Maseroli, E.; Vignozzi, L.; Aversa, A.; Zitzmann, M.; Saad, F.; Mannucci, E.; Maggi, M. Testosterone supplementation and body composition: results from a meta-analysis of observational studies. *J. Endocrinol. Invest.*, **2016**, *39*(9), 967-981. http://dx.doi.org/10.1007/s40618-016-0480-2 PMID: 27241317

[5] Wittert, G.; Bracken, K.; Robledo, K.P.; Grossmann, M.; Yeap, B.B.; Handelsman, D.J.; Stuckey, B.; Conway, A.; Inder, W.; McLachlan, R.; Allan, C.; Jesudason, D.; Fui, M.N.T.; Hague, W.; Jenkins, A.; Daniel, M.; Gebski, V.; Keech, A. Testosterone treatment to prevent or revert type 2 diabetes in men enrolled in a lifestyle programme (T4DM): a randomised, double-blind, placebo-controlled, 2-year, phase 3b trial. *Lancet Diabetes Endocrinol.*, **2021**, *9*(1), 32-45. http://dx.doi.org/10.1016/S2213-8587(20)30367-3 PMID: 33338415

[6] Yassin, A.; Haider, A.; Haider, K.S.; Caliber, M.; Doros, G.; Saad, F.; Garvey, W.T. Testosterone therapy in men with hypogonadism prevents progression from prediabetes to type 2 diabetes: Eight-year data from a registry study. *Diabetes Care*, **2019**, *42*(6), 1104-1111. http://dx.doi.org/10.2337/dc18-2388 PMID: 30862651

[7] Dhindsa, S.; Ghanim, H.; Batra, M.; Dandona, P. Hypogonadotropic hypogonadism in men with diabesity. *Diabetes Care*, **2018**, *41*(7), 1516-1525. http://dx.doi.org/10.2337/dc17-2510 PMID: 29934480

[8] Grossmann, M.; Matsumoto, A.M. A perspective on middle-aged and older men with functional hypogonadism: Focus on holistic management. *J. Clin. Endocrinol. Metab.*, **2017**, *102*(3), 1067-1075. http://dx.doi.org/10.1210/jc.2016-3580 PMID: 28359097

[9] Giagulli, V.A.; Castellana, M.; Carbone, M.D.; Pelusi, C.; Ramunni, M.I.; De Pergola, G.; Guastamacchia, E.; Triggiani, V. Weight loss more than glycemic control may improve testosterone in obese type 2 diabetes mellitus men with hypogonadism. *Andrology*, **2020**, *8*(3), 654-662. http://dx.doi.org/10.1111/andr.12754 PMID: 31919991

[10] Giagulli, V.A.; Castellana, M.; Murro, I.; Pelusi, C.; Guastamacchia, E.; Triggiani, V.; De Pergola, G. The role of diet and weight loss in improving secondary hypogonadism in men with obesity with or without type 2 diabetes mellitus. *Nutrients*, **2019**, *11*(12), 2975. http://dx.doi.org/10.3390/nu11122975 PMID: 31817436

[11] Bhasin, S.; Brito, J.P.; Cunningham, G.R.; Hayes, F.J.; Hodis, H.N.; Matsumoto, A.M.; Snyder, P.J.; Swerdloff, R.S.; Wu, F.C.; Yialamas, M.A. Testosterone therapy in men with hypogonadism: An endocrine society clinical practice guideline. *J. Clin. Endocrinol. Metab.*, **2018**, *103*(5), 1715-1744. http://dx.doi.org/10.1210/jc.2018-00229 PMID: 29562364

[12] Corona, G.; Goulis, D.G.; Huhtaniemi, I.; Zitzmann, M.; Toppari, J.; Forti, G.; Vanderschueren, D.; Wu, F.C.; Corona, G.; Goulis, D.G.; Forti, G.; Behre, H.M.; Punab, M.; Toppari, J.; Krausz, C.; Krausz, C.; Rajpert-De Meyts, E.; Tüttelmann, F.; Isidori, A.M.; Ruiz-Castane, E.; Jezek, D.; Kopa, Z.; Toppari, J.; Simoni, M.; Simoni, M. European academy of andrology (EAA) guidelines on investigation, treatment and monitoring of functional hypogonadism in males: Endorsing organization: European Society of Endocrinology. *Andrology*, **2020**, *8*(5), 970-987. http://dx.doi.org/10.1111/andr.12770 PMID: 32026626

[13] Pelusi, C.; Giagulli, V.A.; Baccini, M.; Fanelli, F.; Mezzullo, M.; Fazzini, A.; Bianchi, N.; Carbone, M.D.; De Pergola, G.; Mastroroberto, M.; Morselli Labate, A.M.; Pasquali, R. Clomiphene citrate effect in obese men with low serum testosterone treated with metformin due to dysmetabolic disorders: A randomized, double-blind, placebo-controlled study. *PLoS One*, **2017**, *12*(9), e0183369. http://dx.doi.org/10.1371/journal.pone.0183369 PMID: 28886024

[14] Krzastek, S.C.; Smith, R.P. Non-testosterone management of male hypogonadism: an examination of the existing literature. *Transl. Androl. Urol.*, **2020**, *9*(S2)(Suppl. 2), S160-S170. http://dx.doi.org/10.21037/tau.2019.11.16 PMID: 32257856

[15] Rastrelli, G.; Maggi, M.; Corona, G. Pharmacological management of late-onset hypogonadism. *Expert Rev. Clin. Pharmacol.*, **2018**, *11*(4), 439-458. http://dx.doi.org/10.1080/17512433.2018.1445969

PMID: 29505313

[16] Giagulli, V.A.; Silvestrini, A.; Bruno, C.; Triggiani, V.; Mordente, A.; Mancini, A. Is there room for SERMs or SARMs as alternative therapies for adult male hypogonadism? *Int. J. Endocrinol.*, **2020**, *2020*, 9649838. http://dx.doi.org/10.1155/2020/9649838 PMID: 32411230

[17] Wu, F.C.; Tajar, A.; Beynon, J.M.; Pye, S.R.; Silman, A.J.; Finn, J.D.; O'Neill, T.W.; Bartfai, G.; Casanueva, F.F.; Forti, G.; Giwercman, A.; Han, T.S.; Kula, K.; Lean, M.E.; Pendleton, N.; Punab, M.; Boonen, S.; Vanderschueren, D.; Labrie, F.; Huhtaniemi, I.T. Identification of late-onset hypogonadism in middle-aged and elderly men. *N. Engl. J. Med.*, **2010**, *363*(2), 123-135. http://dx.doi.org/10.1056/NEJMoa0911101 PMID: 20554979

[18] Giagulli, V.A.; Castellana, M.; Lisco, G.; Triggiani, V. Critical evaluation of different available guidelines for late-onset hypogonadism. *Andrology*, **2020**, *8*(6), 1628-1641. http://dx.doi.org/10.1111/andr.12850 PMID: 32593233

[19] Pelusi, C.; Fanelli, F.; Baccini, M.; Triggiani, V.; Bartolomeo, N.; Carbone, M.D.; De Pergola, G.; Di Dalmazi, G.; Pagotto, U.; Pasquali, R.; Giagulli, V.A. Effect of clomiphene citrate treatment on the Sertoli cells of dysmetabolic obese men with low testosterone levels. *Clin. Endocrinol. (Oxf.)*, **2020**, *92*(1), 38-45. http://dx.doi.org/10.1111/cen.14122 PMID: 31677181

[20] American Diabetes Association. 8. Obesity management for the treatment of type 2 diabetes: Standards of medical care in diabetes-2020. *Diabetes Care*, **2020**, *43*(Suppl. 1), S89-S97. http://dx.doi.org/10.2337/dc20-S008 PMID: 31862751

[21] Rosen, R.C.; Allen, K.R.; Ni, X.; Araujo, A.B. Minimal clinically important differences in the erectile function domain of the International Index of Erectile Function scale. *Eur. Urol.*, **2011**, *60*(5), 1010-1016. http://dx.doi.org/10.1016/j.eururo.2011.07.053 PMID: 21855209

[22] Rosen, R.C.; Riley, A.; Wagner, G.; Osterloh, I.H.; Kirkpatrick, J.; Mishra, A. The international index of erectile function (IIEF): a multidimensional scale for assessment of erectile dysfunction. *Urology*, **1997**, *49*(6), 822-830. http://dx.doi.org/10.1016/S0090-4295(97)00238-0 PMID: 9187685

[23] Mohamed, O.; Freundlich, R.E.; Dakik, H.K.; Grober, E.D.; Najari, B.; Lipshultz, L.I.; Khera, M. The quantitative ADAM questionnaire: a new tool in quantifying the severity of hypogonadism. *Int. J. Impot. Res.*, **2010**, *22*(1), 20-24. http://dx.doi.org/10.1038/ijir.2009.35 PMID: 19657348

[24] Freeman, P.R.; Freeman, P.R. The performance of the two-stage analysis of two-treatment, two-period crossover trials. *Stat. Med.*, **1989**, *8*(12), 1421-1432. http://dx.doi.org/10.1002/sim.4780081202 PMID: 2616932

[25] Thiébaut, R.; Jacqmin-Gadda, H.; Chêne, G.; Leport, C.; Commenges, D. Bivariate linear mixed models using SAS proc MIXED. *Comput. Methods Programs Biomed.*, **2002**, *69*(3), 249-256. http://dx.doi.org/10.1016/S0169-2607(02)00017-2 PMID: 12204452

[26] Bray, G.A.; Heisel, W.E.; Afshin, A.; Jensen, M.D.; Dietz, W.H.; Long, M.; Kushner, R.F.; Daniels, S.R.; Wadden, T.A.; Tsai, A.G.; Hu, F.B.; Jakicic, J.M.; Ryan, D.H.; Wolfe, B.M.; Inge, T.H. The science of obesity management: An endocrine society scientific statement. *Endocr. Rev.*, **2018**, *39*(2), 79-132. http://dx.doi.org/10.1210/er.2017-00253 PMID: 29518206

[27] Pelusi, C.; Fanelli, F.; Baccini, M.; De Pergola, G.; Triggiani, V.; Mezzullo, M.; Fazzini, A.; Di Dalmazi, G.; Petrovic, B.; Paterini, P.; Labate, A.M.M.; Pagotto, U.; Giagulli, V.A. Impact of clomiphene citrate on the steroid profile in dysmetabolic men with low testosterone levels. *Horm. Metab. Res.*, **2021**, *53*(8), 520-528. http://dx.doi.org/10.1055/a-1542-8763 PMID: 34384109

[28] Schulster, M.; Bernie, A.M.; Ramasamy, R. The role of estradiol in male reproductive function. *Asian J. Androl.*, **2016**, *18*(3), 435-440. http://dx.doi.org/10.4103/1008-682X.173932 PMID: 26908066

[29] Wheeler, K.M.; Sharma, D.; Kavoussi, P.K.; Smith, R.P.; Costabile, R. Clomiphene citrate for the treatment of hypogonadism. *Sex. Med. Rev.*, **2019**, *7*(2), 272-276. http://dx.doi.org/10.1016/j.sxmr.2018.10.001 PMID: 30522888

[30] Finkelstein, J.S.; Yu, E.W.; Burnett-Bowie, S.A. Gonadal steroids and body composition, strength, and sexual function in men. *N. Engl. J. Med.*, **2013**, *369*(25), 2457. http://dx.doi.org/10.1056/NEJMoa1206168 PMID: 24350954

[31] Verma, R.; Krishna, A. Effect of tamoxifen on spermatogenesis and testicular steroidogenesis. *Biochem. Biophys. Res. Commun.*, **2017**, *486*(1), 36-42. http://dx.doi.org/10.1016/j.bbrc.2017.02.092 PMID: 28228354

[32] Motofei, I.G.; Rowland, D.L.; Popa, F.; Bratucu, E.; Straja, D.; Manea, M.; Georgescu, S.R.; Paunica, S.; Bratucu, M.; Balalau, C.; Constantin, V.D. A pilot study on tamoxifen sexual side effects and hand preference in male breast cancer. *Arch. Sex. Behav.*, **2015**, *44*(6), 1589-1594. http://dx.doi.org/10.1007/s10508-015-0530-4 PMID: 26108899

[33] Awouters, M.; Vanderschueren, D.; Antonio, L. Aromatase inhibitors and selective estrogen receptor modulators: Unconventional therapies for functional hypogonadism? *Andrology*, **2020**, *8*(6), 1590-1597. http://dx.doi.org/10.1111/andr.12725 PMID: 31696669

[34] Corona, G.; Rastrelli, G.; Morelli, A.; Sarchielli, E.; Cipriani, S.; Vignozzi, L.; Maggi, M. Treatment of functional hypogonadism besides pharmacological substitution. *World J. Mens Health*, **2020**, *38*(3), 256-270. http://dx.doi.org/10.5534/wjmh.190061 PMID: 31496147

[35] Saad, F.; Aversa, A.; Isidori, A.M.; Zafalon, L.; Zitzmann, M.; Gooren, L. Onset of effects of testosterone treatment and time span until maximum effects are achieved. *Eur. J. Endocrinol.*, **2011**, *165*(5), 675-685. http://dx.doi.org/10.1530/EJE-11-0221 PMID: 21753068

[36] Lunenfeld, B.; Mskhalaya, G.; Zitzmann, M.; Arver, S.; Kalinchenko, S.; Tishova, Y.; Morgentaler, A. Recommendations on the diagnosis, treatment and monitoring of hypogonadism in men. *Aging Male*, **2015**, *18*(1), 5-15. http://dx.doi.org/10.3109/13685538.2015.1004049 PMID: 25657080

[37] Hackett, G. The use of questionnaires to assess sexual function. *Trends Urol. Men's Health*, **2017**, *8*(1), 17-20. http://dx.doi.org/10.1002/tre.560

[38] Habous, M.; Giona, S.; Tealab, A.; Aziz, M.; Williamson, B.; Nassar, M.; Abdelrahman, Z.; Remeah, A.; Abdelkader, M.; Binsaleh, S.; Muir, G. Clomiphene citrate and human chorionic gonadotropin are both effective in restoring testosterone in hypogonadism: a short-course randomized study. *BJU Int.*, **2018**, *122*(5), 889-897. http://dx.doi.org/10.1111/bju.14401 PMID: 29772111

[39] Katz, D.J.; Nabulsi, O.; Tal, R.; Mulhall, J.P. Outcomes of clomiphene citrate treatment in young hypogonadal men. *BJU Int.*, **2012**, *110*(4), 573-578. http://dx.doi.org/10.1111/j.1464-410X.2011.10702.x PMID: 22044663

[40] Soares, A.H.; Horie, N.C.; Chiang, L.A.P.; Caramelli, B.; Matheus, M.G.; Campos, A.H.; Marti, L.C.; Rocha, F.A.; Mancini, M.C.; Costa, E.M.F.; Cercato, C. Effects of clomiphene citrate on male obesity-associated hypogonadism: a randomized, double-blind, placebo-controlled study. *Int. J. Obes.*, **2018**, *42*(5), 953-963. http://dx.doi.org/10.1038/s41366-018-0105-2 PMID: 29777228

[41] Chandrapal, J.C.; Nielson, S.; Patel, D.P.; Zhang, C.; Presson, A.P.; Brant, W.O.; Myers, J.B.; Hotaling, J.M. Characterising the safety of clomiphene citrate in male patients through prostate-specific antigen, haematocrit, and testosterone levels. *BJU Int.*, **2016**, *118*(6), 994-1000. http://dx.doi.org/10.1111/bju.13546 PMID: 27226135

[42] Corona, G.; Rastrelli, G.; Vignozzi, L.; Mannucci, E.; Maggi, M. How to recognize late-onset hypogonadism in men with sexual dysfunction. *Asian J. Androl.*, **2012**, *14*(2), 251-259. http://dx.doi.org/10.1038/aja.2011.138 PMID: 22286862