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ORIGINAL ARTICLE

Very low-calorie ketogenic diet rapidly augments testosterone levels in non-diabetic obese subjects

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Abstract

Background: The very low-calorie ketogenic diet (VLCKD) represents an opportunity to attain clinically relevant weight loss in obese patients. Functional hypogonadism represents a frequent hormonal disorder associated with obesity and visceral fat accumulation characterised by low testosterone levels and subnormal luteinising hormone (LH) levels.

Aim: To evaluate the early effects of VLCKD on serum total testosterone (TT) levels in non-diabetic obese patients.

Methods: Twenty-two obese male patients (mean age 39.3 ± 11.7 years, mean body mass index (BMI) 38.2 ± 6.4 kg/m^2) were enrolled and treated for 28 days with VLCKD. Anthropometric and hormonal variables were assessed before, during and after diet intervention.

Results: After 7 and 28 days on a VLCKD, a significant and persistent reduction in body weight, BMI, fat mass, blood glucose, insulin and homeostasis model assessment index was observed compared with baseline. TT significantly increased after 7 days (+35 ± 64 ng/dl) and 28 days (+74 ± 97 ng/dl) on a VLCKD. In addition to TT, a significant increase in serum sex hormone-binding globulin levels was observed after 7 (+2.1 ± 4.1) and 28 days (+7.7 ± 10.0). However, both calculated free testosterone and LH did not change after 7 or 28 days of VLCKD. Following cessation of VLCKD, hypogonadal subjects achieved a higher percentage of total weight loss (8.5% ± 1.5%), a greater reduction in weight (−9.94 ± 1.66 kg), fat mass (−7 ± 2.1 kg) and waist circumference (−6.31 ± 2.65 cm) and a greater improvement in glycaemia (−8.75 ± 10.92 mg/dl) as compared with eugonadal subjects. Furthermore, hypogonadal subjects exhibited a trend of higher TT increase (+98.12 ± 71.51 ng/dl) as compared with eugonadal subjects.

Conclusions: VLCKD results in rapid improvements in TT levels associated with weight loss in male obese non-diabetic subjects, particularly in the presence of obesity-related hypogonadism.

KEYWORDS
hypogonadism, obesity, VLCKD

1 | INTRODUCTION

Obesity and overweight are chronic conditions with a worldwide prevalence that has increased in recent decades. Excessive consumption of high-energy food and a sedentary lifestyle favours the development of adiposity and related metabolic disorders. Obesity increases the incidence of long-term metabolic consequences, such as type 2 diabetes mellitus (T2DM), cardiovascular diseases, dyslipidaemia and hypertension.[1] Recent findings have demonstrated that obesity and visceral fat accumulation are frequently associated with endocrine disorders, such as secondary (functional) hypogonadism in both adults and adolescents.[2] Considering that hyperglycaemia, oxidative stress and inflammation associated with obesity interfere with testosterone production, the new term 'metabolic hypogonadism' has been coined.[3] The increased inactivation of androgens by aromatase into enlarged adipose tissue, the reduction of circulating levels of sex hormone-binding globulin (SHBG) and pro-inflammatory cytokine-induced inhibition of the hypothalamic-pituitary-gonadal (HPG) axis represent key mechanisms involved in the onset of functional hypogonadism.[4] Furthermore, the elevated serum leptin levels detected in obese men appear to contribute to HPG dysfunction, inhibiting gonadotropin releasing hormone (GnRH) pulsatility.[5] Furthermore, hypogonadism is associated with fat accumulation, leading to a vicious cycle in which abnormal adipose tissue expansion impairs testosterone production, resulting in further accumulation of adipose tissue.[6]

Several clinical efforts have been made to counteract obesity with the aim of achieving weight loss and improving body composition. Lifestyle interventions, such as a hypocaloric diet and physical exercise, and metabolic surgery currently represent the preferred treatments for obesity in clinical practice. Metabolic surgery produces relevant weight loss and a related improvement of gonadal function.[7] Similarly, ketogenic diet (KD) protocols have been developed in recent years to attain rapid weight loss, potentially increasing patients' compliance.[8,9] A KD can be classified as either a normocaloric KD, where a high-fat, isoproteic diet (1.2–1.5 g/kg) is provided, or a very low-calorie KD (VLCKD), where the KD is combined with intense caloric restriction[10,11] with a daily carbohydrate intake of less than 30 g and an overall total energy intake of 600–1000 kcal/day.[9] Several trials have suggested the potential effects of KD on testosterone serum levels.[12–19] However, results on early response to VLCKD on HPG function and the mechanism involved have not been fully addressed.

The aim of the current study is to evaluate the early effects of VLCKD on the HPG axis in obese men, focusing on the first 4 weeks of treatment.

2 | METHODS

2.1 | Study design

An observational prospective clinical trial was conducted from May 2019 to November 2021 at the Outpatient Clinic for the Study of Obesity, Unit of Endocrinology, Department of Emergency and Organ Transplantation, University of Bari Aldo Moro. The trial protocol was approved by the Ethics Committee of the Azienda Ospedaliero Universitaria Policlinico di Bari, Bari, Italy and meets the standards of the 7th revision of the Declaration of Helsinki (2013). Each enrolled subject provided written informed consent.

Obese male subjects older than 18 years who were evaluated for weight loss were enrolled. Each patient underwent comprehensive medical history collection, physical examination and laboratory testing. The patients were informed of all therapeutic options for obesity management, and all patients willing to undergo VLCKD were recruited in this study. The exclusion criteria included autoimmune diabetes mellitus, T2DM, chronic renal failure with estimated glomerular filtration rate <60 ml/min/1.73 m^2, active or severe infections, a recent major cardiovascular event, unstable angina, cardiac arrhythmias, frailty, surgery or invasive procedures in the past 48 h, eating disorders and other psychiatric disturbances. Patients with hypogonadism due pituitary or testicular diseases were also excluded.

This manuscript follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.[20]

2.2 | Diet protocol

The VLCKD was based on a very low-calorie diet (600–800 kcal/day) characterised by low content of both carbohydrates (<50 g daily from vegetables) and lipids (~20 g/day). High biological value protein was provided, ranging between 1.2 and 1.5 g per each kg of ideal body weight to preserve lean mass and to meet the minimum daily body requirements. The VLCKD was based on protein preparations of high biological value derived from green peas, eggs, soy and whey. Each protein preparation was provided by ISOMED and was composed of approximately 18 g protein, 4 g carbohydrate and 3 g fat (mainly high-oleic vegetable oils), providing approximately 100–150 kcal.

During the study, patients were allowed to eat four-to-six (depending on ideal body weight) protein preparations and low-carbohydrate vegetables during the 28-day diet.

2.3 | Anthropometric parameters and hormonal measurements

Anthropometric and biochemical parameters were evaluated at enrolment, and on days 7 and 28 from an initiation of the VLCKD. Beta-hydroxybutyrate levels were assessed weekly through a reflex metrics detection system and were maintained between 0.5 and 0.7 mmol/L.

Body weight, free fat mass and fat mass were measured under fasting conditions using a multi-frequency bioelectrical impedance device (InBody 270). Patients underwent the measurements without shoes and with an empty bladder. Each patient underwent blood testing for glucose, insulin, total cholesterol, high-density lipoprotein, triglycerides, creatinine, uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), luteinising hormone (LH), follicle-stimulating hormone (FSH), total testosterone (TT) and SHBG at each visit. An oral glucose tolerance test (OGTT) was performed at baseline.

The homeostasis model assessment (HOMA) index,[21] Matsuda index[22] and disposition index were calculated for the evaluation of insulin resistance, and low-density lipoprotein levels were calculated according to the Friedewald formula.[23] Insulin resistance was defined according to a HOMA index >2.[24] Free testosterone (cFT) levels also were calculated from TT, albumin and SHBG using the Vermeulen equation.[25]

Body mass index (BMI) was defined as weight (kg)/height (m^2) and ideal body weight as that equivalent to the Lorentz formula height-100-[(height-150)/4].[26] The percentage of excess weight loss (%EWL) was calculated using the formula: ([initial weight − follow-up weight]/[initial weight − ideal weight]) × 100. The percentage of total weight loss (%TWL) was calculated using the formula: ([initial weight − follow-up weight]/initial weight) × 100.[27]

During VLCKD, all patients underwent collection of blood samples to assess their electrolytes (e.g. sodium, potassium and chlorine) and beta-hydroxybutyrate levels. Blood samples were collected in the morning (8–10 AM) in a fasting state. Fasting blood samples at baseline and after 24 weeks were used for the measurement of glucose, lipid profile, AST, ALT, CRP and uric acid using an autoanalyser (Dimension Vista 1500 Lab System, Siemens). Serum insulin was measured using a human insulin radioimmunoassay (Linco). HbA1c was measured using an automated HPLC system (D-10 Hemoglobin Analyzer). Complete blood count was measured using an automated cell counter (ADVIA 2120, Siemens). Serum concentrations of TT, FSH and LH were measured by chemiluminescence immunoassay (DiaSorin). SHBG was analysed in the serum by radioimmunoassay (Izotop). Serum levels of TNF-a were measured with an ELISA kit from Thermo Fisher Scientific (TNF-a Human Elisa kit cat. KHC3011).

2.4 | Questionnaires

Signs and symptoms of hypogonadism were evaluated by an administration of validated questionnaires: Androtest (Heinemann et al. 1999), AMS (Corona et al. 2006) and IIEF-5 (Rosen et al. 1999). The results were obtained before and after 28 days of VLCKD.

2.5 | Statistical analysis

Continuous variables are presented as mean ± standard deviation (SD). Differences in continuous variables were assessed using Student's *t*-test or the Mann–Whitney *U*-test, according to normal distribution. All tests of significance were two-sided.

Normal data distribution was assessed using the Kolmogorov–Smirnov test. The primary outcome variables were TT, SHBG and cFT. Each variable was considered per se and as a difference compared to baseline levels (Δ). One-way repeated analysis of variance (ANOVA) was applied to determine the significant differences in selected variables among different time points. Post hoc Bonferroni adjustment analysis was applied to compare the statistical significance for each variable. Paired *t*-tests compared these variables at baseline and after at least 7 and 28 days of VLCKD therapy. A p value of <0.05 was accepted as statistically significant. A trend was assumed for p values ranging from 0.05 to 0.099.

Statistical analysis was performed using RStudio Workbench 2022.02.2.

3 | RESULTS

3.1 | Baseline

Twenty-three patients were initially considered for inclusion; however, one patient was excluded for poor compliance to the VLCKD protocol. In total, 22 patients (mean age 39.3 ± 11.7 years) were enrolled in this study. At enrolment, the subjects had a mean BMI of 38.2 ± 6.4 kg/m^2, with both fasting and post-OGTT blood glucose within the normal range (Table 1). In contrast, the entire cohort exhibited elevated levels of basal insulin and high HOMA index (Table 1), suggesting insulin resistance.

Regarding the HPG axis, low serum TT levels associated with low LH levels were present at baseline in 68.2% of patients, indicating the presence of functional hypogonadal hypogonadism. Both the average TT and cFT detected at baseline confirmed the high rate of hypogonadism related to obesity at baseline (Table 1).

3.2 | Post-VLCKD

After 7 days on VLCKD, a significant reduction in body weight, BMI and fat mass was detected in addition to an increased %EWL ($p < 0.001$) (Table 2). The change in body weight, BMI, fat mass and EWL remained statistically significant after 28 days on VLCKD ($p < 0.001$) (Table 2). These results confirmed the efficacy of VLKCD on body weight reduction, highlighting how this was rapidly achieved already after 7 days of diet. In addition, after 7 and 28 days on VLCKD, a significant decrease in blood glucose, insulin and HOMA index was evident compared with baseline ($p \leq 0.05$) (Table 2). The analysis of circulating levels of TNF-α showed a significant reduction after 28 days but not after 7 days on VLCKD (Table 2).

TABLE 1 Characteristics of the study population

	Whole population	Eugonadism	Hypogonadism	
	n = 22	*n* = 6	*n* = 16	*p*-Value
Age (years)	39.3 (11.7)	37.0 (10.0)	40.2 (12.4)	0.580
Height (cm)	175.3 (6.4)	175.6 (2.9)	175.2 (7.3)	0.916
Body weight (kg)	117.5 (22.3)	112.5 (15.3)	119.4 (24.6)	0.530
BMI (kg/m^2)	38.2 (6.4)	36.5 (4.8)	38.8 (7.0)	0.463
Waist circumference (cm)	113.7 (14.2)	109.3 (12.6)	115.4 (14.8)	0.388
Hip circumference (cm)	120.5 (14.3)	117.2 (11.8)	121.7 (15.3)	0.524
Neck circumference (cm)	43.7 (4.0)	42.2 (2.0)	44.2 (4.4)	0.286
SBP (mm Hg)	136.3 (11.9)	138.7 (11.4)	135.4 (12.3)	0.577
DBP (mmHg)	87.0 (10.4)	83.0 (11.8)	88.5 (9.7)	0.277
HR (bpm)	79.3 (12.9)	79.5 (15.7)	79.2 (12.3)	0.969
FFM (kg)	57.1 (19.3)	62.1 (11.8)	55.2 (21.5)	0.474
FM (kg)	47.0 (15.4)	45.6 (14.3)	47.6 (16.2)	0.790
TBW (kg)	51.7 (7.4)	49.0 (2.9)	52.7 (8.4)	0.304
Fasting blood glucose (mg/dl)	81.8 (9.3)	78.8 (10.0)	82.9 (9.1)	0.369
2h-PG (mg/dl)	119.9 (37.4)	105.2 (11.5)	125.4 (42.4)	0.268
Fasting insulin (mUI/ml)	29.3 (17.8)	27.0 (17.2)	30.2 (18.5)	0.716
2h-PI (mUI/ml)	154.1 (110.6)	111.1 (82.4)	170.2 (117.6)	0.275
Disposition index	4.0 (2.5)	4.8 (3.6)	3.7 (2.1)	0.374
Matsuda index	2.3 (2.1)	3.5 (3.3)	1.9 (1.2)	0.119
HOMA-IR	5.9 (3.7)	5.2 (3.1)	6.2 (3.9)	0.574
OGTT-insulin AUC	20048.1 (12212.3)	14151.2 (9566.1)	22259.4 (12616.2)	0.171
OGTT-glucose AUC	16180.9 (3471.7)	13715.0 (1609.0)	17105.6 (3559.0)	**0.038**
HbA1c (%)	34.3 (3.8)	33.2 (2.5)	34.7 (4.2)	0.419
Total cholesterol (mg/dl)	201.0 (36.9)	198.8 (49.4)	201.8 (33.1)	0.871
HDL (mg/dl)	43.4 (6.1)	43.3 (4.8)	43.4 (6.7)	0.973
Triglycerides (mg/dl)	145.6 (104.5)	110.3 (44.3)	158.8 (118.2)	0.345
LDL (mg/dl)	128.5 (33.9)	133.4 (49.4)	126.6 (28.0)	0.685
Uric acid (mg/dl)	6.0 (1.3)	5.9 (0.9)	6.0 (1.4)	0.847
eGFR (mg/ml/min)	99.3 (17.1)	91.8 (25.9)	101.3 (14.6)	0.334
Sodium (mmol/L)	139.9 (1.9)	139.8 (2.1)	139.9 (1.9)	0.965
AST (UI/L)	25.2 (6.7)	25.8 (5.2)	25.0 (7.3)	0.803
ALT (UI/L)	51.9 (25.2)	37.0 (8.3)	57.9 (27.3)	0.086
γ-gt (UI/L)	45.6 (26.0)	32.8 (7.6)	51.1 (29.3)	0.155
Rbc (10^3/ml)	5.2 (0.6)	5.7 (1.0)	5.1 (0.4)	0.062
Haematocrit (%)	44.0 (2.5)	45.7 (2.3)	43.5 (2.4)	0.079
Hb (g/dl)	14.9 (0.8)	15.0 (1.0)	14.8 (0.7)	0.758
TNF-α	9.4 (4.5)	9.7 (5.1)	9.3 (4.4)	0.848
FSH (UI/L)	4.6 (3.9)	5.7 (4.4)	4.2 (3.7)	0.421
LH (UI/L)	3.0 (1.4)	3.1 (1.9)	2.9 (1.3)	0.781
Total testosterone (ng/dl)	250.8 (112.2)	402.0 (57.2)	194.1 (62.9)	<**0.001**
cf-Testosterone (pg/ml)	6.7 (3.5)	10.6 (4.0)	5.2 (1.8)	<**0.001**
SHBG (nmol/L)	20.3 (8.9)	23.5 (11.5)	19.1 (7.8)	0.310

(Continues)

TABLE 1 (Continued)

	Whole population	Eugonadism	Hypogonadism	
	n = 22	*n* = 6	*n* = 16	*p*-Value
LH/T ratio	1.4 (1.0)	0.8 (0.5)	1.6 (1.1)	0.082

Note: Values shown in the table are means (SD) (unpaired Student's *t*-test between eugonadal and hypogonadal subjects). Bold font indicates $p < 0.05$.Abbreviations: 2h-PG, post-prandial glucose 120 min after oral glucose tolerance test; 2h-PI, post-prandial insulin 120 min after oral glucose tolerance test; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under curve; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FFM, free fat mass; FM, fat mass; FSH, follicle stimulating hormone; Hb, haemoglobin; HbA1c, glycated haemoglobin A1c; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; HR, heart rate; LH, luteinising hormone; LDL, low-density lipoprotein; OGTT, oral glucose tolerance test; Rbc, red blood cells; SHBG, sex hormone-binding globulin; SBP, systolic blood pressure.

TABLE 2 Changes in anthropometric and biochemical parameters in the study population on days 7 and 28 versus baseline

	Baseline	Day 7 Δ	*p*-Value versus baseline	Day 28 Δ	*p*-Value versus baseline	*p*-Value versus day 7
Body weight (kg)	112.4 (19.9)	**−3.7 (1.3)**	<0.001	**−9.3 (1.9)**	<0.001	<0.001
BMI (kg/m^2)	36.4 (5.0)	**−1.2 (0.5)**	<0.001	**−3.0 (0.7)**	<0.001	<0.001
EWL (%)	//	**8.5 (3.7)**	<0.001	**21.3 (7)**	<0.001	<0.001
TWL (%)	//	**3.2 (1.1)**	<0.001	**8.0 (1.7)**	<0.001	<0.001
Waist circumference (cm)	110.2 (11.9)	**−1.6 (3.5)**	0.046	**−5.4 (3.2)**	<0.001	<0.001
FM (kg)	42.6 (13.0)	**−1.9 (1.8)**	<0.001	**−6.4 (2.1)**	<0.001	<0.001
Fasting blood glucose (mg/dl)	81.8 (9.3)	**−7.2 (10.7)**	0.005	**−5.8 (11.2)**	0.002	0.440
Fasting insulin (mUI/ml)	27.8 (18.1)	**−13.0 (16.4)**	0.005	**−15.0 (13.1)**	0.001	0.094
HOMA-IR	5.6 (3.7)	**−2.8 (3.7)**	0.003	**−3.1 (2.8)**	<0.001	0.310
TNF-α (pg/ml)	9.4 (4.5)	−0.4 (4.1)	0.647	**−2.3 (4.0)**	0.044	0.013
Total testosterone (ng/dl)	250.8 (112.2)	**+35.4 (64.1)**	0.017	**+74.3 (97.3)**	0.002	0.033
cF-Testosterone (pg/ml)	5.9 (1.6)	+0.3 (2.0)	0.447	+2.3 (8.4)	0.213	0.285
SHBG (nmol/L)	21.9 (10.8)	**+2.1 (4.1)**	0.026	**+7.7 (10.0)**	0.002	0.003
LH (UI/L)	3.3 (1.5)	−0.1 (1.0)	0.500	−0.1 (1.0)	0.900	0.640
LH/T ratio	1.4 (1.0)	**−0.3 (0.6)**	0.036	**−0.5 (1.0)**	0.032	0.218

Note: Values shown in the table are means (SD) (repeated measures ANOVA and Post hoc paired Student's *t*-test). Bold font indicates $p < 0.05$.Abbreviations: BMI, body mass index; cf-Testosterone, calculated free testosterone; EWL, excess weight loss; HbA1c, glycated haemoglobin A1c; FM, fat mass; HOMA-IR, homeostatic model assessment for insulin resistance; LH, luteinising hormone; SHBG, sex hormone-binding globulin; TNF-α, tumour necrosis factor;TWL, total weight loss.

TT significantly increased after 7 days (Δ +35 ± 64 nmol/L) and 28 days (Δ +74 ± 97 nmol/L) on VLCKD. In addition to TT, a significant increase in serum SHBG level was observed after 7 and 28 days ($p < 0.01$) (Table 2, Figure 1). Neither cFT nor LH changed significantly after 7 and 28 days on VLCKD compared to baseline (Table 2, Figure 2). However, the LH/T ratio decreased significantly during the 4 weeks of intervention (Table 2). The significant variation in TT after VLCKD was used to calculate a posteriori the statistical power of the analysis. A post hoc analysis revealed a power of 99% and $p < 0.05$ to establish an effect size of 0.5 for TT, which means a variation of 0.5 SD above the average subject at day 0.

The increase in TT was correlated with reduction in blood glucose on day 7. Moreover, TT increase was associated with both body weight reduction and fat mass loss (Figure 2), independent of their baseline values (data not shown).

Signs and symptoms of hypogonadism derived from the questionnaires (i.e. Androtest, AMS and IIEF-5) administered before and after 28 days on VLCKD showed a significant amelioration only for somatic domain of AMS (−3.4 ± 5.4; $p = 0.019$) (Table S1)

After the stratification of the cohort according to gonadal status based on a baseline TT of 300 ng/dl, a significant difference in glucose levels during OGTT was appreciated with higher levels in the hypogonadal compared to the eugonadal subjects (Table 1, Figure S1). At the end of VLCKD, hypogonadal subjects exhibited also higher %TWL, greater reduction in weight, fat mass and waist circumference and a larger improvement in glycaemia as compared with eugonadal subjects

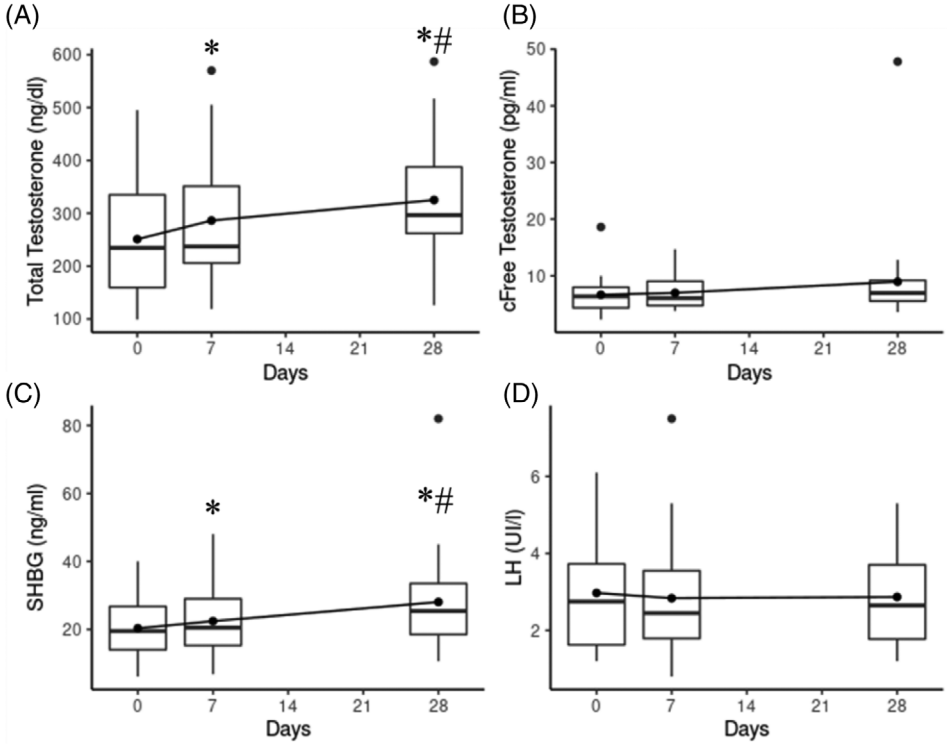

FIGURE 1 Hormonal parameters boxplots on days 0, 7 and 28 of very low-calorie ketogenic diet (VLCKD): (A) total testosterone, (B) calculated free testosterone, (C) sex hormone-binding globulin (SHBG) and (D) luteinising hormone (LH). *$p \leq 0.05$ versus day 0; #$p \leq 0.05$ versus day 7

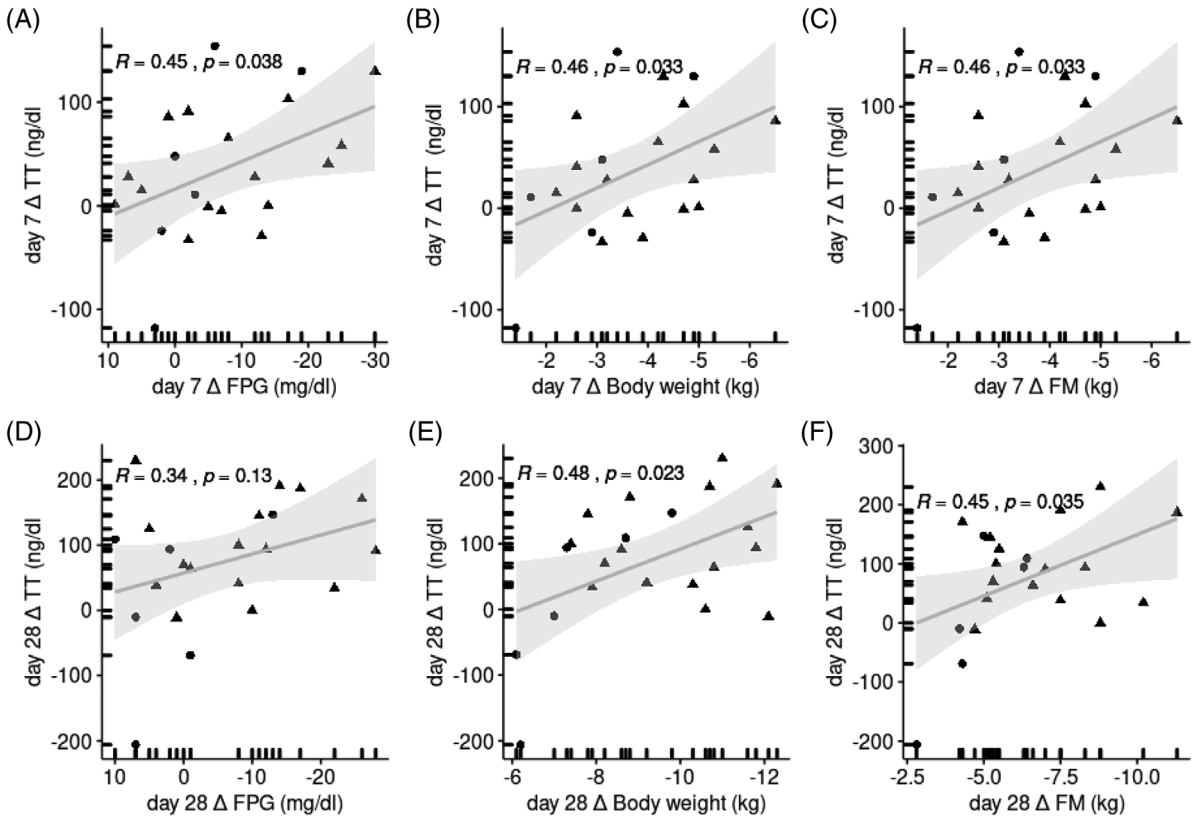

FIGURE 2 Total testosterone (TT) variation according to fasting plasma glucose (FPG), body weight and fat mass (FM) variation: (A–C) TT variation scatterplot according to FPG, body weight and FM variation at day 7 of very low-calorie ketogenic diet (VLCKD); (D–F) TT variation scatterplot according to FPG, body weight and FM variation at day 7 of VLCKD

TABLE 3 Changes in anthropometric and biochemical parameters on day 28 versus baseline according to gonadal status at baseline

	Eugonadism (6)	Hypogonadism (16)	*p*-Value
EWL (%)	19.5 (8.8)	22.0 (6.3)	0.455
Body weight (kg)	−7.52 (1.46)	−9.94 (1.66)	**0.005**
BMI (kg/m^2)	−2.45 (0.54)	−3.26 (0.63)	**0.012**
TWL (%)	6.8 (1.9)	8.5 (1.5)	**0.046**
Waist circumference (cm)	−3.08 (3.56)	−6.31 (2.65)	**0.031**
FM (kg)	−4.8 (1.4)	−7.0 (2.1)	**0.031**
Fasting blood glucose (mg/dl)	+2.00 (8.34)	−8.75 (10.92)	**0.042**
Fasting insulin (mUI/ml)	−15.95 (14.08)	−14.58 (13.22)	0.834
HOMA-IR	−2.88 (2.34)	−3.25 (2.97)	0.791
Total testosterone (ng/dl)	+10.83 (133.36)	+98.12 (71.51)	0.059
cf-Testosterone (pg/ml)	+4.48 (16.75)	+1.49 (1.68)	0.471
SHBG (nmol/L)	+8.58 (16.97)	+7.41 (6.54)	0.812
LH (UI/L)	+0.17 (1.13)	−0.21 (1.01)	0.463
LH/T ratio	0.0 (0.4)	−0.6 (1.1)	0.159
TNF-α (pg/ml)	−3.7 (4.6)	−1.7 (3.8)	0.325

Note: Values shown in the table are means (SD) (unpaired Student's *t*-test). Bold font indicates $p < 0.05$.Abbreviations: BMI, body mass index; cf-Testosterone, calculated free testosterone; EWL, excess weight loss; FM, fat mass; HbA1c, glycated haemoglobin A1c; HOMA-IR, homeostatic model assessment for insulin resistance; LH, luteinising hormone; SHBG, sex hormone-binding globulin; TNF-α, tumour necrosis factor;TWL, total weight loss.

(Table 3). Furthermore, hypogonadal subjects exhibited higher numerical increase in TT, which was not statistically significant ($p = 0.059$). A tendency for inverse correlation between TT increase and basal levels of TT was also apparent ($r = -0.4$, $p = 0.069$) (Table 3, Figure 3).

4 | DISCUSSION

We investigated the effects of VLKCD on serum testosterone levels in obese men. A rapid increase in TT after 7 days on VLKCD was evident and persisted until day 28 of the dietary regimen. Interestingly, SHBG also was increased, with a final net stability of cFT. According to recent studies,[13,14] HPG improvement after VLCKD parallels the effectiveness of this diet on body weight, FM, waist circumference and glycaemia reduction. However, to our knowledge, no previous studies investigated the early effects of VLCKD on the HPG axis in male patients with obesity.

A bidirectional relationship between body weight excess and hypogonadism has long been demonstrated.[19,28–30] The mechanism behind this connection is not completely understood, and several different hypotheses have been proposed. First, insulin resistance typically observed in obese patients is associated with reduced serum SHBG levels, higher testosterone levels with increased aromatisation in fat tissue, and reduced bioavailable steroids.[31] Second, the increased aromatase activity observed in supernumerary adipocytes could lead to a higher degree of testosterone conversion to oestradiol, leading to relative hyperoestrogenism that may subsequently reduce serum testosterone levels through its inhibitory effect on LH secretion.[32] Third, elevated leptin levels observed in obese subjects could inhibit GnRH pulsatility, leading to hypogonadotropic hypogonadism.[5] Finally, the role of inflammation in linking hypogonadism and obesity has been recently proposed.[33,34] In the context of this complex connection, body weight reduction is expected to restore HPG axis physiology regardless of the method applied to reach this goal. Indeed, several studies have demonstrated the restoration of serum testosterone levels after weight loss achieved by lifestyle interventions, weight-lowering drugs or bariatric surgery.[4] Whether diet-induced ketosis may favour an additional increase in TT compared to non-KD is not yet known.[35] Therefore, we could not exclude that the observed TT increase in this study could be attributable to the rapid weight loss rather than to VLCKD per se, as the intense calorie restriction is known to be associated with an increase in TT.[36] A direct head-to-head comparison between the effects of VLCKD and very low-calorie diet, or bariatric surgery, respectively, on plasma androgens and sexual function has not yet been reported.

We confirmed the clinical efficacy of VLCKD, demonstrating for the first time a rapid increase in TT that was already evident on day 7 of the diet, although without significant effect on LH serum concentrations. The LH/T ratio is commonly defined as a marker of testicular function.[37–39] In this study, the LH/T ratio decreased significantly during the period of VLCKD intervention, suggesting functional improvement in testicular function and HPG axis.[40]

A glucose load or a mixed meal transiently but significantly lowers TT levels in healthy, non-diabetic eugonadal men,[41] indicating that nutrients may reduce TT. In our study, a strong correlation between blood glucose reduction and TT increase was evident, suggesting that the restriction of carbohydrates (and food quantity) followed by robust blood glucose reduction may abrogate the expected TT decrease induced by feeding. Moreover, several studies have demonstrated that hyperglycaemia may hamper steroidogenesis and testis function by triggering oxidative stress and inflammation,[42,43] and in this study, higher glucose levels during OGTT were observed in hypogonadal subjects as compared with eugonadal subjects. Thus, it is possible that the efficacy of VLCKD in improving the HPG axis (i.e. higher TT, stable LH and higher LH/TT ratio) could be also explained by the reduction of glycaemia.

VLCKD is known to ameliorate liver function,[44–46] reducing intrahepatic fat content,[47] and increasing the insulin sensitivity index.[48] In obese patients with T2DM, the reduction in carbohydrate intake has been associated with an early and significant decrease in hepatic triacylglycerol content. Consequently, higher suppression of hepatic glucose production was observed because of improved hepatic insulin sensitivity.[49] Higher hepatic insulin sensitivity is typically associated

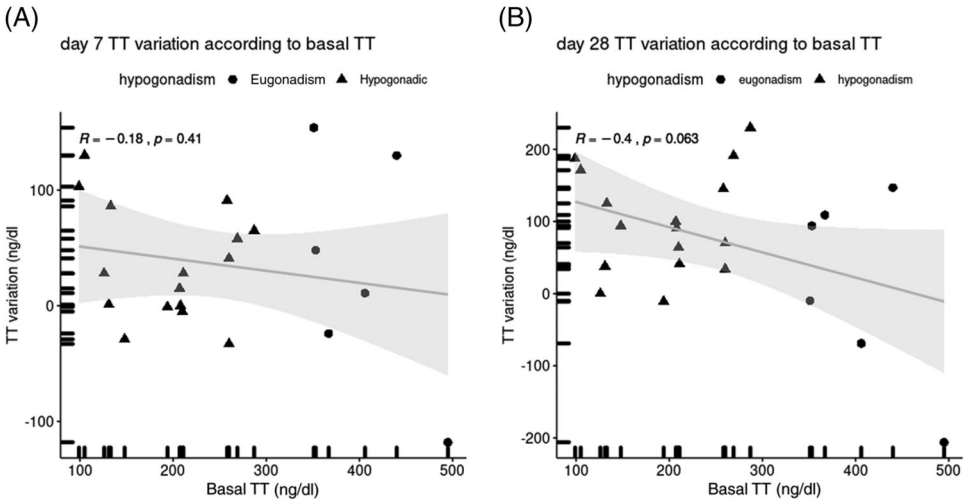

FIGURE 3 Total testosterone (TT) variation according to basal TT: (A) TT variation scatterplot on day 7 of very low-calorie ketogenic diet (VLCKD) according to basal TT; (B) TT variation scatterplot on day 28 of VLCKD according to basal TT

with lower fasting plasma glucose and plasma insulin levels, as also observed in this study in which insulinaemia was reduced by ~50% after 4 weeks of VLCKD. Cross-sectional studies in men have suggested that SHBG may be not related to fasting blood glucose, HbA1c or lipid levels, but rather to intrahepatic fat.[50,51] Indeed, it has been estimated that ~20% of the increase in serum SHBG is mediated by a reduction in intrahepatic lipid content.[52] Therefore, the VLCKD-related increase in SHBG may potentially reflect the beneficial effect on liver metabolism and may, at least in part, contribute to the early increase of TT.

Accumulating evidence reported that obesity-related pro-inflammatory milieu is often associated with reduced levels of testosterone. Both in vivo and in vitro studies observed that high concentrations of IL-6, IL-1β and TNF-α affect the hypothalamus-pituitary-testis axis activity by suppressing the release of GnRH, LH and FSH, and this is followed by TT reduction. [53,54] Particularly, VLCKD has been demonstrated to have anti-inflammatory benefits in terms of reduction of serum TNF-α levels.[55] Indeed, a significant reduction of this cytokine was observed only after 28 days of VLCKD. Moreover, TT variation was not significantly related to the TNF-α decrease, and only a trend to an inverse relation was observed (data not shown) suggesting a marginal contribution of the improvement in inflammation obtained with VLCKD on TT increase.

Interestingly, hypogonadal subjects on VLCKD achieved greater advantages in terms of reduction in body weight, fat mass, waist circumference and fasting plasma glucose compared with eugonadal subjects. The mechanism underlying this phenomenon is unclear, as hypogonadism is known to be associated with increased fat mass[6,33,34] and may potentially counteract the VLCKD-induced fat mass reduction. Moreover, low serum testosterone levels may contribute to fatigue and inertia, making it more difficult to engage in VLCKD.[56] We speculate that the greater metabolic response in hypogonadal men may be favoured by the higher TT increase as compared with eugonadal men. This is also in-line with previous studies indicating the role of testosterone in improving glycaemia,[57,58] waist circumference[59] and fat mass[60] in hypogonadal men with obesity during androgen replacement therapy.[61]

In this study, we did not obtain data on β-oestradiol. However, it is well-known that β-oestradiol is synthesised mainly from androgen precursors by the enzyme aromatase in adipocytes.[62,63] Indeed, extra-glandular aromatisation of circulating androgen precursors is the main source of oestrogen in men,[64,65] and aromatase activity is dependent on body fat mass.[66] The increase in aromatase activity leads to a higher conversion of testosterone to oestradiol, resulting in decreased bioavailable testosterone. As our intervention resulted in major decreases in body fat, the increase in the T/β-oestradiol ratio is to be expected in the light of the substantial decrease in aromatase activity in adipocytes.

In this study, the rapid TT increase was not accompanied by an increase in bioavailable testosterone, and the increase in TT following VLKCD was not accompanied by a substantial increase in cFT, suggesting a modest clinical benefit at this time point of intervention. However, the somatic domain of the AMS questionnaire, dealing with overall quality of life and strength, displayed a significant amelioration even although this was not related to TT variation (data not shown). In contrast, both psychological (e.g. mood) and sexual domain (e.g. erectile function and sexual desire) of AMS and IIEF-5 showed comparable values before and after VLCKD. This is not surprising as maximal effects obtained from testosterone replacement therapy (TRT) on symptoms of hypogonadism are attained with different interval periods, usually after 3–6 months of TRT and may take even up to 1 year in individual cases.[67,68] Thus, the onset of the clinical effects of increased testosterone may require more time[67] and may not be evident after only 1 month on VLCKD. The European Male Aging Study reported that testosterone undergoes fluctuations by lifestyle factors according to the extent of weight changes. In the setting of modest weight loss (<15%), a slight increase in TT

(+2 nmol/L) was observed, probably because of an increase in SHBG levels, whereas cFT did not change. However, with greater weight loss (>15%), TT was also increased (+5.75 nmol/L) and was associated with significantly higher cFT levels (+51.78 pmol/L). This was likely because of an activation of the HPG axis, as evidenced by a significant increase in LH release (+2 U/L). We infer that VLKCD requires a longer time than 4 weeks to induce a substantial weight loss, as evidenced by previous experiences demonstrating mean reductions of 10–15 kg when VLCKD was carried out for more than 4 weeks.[69] Indeed, full restoration of the HPG axis was demonstrated in a previous study with an exposure of patients to VLCKD for longer times.[14]

5 | CONCLUSIONS

Obesity plays a critical role in the development of functional hypogonadism in males. Moreover, hypogonadism facilitates the expansion of fat mass, typically in the visceral compartment. In this context, rapid reduction of body weight, fat mass, waist circumference and blood glucose achieved with VLCKD resulted in the prompt amelioration of serum testosterone levels, especially in hypogonadal subjects. The results of this study suggest that VLCKD represents an effective and rapid therapeutic tool against obesity and obesity-related hypogonadism in non-diabetic males.

AUTHOR CONTRIBUTIONS

Conceptualisation: Angelo Cignarelli and Eleonora Conte. *Methodology*: Angelo Cignarelli, Eleonora Conte and Simona Di Leo. *Recruitment of the subjects*: Angelo Cignarelli, Eleonora Conte, Fiorella Giordano and Simona Di Leo. *Data collection*: Angelo Cignarelli, Eleonora Conte, Valentina Annamaria Genchi and Fiorella Giordano. *Statistical analysis*: Angelo Cignarelli and Daniele Santi. *Writing – original draft preparation*: Angelo Cignarelli, Valentina Annamaria Genchi and Daniele Santi. *Revision of the article and contribution to the discussion*: Annalisa Natalicchio, Luigi Laviola, Francesco Giorgino and Sebastio Perrini. *Supervision*: Francesco Giorgino and Sebastio Perrini. *Gave final approval of the version to be published*: Francesco Giorgino. *Read and agreed to the published version of the manuscript*: All authors.

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CONFLICTS OF INTEREST

L.L. provided advisory services to Abbott, AstraZeneca, Boehringer Ingelheim, Eli Lilly, Novo Nordisk, Roche Diabetes Care, Sanofi and Takeda. F.G. provided advisory services to AstraZeneca; Eli Lilly; Novo Nordisk; Roche Diabetes Care, Sanofi, served as a consultant for Boehringer Ingelheim, Lifescan, Merck Sharp & Dohme, Sanofi, AstraZeneca, Medimmune, Roche Diabetes Care, Sanofi, Medtronic and received research support from Eli Lilly and Roche Diabetes Care. Other authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The participants of this study did not give written consent for their data to be shared publicly, so due to the sensitive nature of the research supporting data is not available.

REFERENCES

1. De Lorenzo A, Gratteri S, Gualtieri P, Cammarano A, Bertucci P, Di Renzo L. Why primary obesity is a disease. *J Transl Med*. 2019;17(1):169.
2. Handakas E, Lau CH, Alfano R, et al. A systematic review of metabolomic studies of childhood obesity: state of the evidence for metabolic determinants and consequences. *Obes Rev*. 2022;23(Suppl1):e13384.
3. Grossmann M. Hypogonadism and male obesity: focus on unresolved questions. *Clin Endocrinol (Oxf)*. 2018;89(1):11-21.
4. Genchi VA, Rossi E, Lauriola C, et al. Adipose tissue dysfunction and obesity-related male hypogonadism. *Int J Mol Sci*. 2022;23(15):8194.
5. Chen X, Xiao Z, Cai Y, Huang L, Chen C. Hypothalamic mechanisms of obesity-associated disturbance of hypothalamic-pituitary-ovarian axis. *Trends Endocrinol Metab*. 2022;33(3):206-217.
6. Carrageta DF, Oliveira PF, Alves MG, Monteiro MP. Obesity and male hypogonadism: tales of a vicious cycle. *Obes Rev*. 2019;20(8):1148-1158.
7. Corona G, Rastrelli G, Monami M, et al. Body weight loss reverts obesity-associated hypogonadotropic hypogonadism: a systematic review and meta-analysis. *Eur J Endocrinol*. 2013;168(6):829-843.
8. Paoli A, Rubini A, Volek JS, Grimaldi KA. Beyond weight loss: a review of the therapeutic uses of very-low-carbohydrate (ketogenic) diets. *Eur J Clin Nutr*. 2013;67(8):789-796.
9. Caprio M, Infante M, Moriconi E, et al. Very-low-calorie ketogenic diet (VLCKD) in the management of metabolic diseases: systematic review and consensus statement from the Italian Society of Endocrinology (SIE). *J Endocrinol Invest*. 2019;42(11):1365-1386.
10. Basolo A, Magno S, Santini F, Ceccarini G. Ketogenic diet and weight loss: is there an effect on energy expenditure. *Nutrients*. 2022;14(9):1814.
11. Tsai AG, Wadden TA. The evolution of very-low-calorie diets: an update and meta-analysis. *Obesity (Silver Spring, Md)*. 2006;14(8):1283-1293.
12. Durkalec-Michalski K, Nowaczyk PM, Główka N, Ziobrowska A, Podgórski T. Is a four-week ketogenic diet an effective nutritional strategy in CrossFit-trained female and male athletes. *Nutrients*. 2021;13(3):864.
13. La Vignera S, Cannarella R, Galvano F, et al. The ketogenic diet corrects metabolic hypogonadism and preserves pancreatic ß-cell function in overweight/obese men: a single-arm uncontrolled study. *Endocrine*. 2021;72(2):392-399.
14. Mongioì LM, Cimino L, Condorelli RA, et al. Effectiveness of a very low calorie ketogenic diet on testicular function in overweight/obese men. *Nutrients*. 2020;12(10):2967.
15. Paoli A, Cenci L, Pompei P, et al. Effects of two months of very low carbohydrate ketogenic diet on body composition, muscle strength, muscle area, and blood parameters in competitive natural body builders. *Nutrients*. 2021;13(2):374.
16. Vidić V, Ilić V, Toskić L, Janković N, Ugarković D. Effects of calorie restricted low carbohydrate high fat ketogenic vs. non-ketogenic diet

on strength, body-composition, hormonal and lipid profile in trained middle-aged men. *Clin Nutr*. 2021;40(4):1495-1502.
17. Wilson JM, Lowery RP, Roberts MD, et al. Effects of ketogenic dieting on body composition, strength, power, and hormonal profiles in resistance training men. *J Strength Cond Res*. 2020;34(12):3463-3474.
18. Zajac A, Poprzecki S, Maszczyk A, Czuba M, Michalczyk M, Zydek G. The effects of a ketogenic diet on exercise metabolism and physical performance in off-road cyclists. *Nutrients*. 2014;6(7):2493-2508.
19. Furini C, Spaggiari G, Simoni M, Greco C, Santi D. Ketogenic state improves testosterone serum levels-results from a systematic review and meta-analysis [published online ahead of print, 2022 Sep 23]. *Endocrine*. 2022; https://doi.org/10.1007/s12020-022-03195-5
20. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Ann Intern Med*. 2007;147(8):573-577.
21. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28(7):412-419.
22. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care*. 1999;22(9):1462-1470.
23. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18(6):499-502.
24. Tang Q, Li X, Song P, Xu L. Optimal cut-off values for the homeostasis model assessment of insulin resistance (HOMA-IR) and pre-diabetes screening: Developments in research and prospects for the future. *Drug Discov Ther*. 2015;9(6):380-385.
25. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab*. 1999;84(10):3666-3672.
26. Budzyński J, Tojek K, Czerniak B, Banaszkiewicz Z. Scores of nutritional risk and parameters of nutritional status assessment as predictors of in-hospital mortality and readmissions in the general hospital population. *Clin Nutr*. 2016;35(6):1464-1471.
27. Brethauer SA, Kim J, el Chaar M, et al. Standardized outcomes reporting in metabolic and bariatric surgery. *Surg Obes Relat Dis*. 2015;11(3):489-506.
28. Dandona P, Dhindsa S, Chaudhuri A, Bhatia V, Topiwala S, Mohanty P. Hypogonadotrophic hypogonadism in type 2 diabetes, obesity and the metabolic syndrome. *Curr Mol Med*. 2008;8(8):816-828.
29. Dhindsa S, Miller MG, McWhirter CL, et al. Testosterone concentrations in diabetic and nondiabetic obese men. *Diabetes Care*. 2010;33(6):1186-1192.
30. Corona G, Monami M, Rastrelli G, et al. Testosterone and metabolic syndrome: a meta-analysis study. *J Sex Med*. 2011;8(1):272-283.
31. van Hulsteijn LT, Pasquali R, Casanueva F, et al. Prevalence of endocrine disorders in obese patients: systematic review and meta-analysis. *European J Endocrinol*. 2020;182(1):11-21.
32. Yuxin L, Chen L, Xiaoxia L, et al. Research progress on the relationship between obesity-inflammation-aromatase axis and male infertility. *Oxid Med Cell Longev*. 2021;2021:6612796.
33. Corona G, Rastrelli G, Morelli A, et al. Treatment of functional hypogonadism besides pharmacological substitution. *World J Mens Health*. 2020;38(3):256-270.
34. Corona G, Rastrelli G, Vignozzi L, et al. The role of testosterone treatment in patients with metabolic disorders. *Expert Rev Clin Pharmacol*. 2021;14(9):1091-1103.
35. Onfiani G. VLCD versus VLCKD for obese male patients with hypogonadism: considerations about the recent systematic review and consensus statement published by the Italian Society of Endocrinology. *Eat Weight Disord*. 2022;27:2955-2956.
36. Schulte DM, Hahn M, Oberhäuser F, et al. Caloric restriction increases serum testosterone concentrations in obese male subjects by two distinct mechanisms. *Horm Metab Res*. 2014;46(4):283-286.
37. Aleem M, Padwal V, Choudhari J, Balasinor N, Gill-Sharma MK. Sperm protamine levels as indicators of fertilising potential in sexually mature male rats. *Andrologia*. 2008;40(1):29-37.
38. Bandak M, Aksglaede L, Juul A, Rørth M, Daugaard G. The pituitary-Leydig cell axis before and after orchiectomy in patients with stage I testicular cancer. *Eur J Cancer*. 2011;47(17):2585-2591. https://doi.org/10.1016/j.ejca.2011.05.026
39. Rannikko A, Petas A, Raivio T, Jänne OA, Rannikko S, Adlercreutz H. The effects of short-term oral phytoestrogen supplementation on the hypothalamic-pituitary-testicular axis in prostate cancer patients. *Prostate*. 2006;66(10):1086-1091.
40. de Mattos K, Viger RS, Tremblay JJ. Transcription factors in the regulation of Leydig cell gene expression and function. *Front Endocrinol*. 2022;13:881309.
41. Gagliano-Jucá T, Li Z, Pencina KM, et al. Oral glucose load and mixed meal feeding lowers testosterone levels in healthy eugonadal men. *Endocrine*. 2019;63(1):149-156.
42. Naas H, de Oliveira AA, Karpova T, Nunes KP. Toll-like receptor 4 (TLR4) as a possible pathological mechanism in hyperglycemia-associated testicular dysfunction. *Med Hypotheses*. 2019;127:116-119.
43. Kannan A, Mariajoseph-Antony LF, Panneerselvam A, et al. Aquaporin 9 regulates Leydig cell steroidogenesis in diabetes. *Syst Biol Reprod Med*. 2022;68(3):213-226.
44. Herrington GJ, Peterson JJ, Cheng L, et al. The use of very low-calorie diets in subjects with obesity complicated with nonalcoholic fatty liver disease: a scoping review. *Obes Sci Pract*. 2022;8(4):510-524.
45. Talavera-Urquijo E, Rodríguez-Navarro S, Beisani M, et al. Morpho-functional changes after sleeve gastrectomy and very low calorie diet in an animal model of non-alcoholic fatty liver disease. *Obes Surg*. 2018;28(1):142-151.
46. Watanabe M, Tozzi R, Risi R, et al. Beneficial effects of the ketogenic diet on nonalcoholic fatty liver disease: a comprehensive review of the literature. *Obes Rev*. 2020;21(8):e13024.
47. Lewis MC, Phillips ML, Slavotinek JP, Kow L, Thompson CH, Toouli J. Change in liver size and fat content after treatment with Optifast very low calorie diet. *Obes Surg*. 2006;16(6):697-701.
48. Shah K, Stufflebam A, Hilton TN, Sinacore DR, Klein S, Villareal DT. Diet and exercise interventions reduce intrahepatic fat content and improve insulin sensitivity in obese older adults. *Obesity (Silver Spring, Md)*. 2009;17(12):2162-2168.
49. Lim EL, Hollingsworth KG, Aribisala BS, Chen MJ, Mathers JC, Taylor R. Reversal of type 2 diabetes: normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol. *Diabetologia*. 2011;54(10):2506-2514.
50. Bonnet F, Velayoudom Cephise FL, Gautier A, et al. Role of sex steroids, intrahepatic fat and liver enzymes in the association between SHBG and metabolic features. *Clin Endocrinol (Oxf)*. 2013;79(4):517-522.
51. Tchernof A, Labrie F, Bélanger A, et al. Relationships between endogenous steroid hormone, sex hormone-binding globulin and lipoprotein levels in men: contribution of visceral obesity, insulin levels and other metabolic variables. *Atherosclerosis*. 1997;133(2):235-244.
52. Telgenkamp I, Kusters Y, Schalkwijk CG, et al. Contribution of liver fat to weight loss-induced changes in serum hepatokines: a randomized controlled trial. *J Clin Endocrinol Metab*. 2019;104(7):2719-2727.
53. Tsilidis KK, Rohrmann S, McGlynn KA, et al. Association between endogenous sex steroid hormones and inflammatory biomarkers in US men. *Andrology*. 2013;1(6):919-928.
54. Bobjer J, Katrinaki M, Tsatsanis C, Lundberg Giwercman Y, Giwercman A. Negative association between testosterone concentration and

inflammatory markers in young men: a nested cross-sectional study. *PLoS One*. 2013;8(4):e61466.
55. Monda V, Polito R, Lovino A, et al. Short-term physiological effects of a very low-calorie ketogenic diet: effects on adiponectin levels and inflammatory states. *Int J Mol Sci*. 2020;21(9):3228.
56. O'Connor DB, Archer J, Wu FC. Effects of testosterone on mood, aggression, and sexual behavior in young men: a double-blind, placebo-controlled, cross-over study. *J Clin Endocrinol Metab*. 2004;89(6):2837-2845.
57. Saad F. Testosterone therapy and glucose homeostasis in men with testosterone deficiency (Hypogonadism). *Adv Exp Med Biol*. 2017;1043:527-558.
58. Haider KS, Haider A, Saad F, et al. Remission of type 2 diabetes following long-term treatment with injectable testosterone undecanoate in patients with hypogonadism and type 2 diabetes: 11-year data from a real-world registry study. *Diabetes Obes Metab*. 2020;22(11):2055-2068.
59. Haider A, Yassin A, Doros G, Saad F. Effects of long-term testosterone therapy on patients with "diabesity": results of observational studies of pooled analyses in obese hypogonadal men with type 2 diabetes. *Int J Endocrinol*. 2014;2014:683515.
60. Isidori AM, Giannetta E, Greco EA, et al. Effects of testosterone on body composition, bone metabolism and serum lipid profile in middle-aged men: a meta-analysis. *Clin Endocrinol (Oxf)*. 2005;63(3):280-293.
61. Mangolim AS, Brito LAR, Nunes-Nogueira VDS. Effectiveness of testosterone replacement in men with obesity: a systematic review and meta-analysis. *Eur J Endocrinol/European Federation of Endocrine Societies*. 2021;186(1):123-135.
62. de Ronde W, Pols HA, van Leeuwen JP, de Jong FH. The importance of oestrogens in males. *Clin Endocrinol (Oxf)*. 2003;58(5):529-542.
63. Kley HK, Deselaers T, Peerenboom H, Krüskemper HL. Enhanced conversion of androstenedione to estrogens in obese males. *J Clin Endocrinol Metab*. 1980;51(5):1128-1132.
64. Merlotti D, Gennari L, Stolakis K, Nuti R. Aromatase activity and bone loss in men. *J Osteoporos*. 2011;2011:230671.
65. Rochira V, Santi D, Carani C. Pathophyshyology of estrogen action in men. In: Nieschlag E, Behre HM, eds. *Testosterone*. 4th ed. Cambridge University Press 2012:421-436.
66. Schindler AE, Ebert A, Friedrich E. Conversion of androstenedione to estrone by human tissue. *J Clin Endocrinol Metab*. 1972;35(4):627-630.
67. Saad F, Aversa A, Isidori AM, Zafalon L, Zitzmann M, Gooren L. Onset of effects of testosterone treatment and time span until maximum effects are achieved. *Eur J Endocrinol/European Federation of Endocrine Societies*. 2011;165(5):675-685.
68. Yassin AA, Saad F. Improvement of sexual function in men with late-onset hypogonadism treated with testosterone only. *J Sex Med*. 2007;4(2):497-501.
69. Castellana M, Conte E, Cignarelli A, et al. Efficacy and safety of very low calorie ketogenic diet (VLCKD) in patients with overweight and obesity: a systematic review and meta-analysis. *Rev Endocr Metab Disord*. 2020;21(1):5-16.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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