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Correspondence:
Vito A. Giagulli, Outpatient Clinic for Endocrinology and Metabolic Diseases, Conversano Hospital, ASL Bari, Via De Amicis, 70014 Conversano, Italy.
E-mail vitogiagulli@alice.it

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Adding liraglutide to lifestyle changes, metformin and testosterone therapy boosts erectile function in diabetic obese men with overt hypogonadism

[1,2]V. A. Giagulli, [3]M. D. Carbone, [1]M. I. Ramunni, [2]B. Licchelli, [4]G. De Pergola, [5]C. Sabbà, [2]E. Guastamacchia and [2]V. Triggiani

[1]Outpatient Clinic for Endocrinology and Metabolic Diseases, Conversano Hospital, Conversano, [2]Endocrinology and Metabolic Diseases, University of Bari, Bari, [3]Institute of Clinical and Hormonal Research, Foggia, [4]Nutrition Outpatient Clinic, Clinical Oncology Unit, and [5]Rare Diseases Center, University of Bari, Bari, Italy

SUMMARY

The aim of this retrospective observational study was to evaluate whether adding liraglutide to lifestyle changes, metformin (Met) and testosterone replacement therapy (TRT), by means of improving weight and glycaemic control, could boost erectile function in type 2 diabetic obese men with overt hypogonadism and erectile dysfunction (ED) in a 'real-life setting'. Forty-three obese, diabetic and hypogonadal men (aged 45–59 years) were evaluated because of complaining about the recent onset of ED. They were subdivided into two groups according to whether hypogonadism occurred after puberty (G1; $n = 30$: 25 with dysfunctional hypogonadism and 5 with acquired hypogonadotropic hypogonadism) or before puberty (G2; $n = 13$: 10 with Klinefelter's syndrome and 3 with idiopathic hypogonadotropic hypogonadism). Both G1 and G2 patients were given a combination of testosterone (T) [testosterone undecanoate (TU) 1000 mg/every 12 weeks] and Met (2000–3000 mg/day) for 1 year. In the poor responders (N) to this therapy in terms of glycaemic target (G1N: $n = 16$; G2N: $n = 10$), liraglutide (L) (1.2 μg/day) was added for a second year, while the good responders (Y) to T + Met (G1Y: 14/30 and G2Y: 3/13) continued this two drugs regimen therapy for another year. All patients were asked to fill in the International Index of Erectile Function (IIEF 15) questionnaire before starting TU plus Met (T1) and after 12 months (T2) and 24 months (T3) of treatment. Patients underwent a clinical examination and a determination of serum sex hormone binding globulin (SHBG), total testosterone (T) and glycosylated haemoglobin (HbA1c) at T1, T2 and T3. At T2, each patient obtained an improvement of ED ($p < 0.01$) and of the metabolic parameters without reaching, however, the glycaemic goals [HbA1c = >7.5% (>58 mmol/mol)], while T turned out to be within the range of young men. L added to TU and Met regimen in G1N and G2N allowed these patients to reach not only the glycaemic target [HbA1c = <7.5% (<58 nmol/mol)] and a significant reduction in body weight ($p < 0.01$), but also a further increase in SHBG ($p < 0.05$) and T ($p < 0.01$) plasma levels as well as a significant increment of IIEF score (T3). Conversely, at T3 G1Y and G2Y, who received the combined therapy with TRT and Met for the second year, showed a partial failure of that treatment given that there was no improvement of the IIEF score and they showed a significant rise in serum HbA1c ($p < 0.05$) and weight ($p < 0.04$) compared with the assessments at T2. These results suggest that TRT could improve clinical and metabolic parameters in obese, type 2 diabetic men with ED and overt hypogonadism (independently of when T deficit occurred). Furthermore, in case of insufficient metabolic control the addition of L to TRT and Met regimen allows to achieve serum T levels in the range of healthy men, as well as to reach glycaemic target and to lower weight, leading to a considerable improvement of ED.

INTRODUCTION

Diabetes mellitus, obesity, hypertension and dyslipidemia have been reported as main risk factors for developing erectile dysfunction (ED) in men with or without overt hypogonadism (Seftel *et al.*, 2004; Lewis *et al.*, 2010). The treatment of these conditions can effectively improve ED in these patients (Glina *et al.*, 2013).

Men with both pre-pubertal (i.e. Klinfelter syndrome) and post-pubertal onset hypogonadism could develop type 2 diabetes mellitus (T2DM) during their life span (Pei *et al.*, 1998;

Bojesen *et al.*, 2006). Recently several studies and a meta-analysis have highlighted the relationship between hypogonadism, metabolic syndrome (MetS), T2DM (Andersson *et al.*, 1994; Dhindsa *et al.*, 2004; Ding *et al.*, 2006; Kapoor *et al.*, 2007; Grossmann *et al.*, 2008) and ED in adult men (Jones *et al.*,2011; O'Connor *et al.*, 2011; Hackett *et al.*, 2013). Furthermore, testosterone replacement therapy (TRT) leads to improve insulin sensitivity and metabolic control in T2DM obese men with hypotestosteronemia (Boyanov *et al.*, 2003; Kapoor *et al.*, 2006; Corona *et al.*, 2011; Hackett *et al.*, 2013; Juang *et al.*, 2014). Hence, low serum testosterone (T) might play a key role in determining not only ED but also metabolic diseases in adult men.

Both age and obesity (Kaufman & Vermeulen, 2005) have been recognized as key drivers for metabolic diseases and hypogonadism, especially in adult and ageing men. Indeed, by means of different mechanisms, these conditions have both a negative effect on the hypothalamic–pituitary–testicular axis, leading to a reduction in serum T (Giagulli *et al.*, 1994; Wu *et al.*, 2008). However, at variance with age, obesity is a modifiable factor, and when obese subjects lose weight, T levels increase (Corona *et al.*, 2013). Recently, in uncontrolled studies conducted in a large number of hypogonadal men with obesity and/or T2DM, long duration TRT was shown to result in both a significant fat loss and the achievement of stable metabolic control (Saad *et al.*, 2013; Zitzmann *et al.*, 2013). Therefore, obesity and hypogonadism can be regarded as the main clinical factors underlying metabolic diseases that should be corrected to obtain a stable metabolic control in these patients.

Different classes of anti-diabetic drugs, often in combination and in add-on therapy regimen, should be used, as recommended by different international scientific societies, to obtain a stable metabolic control in T2DM patients (Handelsman *et al.*, 2015; Inzucchi *et al.*, 2015). At variance with other anti-diabetic compounds (i.e. sulfonylureas, thiazolidinediones, insulin, etc.), glucagon-like peptide-1 (GLP-1) agonists have both efficacious glucose-lowering properties and a sustained weight-reducing effect in T2DM subjects, even more than metformin (Met) (Robinson *et al.*, 2013; Inzucchi *et al.*, 2015).

The main purpose of this retrospective observational study was to assess whether adding liraglutide (L) (a long-acting GLP-1 agonist) to lifestyle changes and Met and TRT regimen, by means of a stable metabolic control and a reduction in body weight, could lead to an improvement of hypogonadism and ED in adult and middle-aged obese men affected by both T2DM and pre- or post-pubertal onset hypogonadism.

MATERIALS AND METHODS

Participants' inclusion criteria

Patients considered for this retrospective observational study were selected on the basis of their clinical records among the T2DM men attending our outpatient clinic for endocrinology and metabolic diseases. We selected men who were suffering from obesity, as well as from overt hypogonadism and recent onset of ED.

All participants had to be affected both by T2DM without a the good glycaemic control [glycosylated haemoglobin (HbA_1c) >8%] (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, Cheng AY, 2013; International Diabetes Federation Guideline Development Group, 2014, Handelsman *et al.*, 2015; Inzucchi *et al.*, 2015) and by obesity [body mass index (BMI) >30 kg/m^2) (Apovian *et al.*, 2015; Handelsman *et al.*, 2015). To qualify for our study, men were required to have two early morning T values <300 ng/dL and calculated free T (FT) levels <65 pg/mL (Wang *et al.*, 2008; Bhasin *et al.*, 2010), whereas, those with two serum T levels <150 ng/dL were assessed if they were suffering from the organic forms of pre- or post-pubertal onset of hypogonadism (Wang *et al.*, 2008; Bhasin *et al.*, 2010). Finally, men were selected if they complained of ED, showing a score <14 on the International Index of Erectile Function Questionnaire (IIEF 15; Rosen *et al.*, 2002).

From 396 T2DM men attending our outpatient clinics for metabolic diseases for the first time in the last 2 years and whose diagnosis dated back between 3 and 5 years, a dysfunctional overt hypogonadism with low-normal gonadotophins levels (LH = 6.0 ± 2.8 UI/L), a condition related to the diabetes itself (Wang *et al.*, 2008; Bhasin *et al.*, 2010), was diagnosed in 25 patients (aged 48–60). Furthermore, they were obese, had serum HbA_1c >8% and complained of ED. Additionally, 5 obese men (aged 50–62) suffering from a severe form of adult secondary hypogonadotropic hypogonadism (LH = 1.8 ± 2.5 UI/L) due to a non-functioning pituitary adenoma (n= 4) or to head trauma (n = 1) whose diagnosis of hypogonadism had been made more than 9 years earlier, while they were diagnosed as having T2DM almost 3 years earlier, were also included in the study, given that they had HbA_1c >8% and complained of ED. These patients along with the previous 25 diabetic men with hypogonadism formed the first group (G1 = patients with post-pubertal onset hypogonadism). Until the visit considered as T1 in the study (just before the administration of the TU + Met combined therapy), 3 out of 30 had been treated with sulfonylureas, 4 out of 30 with glinide, 2 out of 30 with alpha-glucosidase inhibitors and 18 out of 30 with Met, while 3 out of 30 received a diet regimen only. Three out of 4 subjects affected by non-functioning adenoma who underwent trans-sphenoidal surgery were treated with hydrocortisone (15–25 mg/day in divided doses) and levothyroxine (1.5–1.8 μg/kg/day) because of their secondary deficits (Giagulli & Carbone, 2006).

The second group of participants to our study (G2 = diabetic men with pre-pubertal onset hypogonadism) was obtained from 79 men affected by pre-pubertal onset hypogonadism (52/79 subjects with Klinfelter's syndrome and 27/79 with hypogonadonatropic hypogonadism) attending our outpatient clinic for endocrine diseases. A total of 13 obese men (aged 45–58) affected by pre-pubertal onset hypogonadism (n = 10 Klinefelter's syndrome and n = 3 with idiopathic pre-pubertal hypogonadotropic hypogonadism who showed LH ≥10 UI/L and LH = 1.0 ± 1.1 UI/L at the time of their diagnosis, respectively) with a diagnosis of T2DM dating 2–4 years, were included. All subjects, moreover, showed a poor metabolic control (HbA_1c >8%) and also complained of ED. Four out of 13 have been treated with alpha-glucosidase inhibitors and 6 out of 13 with Met, while 3 out of 13 received a diet regimen only.

All subjects with pre- and post-pubertal onset hypogonadism were examined by a single physician and their clinical history and their physical examination were addressed to evaluate specific signs and symptoms characterizing metabolic diseases and hypogonadism. BMI (kg/m^2) and waist circumference (WC = cm) were assessed. Digital rectal examination (DRE) was

performed to evaluate the prostate. Testis size was assessed using the Prader orchidometer. Systolic (SBP) and diastolic (DBP; average of two separate measurements) blood pressures were determined in a sitting position after at least 10-min rest (ESH/ESC Task Force, 2013).

All hypogonadal diabetic subjects belonging to the first group (G1) presented full developed secondary sexual characteristics, while the participants with pre-pubertal onset hypogonadism showed different degrees of incomplete puberty (i.e. scanty hairs and, as in Klinefelter subjects, the pubic hair with the triangle upwards) and typical signs of pre-pubertal onset hypogonadism such as long upper and lower limbs (hypogonadotropic hypogonadism). All the patients were affected by overt abdominal obesity (BMI >30; WC >100 cm) and T2DM (Apovian *et al.*, 2015; Handelsman *et al.*, 2015; National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), 2002).

The diagnosis of Klinefelter's syndrome was made according to the clinical (i.e. small, firm testes, gynaecomastia, infertility, etc.), hormonal (Follicle Stimulating Hormone ≥10 UI/L) and genetic criteria. Indeed, 9/10 Klinefelter men showed a classic karyotype defect (47, XXY), while the last one had a 46XY/47XXY karyotype (Groth *et al.*, 2013).

The diagnosis of secondary hypogonadism was made based on both clinical signs and hormonal parameters (i.e. T, gonadotrophins, etc.) as previously described (Giagulli & Carbone, 2006). However, all of those participants with secondary hypogonadism who had serum T levels below 150 ng/dL received a cerebral nuclear magnetic resonance imaging, as advised by major Scientific International Societies (Wang *et al.*, 2008; Bhasin *et al.*, 2010), with the aim of identifying adult onset forms due to pituitary adenoma.

Exclusion criteria consisted in the fact that none of the patients in both groups presented peripheral somatic neuropathy, severe obstructive sleep apnoea or kidney, liver, heart and lung failure. Nobody had acute cardiovascular events been reported in their medical history nor a family history of prostate or breast cancer. Serum prostate-specific antigen (PSA) levels and haematocrit values had not to be above 4 ng/mL and 55%, respectively.

Twelve out of 30 subjects belonging to the G1 group suffered from hypertension and, in particular, in 6/12 patients the hypertension were diagnosed between 3 and 5 years before the diagnosis of diabetes and one patient experienced diabetic retinopathy. Six out of 12 were treated with angiotensin-converting enzyme inhibitors, 2/12 with angiotensin-receptor blockers and, finally, 4/12 with calcium channel blockers. Six out of 30 participants of this group were ex-smokers. Finally, 9/30 subjects were given statins according to National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) (2002).

All men with pre-pubertal onset hypogonadism and the five adult hypogonadal subjects with pituitary organic pathology had been previously treated with esters of T intramuscularly (200 mg/ampoule) every 3 weeks since the time of the diagnosis. These patients were switched to the long-acting parenteral T undecanoate compound (1000 mg/ampoule) (TU) given every 12 weeks, according to the most recent evidence (Giagulli *et al.*, 2011a; Wang *et al.*, 2011).

Setting, design overview and therapeutic strategy

This 2-year retrospective observational study was a single physician's experience (VA Giagulli) conducted at a secondary referral endocrinology centre (Outclinic patients, Conversano Hospital, Bari, Italy). All data were obtained from our outpatient clinical records. The study protocol agreed with the 2000 version of the Declaration of Helsinki and was approved by the Local Ethical Committee (September, 2014: $n = 4545$) of the University of Bari. All participants signed an informed consent at the time of the first clinical visit as recommended by good clinical practise.

In particular, we used a patient-centred approach with a tailored treatment according to the clinical conditions of our participants as to the recommendations of state-of-the-art international guidelines (Apovian *et al.*, 2015; Handelsman *et al.*, 2015; Inzucchi *et al.*, 2015). In addition, we considered the possibility of negative effects of drug–drug interactions, given that our patients did not have a single clinical condition, being affected by multi-morbidity (Bourgeois *et al.*, 2010). Furthermore, the initial prescription of Met as well as of the further drug (add-on therapy) was made according to the rules of prescriptions and redeemability of the Italian Agency for Drug Administration (http://www.agenziafarmaco.gov.it/).

Therefore, pursuing the goal of obtaining weight loss and improving both the metabolic control and the sexual activity, our diabetic hypogonadal men were treated with 2000–3000 mg/day (in three divided doses after the main meals) of Met and TU 1000 mg deeply intramuscular given every 12 weeks as previously reported (Giagulli *et al.*, 2011b). Furthermore, both groups of participants were given a 1500 ± 200 kcal Mediterranean diet (50% carbohydrate, 20% protein and 30% fat) for the whole duration of the study and had to walk a brisk pace three times a week for a cumulative time of 150 min a week, as generally recommended (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, Cheng AY, 2013; Inzucchi *et al.*, 2015). Moreover, a validated multi-dimensional self-report instrument for the evaluation of male sex function known as IIEF 15 (Rosen *et al.*, 2002) were administered to our patients given that they complained of ED. To verify their diet adherence and the metabolic control, each patient was controlled by means of an interview and a clinical visit along with biochemical parameters evaluation every 3 months as it is usually done in our clinical practise. Everyone declared to have been compliant to the diet guidelines. For all subjects [16/30 for G1 (G1N) and 10/13 for G2 (G2N)] who did not achieve after 12 months the glycaemic target [HbA1c ≤7% (≤53 nmol/mol)] as recommended by the International Scientific Societies for the diabetic adults with short duration of diabetes and long life expectancy, and no significant cardiovascular diseases (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, Cheng AY, 2013; Handelsman *et al.*, 2015; Inzucchi *et al.*, 2015) as our patients were, liraglutide (1.2 μg/day) (L) at bed time was added to previous therapeutic regimen for a further 12 months (Handelsman *et al.*, 2015; Inzucchi *et al.*, 2015). Conversely, those subjects who obtained a stable metabolic control [14/30 for G1 (G1Y)

and 3/13 for G2 (G2Y)] continued to be treated with the previous therapy regimen (TRT + Met).

Considering the time when our patients filled in their sexual function questionnaires (IIEF 15), we decided to schedule our points in time for our clinical survey as follows: the day when they were administered the IIEF 15 questionnaire for the first time before starting TU treatment was regarded as the basal condition (first point in time: T1); after the first 12 months of combined TU and Met therapy when they were asked to fill in the IIEF 15 for the second time as the second point in time (T2); the end of the study was fixed after a further 12 months period of TU and Met therapy with (poor responders) or without (good responders) the addition of L, when IIEF 15 test was administered for the third time (third point in time: T3). Moreover, we analyzed measurements of serum T, sex hormone binding globulin (SHBG), PSA, glycosylated haemoglobin (HbA1c), haemoglobin (Hb), glycaemia (Gly), total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL) as well as SBP and DBP and anthropometric parameters (height, weight, WC and BMI) at every time point of the survey (T1, T2 and T3). Finally, the whole groups of hypogonadal men underwent DRE to assess some possible abnormal variation in the prostate volume at the scheduled times (T1, T2 and T3), respectively.

Methods

Blood samples were drawn between 08.00 and 09.00 h after an overnight fast, and the plasma was kept at −20°C until routinely processing.

Serum LH, FSH, T, SHBG and PSA levels were assessed by commercial immunometric assays (Immulite, EURO/DPC, UK). As previously described (Giagulli & Carbone, 2006; Giagulli *et al.*, 2011b), our normal ranges were 7.5 ± 2.6 UI/L for LH, 6.6 ± 2.5 UI/L for FSH, 450 ± 90 ng/dL for T (to convert ng/dL into nM/L multiply by 0.0347) and 45.4 ± 5 nmol/L for SHBG, respectively. Moreover, all these methods showed an intra- and inter-assay coefficient of variation <8 and <10%, respectively. Both serum FT and bioavailable T (BioT) fractions were calculated according to *Vermeulen*'s formula (Vermeulen *et al.*, 1999) and our normal calculated values were 12 ± 2.2 ng/dL for FT and 275 ± 65 ng/dL for BioT, respectively (Giagulli & Carbone, 2006; Giagulli *et al.*, 2011b).

HbA1c was measured by ionic-exchange chromatography-based automated HPLC analyzer (ADAMS A1c Menarini HA-8160, Firenze, Italy) (Sacks *et al.*, 2011). Plasma Gly levels were determined by the glucose oxidase method (Sclavo, Siena, Italy), while serum lipids (TC, TG and HDL) levels were determined by an automatic colorimetric method (Hitachi; Boehringer Mannheim, Mannheim, Germany), as previously reported (De Pergola *et al.*, 1993, 2013). Low-density lipoprotein cholesterol (LDL) was calculated using Friedewald's formula: LDL = TC − (HDL + TG/5). Serum Gly, TC and TG were reported in mg/dL (to convert into mM/L it should be multiplied by 0.0555, 0.0259 and 0.0113, respectively).

Statistics

Since no variables in the study turned out to be skewed, all data were given as the mean of the raw data ± SD unless otherwise stated.

Results were analyzed using non-parametric statistics, and differences between the two groups of pre- and post-pubertal hypogonadal diabetic patients were evaluated using the Mann–Whitney *U*-test. The paired two-tailed Student's *t*-test was performed to compare differences within group between time points.

An 'a priori' 5% was deemed statistically significant. Statistical analysis was performed by SPSS version 10.0 (Chicago, IL, USA).

RESULTS

In baseline condition (T1) the demographical and the clinical characteristics as well as the plasma metabolic parameters and hormonal levels of both groups showed some statistical difference that can be summarized as follows: the age (G1 = 53.4 ± 4.4 vs. G2 = 50.6 ± 4.3 years; $p < 0.01$), the duration of diabetes (G1 = 3.8 ± 0.8 vs. G2 = 3.0 ± 0.8 years; $p < 0.05$), serum Gly (G1 = 201.0 ± 22.0 vs. G2 = 184.4 ± 30.8 mg/dL; $p < 0.05$) and DBP values (G1 = 88.9 ± 4.4 vs. G2 = 85.8 ± 4.7 mm/Hg; $p < 0.05$) were statistically higher in G1 compared with G2; serum TC (G1 = 228.5 ± 20.4 vs. G2 = 193.7 ± 14.5 mg/dL; $p < 0.01$) and serum calculated LDL (G1 = 149.6 ± 19.7 vs. G2 = 111.0 ± 15.9 mg/dL; $p < 0.05$) were higher in G1, while serum TG (G1 = 198.3 ± 25.4; G2 = 289.41 ± 143.8 mg/dL; $p < 0.01$) was significantly higher in G2; finally, the IIEF score was statistically higher in G2 (G1 = 12.2 ± 2.3 vs. G2 = 14.0 ± 2.0; $p < 0.05$) compared with G1 (Tables 1 and 4).

The demographical, physical, metabolic and hormonal parameters of either groups of participants (G1 and G2) at the first point in time (T1) and after 12 months of combined therapy with Met (2000–3000 mg/day) and TU (1000 mg i.m. every 12 weeks) (T2) are shown in Tables 1 and 4, respectively. With the exception of DBP values and circulating HDL, BMI and WC and the metabolic parameters (serum Gly, HbA1c, TC, TG and calculated LDL) were significantly decreased, whereas plasma

Table 1 Clinical characteristics, metabolic and hormonal parameters in post-pubertal onset hypogonadal subjects (G1) at the observational starting point (T1) and after 12 months (T2) of therapy with Met and TU (1000 mg/12 week)

G1 (n = 30)	T1	T2	p
Age (years)	53.5 ± 4.4	–	–
Duration of diabetes (years)	3.8 ± 0.8	–	–
Height (cm)	171.1 ± 5.2	–	–
Weight (kg)	100.0 ± 8.9	96.5 ± 7.7	**<0.01**
BMI (kg/m^2)	34.2 ± 2.4	32.5 ± 2.7	**<0.01**
WC (cm)	104.1 ± 6.2	98.8 ± 5.7	**<0.01**
SBP (mmHg)	150.7 ± 13.4	148.6 ± 11.3	**<0.05**
DBP (mmHg)	88.9 ± 4.4	88.5 ± 3.9	<0.58
Gly (mg/dL)	201.0 ± 22.0	148.4 ± 18.7	**<0.001**
HbA1c (%)	8.8 ± 0.6	7.8 ± 0.6	**<0.001**
TC (mg/dL)	228.5 ± 20.4	219.7 ± 17.6	**<0.01**
TG (mg/dL)	198.3 ± 25.4	185.0 ± 23.6	**<0.001**
HDL (mg/dL)	38.9 ± 2.7	39.1 ± 2.5	<0.71
LDL (mg/dL)	149.6 ± 19.7	144.1 ± 16.3	**<0.01**
T (ng/dL)	278.4 ± 23.7	464.8 ± 63.8	**<0.001**
SHBG (nmol/L)	36.3 ± 3.5	37.3 ± 3.2	**<0.05**
FT (ng/dL)	5.2 ± 0.6	8.7 ± 1.5	**<0.001**
BioT (ng/dL)	120.5 ± 14.5	204.7 ± 35.1	**<0.001**
IIEF (score)	12.2 ± 2.3	14.5 ± 1.8	**<0.01**

Results were expressed as mean ± SD. Statistically significant p values are given in bold. Met, metformin; TU, testosterone undecanoate; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; Gly, glycaemia; HbA1c, glycosylated haemoglobin; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; T, testosterone; SHBG, sex hormone binding globulin; FT, free T; BioT, bioavailable T; IIEF, International Index of Erectile Function.

SHBG, T levels and calculated BioT and FT as well as IIEF values proved to be statistically increased at the end of 1 year of the combined TU and Met treatment (T2).

Tables 2 and 3 report the physical characteristics, the plasma metabolic parameters and hormonal levels of the two G1 subgroups subdivided in accordance with the achievement of glycaemic targets [recommended by the International Societies for the treatment of T2DM in adults (Handelsman *et al.*, 2015; Inzucchi *et al.*, 2015)]: those participants who did not achieve the glycaemic target (G1N; Table 2) after 1 year of combined therapy with Met and TU and those who did (G1Y; Table 3). On the other hand, Table 5 shows the physical characteristics, plasma metabolic parameters and hormonal levels of G2 subgroup who did not achieve the Gly targets (G2N) after 1 year of the combined therapy regimen (Met and TU).

Anthropometric parameters and clinical characteristics

TU, in combination with Met (T2) significantly lowered weight, WC, BMI and SBP in all the patients. The addition of L in poor responders (T3) improved these parameters in both groups (G1N and G2N) (Tables 2 and 5). Conversely, in G1Y participants at the end of the observation (T3) both weight (+0.8 ± 0.9%; $p < 0.04$) and WC (+1.6 ± 1.3%; $p < 0.01$) significantly increased while SBP and DBP values were higher than those assessed at T2 and very close to the measures at T1 (Table 3). Three participants of G2 (G2Y) who achieved the glycaemic goals for adult subjects with T2DM (Inzucchi *et al.*, 2015; Handelsman *et al.*, 2015) presented a slight rise in weight, WC, BMI and DBP values after 2 years of Met and TU combined therapy at the end of the study (T3) in comparison with second point in time (T2) (data not shown).

Glycaemic and lipid parameters

Although in G1N and G2N the combined therapy with Met and TU significantly improved both serum Gly, HbA1c and plasma lipid parameters such as calculated serum LDL and TG in comparison with baseline characteristics (T1) (Tables 2 and 5), they did not fulfil those criteria stated by major International Societies for the treatment of adult diabetic subjects (Inzucchi *et al.*, 2015; Handelsman *et al.*, 2015). As a consequence, the addition of L (1.2 μg/day) to the previous treatment allowed a further reduction in HbA1c up to 7.3% at T3, which is generally accepted as good metabolic control in adult diabetic subjects (Tables 2 and 5). In addition, both circulating TC and TG as well as calculated LDL were improved with the addition of the L therapy (T3) in both groups (G1N and G2N) (Tables 2 and 5).

Conversely, G1Y patients who obtained a significant reduction in lipid parameters and in particular, reached the Gly target [HbA1c %: 7.0 ± 0.1 (T2) vs. 8.2 ± 0.3 (T1); $p < 0.01$] by means of combined therapy with TU and Met at T1, presented a statistically raise in Gly (+7.5 ± 9.1 mg/dL; $p < 0.05$) and plasma HbA1c levels (+0.2 ± 0.1% (T3); $p < 0.05$) at the end of our study (T3) (Table 3).

Finally, plasma levels of HbA1c in the three subjects belonging to G3 group who kept on taking Met and TU regimen for 2 years as they reached the glycaemic target at T2, experienced a rise in circulating TC and TG as well as in HbA1c of about 0.5% at the end of our study (data not reported).

Hormonal parameters and IIEF

Testosterone undecanoate treatment allowed to obtain plasma levels of T and of its active biological fractions (FT and BioT) in the normal range of adult healthy men (Wang *et al.*, 2008; Bhasin *et al.*, 2010) in the whole group of participants (G1N, G1Y and G2N) until the end of our study (Tables 2, 3 and 5). Serum SHBG, however, was statistically increased at T2 (+1.1 ± 1.7%; $p < 0.05$) and T3 (+1.5 ± 1.0%; $p < 0.01$) in G1N and G2N (Tables 2 and 5), while it was higher than the values determined at T1 in G1Y men only at T2 (+1.5 ± 1.3%; $p < 0.02$) but not at T3 (Table 3).

Combined therapy with TU and Met significantly improved the IIEF score in adult obese men with T2DM and pre-pubertal onset hypogonadism (+2.4 ± 2.2%; $p < 0.05$ in G1N;

Table 2 Clinical characteristics, metabolic and hormonal parameters of the subgroup of poor responders among the post-pubertal onset hypogonadal men (G1N; $n = 16$) at the observational starting time point (T1), after Met plus TU for 12 months (T2) and after the addition of L (1.2 μg/day) for further 12 months (T3)

G1N ($n = 16$)	T1	T2	p1 (p)	T3	p2 (p)
Age (years)	52.7 ± 4.5	–	–	–	–
Duration of diabetes (years)	3.8 ± 0.8	–	–	–	–
Height (cm)	171.2 ± 5.7	–	–	–	–
Weight (kg)	102.7 ± 9.1	99.0 ± 7.6	**<0.01**	93.7 ± 6.3	**<0.01**
BMI (kg/m^2)	35.2 ± 2.3	34.0 ± 3.1	**<0.01**	32.6 ± 2.0	**<0.01**
WC (cm)	103.7 ± 7.0	99.1 ± 6.2	**<0.01**	92.5 ± 5.3	**<0.001**
SBP (mmHg)	155.8 ± 13.7	150.1 ± 12.0	**<0.01**	145.7 ± 9.6	**<0.001**
DBP (mmHg)	86.4 ± 4.5	85.5 ± 4.2	<0.07	82.5 ± 2.6	**<0.01**
Gly (mg/dL)	180.4 ± 24.8	155.5 ± 19.7	**<0.001**	130.3 ± 15.6	**<0.001**
HbA1c (%)	9.1 ± 0.4	8.3 ± 0.3	**<0.001**	7.3 ± 0.3	**<0.001**
TC (mg/dL)	226.6 ± 20.8	216.8 ± 16.8	**<0.01**	206.9 ± 10.8	**<0.01**
TG (mg/dL)	202.8 ± 28.6	190.1 ± 27.3	**<0.001**	175.4 ± 19.4	**<0.001**
HDL (mg/dL)	38.6 ± 2.8	39.0 ± 2.6	<0.61	39.6 ± 3.0	<0.07
LDL (mg/dL)	147.3 ± 21.3	133.6 ± 15.4	**<0.001**	125.5 ± 10.7	**<0.001**
T (ng/dL)	285.8 ± 25.0	466.1 ± 63.6	**<0.001**	481.7 ± 57.3	**<0.001**
SHBG (nmol/L)	36.0 ± 3.2	37.1 ± 2.8	**<0.05**	39.1 ± 2.2	**<0.01**
FT (ng/dL)	5.4 ± 0.6	8.7 ± 1.6	**<0.001**	9.0 ± 1.3	<0.13
BioT (ng/dL)	124.6 ± 13.4	204.0 ± 37.1	**<0.001**	211.1 ± 30.0	<0.14
IIEF (score)	12.2 ± 2.2	14.6 ± 1.7	**<0.05**	19.9 ± 2.0	**<0.001**

Statistically significant p values are reported in bold. p1, significance T2 vs. T1; p2, significance T3 vs. T2; Met, metformin; TU, testosterone undecanoate; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; Gly, glycaemia; HbA1c, glycosylated haemoglobin; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; T, testosterone; SHBG, sex hormone binding globulin; FT, free T; BioT, bioavailable T; IIEF, International Index of Erectile Function.

Table 3 Clinical characteristics, metabolic and hormonal parameters of good responders among patients belonging to the post-pubertal onset hypogonadal group (G1Y; n= 14) at starting time point (T1), after 12 months of Met and TU therapy (T2) and after continuing the same therapy regimen (Met and TU) for further 1 year. (T3)

G1Y (n = 14)	T1	T2	p1 (p)	T3	p2 (p)
Age (years)	55.0 ± 4.0	–	–	–	–
Duration of diabetes (years)	3.8 ± 0.9	–	–	–	–
Height (cm)	170.9 ± 4.4	–	–	–	–
Weight (kg)	95.0 ± 6.2	91.4 ± 5.4	**<0.01**	92.2 ± 6.0	**<0.04**
BMI (kg/m^2)	32.5 ± 1.4	31.1 ± 1.2	**<0.01**	31.4 ± 1.3	<0.16
WC (cm)	102.2 ± 2.3	94.3 ± 2.6	**<0.01**	95.9 ± 2.7	**<0.01**
SBP (mmHg)	141.5 ± 5.8	138.5 ± 6.7	**<0.01**	141.0 ± 8.1	<0.06
DBP (mmHg)	83.0 ± 3.5	82.5 ± 2.6	<0.32	84.0 ± 3.9	<0.18
Gly (mg/dL)	165.2 ± 10.9	138.3 ± 10.3	**<0.01**	145.8 ± 10.9	**<0.05**
HbA1c (%)	8.2 ± 0.3	7.0 ± 0.1	**<0.01**	7.5 ± 0.2	**<0.05**
TC (mg/dL)	231.8 ± 20.3	213.5 ± 19.8	**<0.01**	208.4 ± 14.1	<0.44
TG (mg/dL)	190.2 ± 16.6	175.9 ± 11.2	**<0.01**	183.9 ± 10.7	<0.17
HDL (mg/dL)	39.6 ± 2.5	40.4 ± 2.3	<0.07	41.1 ± 1.8	<0.42
LDL (mg/dL)	153.9 ± 16.6	140.6 ± 17.7	**<0.01**	132.3 ± 12.5	<0.28
T (ng/dL)	265.0 ± 13.9	462.4 ± 67.4	**<0.01**	463.3 ± 66.0	<0.68
SHBG (nmol/L)	37.0 ± 4.1	38.5 ± 4.0	**<0.02**	38.5 ± 3.5	<0.74
FT (ng/dL)	4.9 ± 0.6	8.7 ± 1.4	**<0.01**	8.8 ± 1.6	<0.80
BioT (ng/dL)	113.0 ± 14.0	206.1 ± 33.1	**<0.01**	206.8 ± 37.0	<0.65
IIEF (score)	12.2 ± 2.5	14.7 ± 2.0	**<0.05**	14.3 ± 1.9	<0.44

Statistically significant p values are given in bold. p1, significance T2 vs. T1; p2, significance T3 vs. T2; Met, metformin; TU, testosterone undecanoate; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; Gly, glycaemia; HbA1c, glycosylated haemoglobin; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; T, testosterone; SHBG, sex hormone binding globulin; FT, free T; BioT, bioavailable T; IIEF, International Index of Erectile Function.

+2.5 ± 3.6%; $p < 0.05$ in G1Y and +2.5 ± 1.8%; $p < 0.01$) at T2 in comparison with baseline condition (Tables 2, 3 and 5), while in those patients belonging to the G1Y group it did not at the third point in time (T3) (Table 3). However, anti-glycaemic two drugs combination (Met and L) treatment plus TU regimen led to an almost complete resolution of ED (Rosen *et al.*, 2002) in the G1N and G2N subjects at third point in time (T3) of our study (Table 2 and 5).

Safety

No significant variation in prostate volume was found at DRE in all participants. Conversely, a significant rise in haematocrit values (G1 + 2.56 ± 0.90%, $p \leq 0.05$; G2 + 2.67 ± 0.89% $p = <0.05$) and in circulating PSA levels both in G1 (+0.35 ± 0.22 ng/mL $p \leq 0.01$) and in G2 (+0.32 ± 0.24 ng/mL $p \leq 0.05$) was reported (data not shown). However, these parameters remained stable within the normal range throughout the observational study in both groups.

None complained of the major adverse cardiovascular events or of prostate cancer throughout the entire period of the observation.

Finally, only two participants belonging to G1 group complained of vomiting and nausea during the first month of therapy with L which, however, did not cause the dropout of this treatment.

DISCUSSION

It is generally accepted that obesity, dyslipidemia, hypertension, MetS and diabetes mellitus are primary risk factors for the ED development (Seftel *et al.*, 2004; Lewis *et al.*, 2010; Glina *et al.*, 2013). In addition, to date, accumulating evidence has given weight to define a subtle link among hypogonadism, obesity, MetS with or without T2DM (Andersson *et al.*, 1994; Pei *et al.*, 1998; Dhindsa *et al.*, 2004; Bojesen *et al.*, 2006; Ding *et al.*, 2006; Kapoor *et al.*, 2007; Grossmann *et al.*, 2008; O'Connor *et al.*, 2011; Glina *et al.*, 2013) and ultimately vascular diseases including ED in men (Montorsi *et al.*, 2006). As a result, ED could be regarded as the main clinical sign whose pathophysiological mechanism may involve both serum T deficit and metabolic disorders in men.

In diabetic men, the duration of the disease, age and peripheral neuropathy are directly associated with ED severity, as does poor glycaemic control (Romero *et al.*, 2000; Wesslle *et al.*, 2011). Furthermore, although the majority of previous uncontrolled studies (Boyanov *et al.*, 2003; Kapoor *et al.*, 2006; Saad *et al.*, 2013; Zitzmann *et al.*, 2013), some of those controlled (Jones *et al.*, 2011; Hackett *et al.*, 2013, 2014) and a meta-analysis (Corona *et al.*, 2011) highlighted that T supplementation may reduce body weight and improve glycaemic control in obese men with plasma T levels in the low–normal range (Wang *et al.*, 2008, 2011; Bhasin *et al.*, 2010) and with or without T2DM, the contribution of TRT in obese diabetic men with T deficit and ED related to the weight loss, the improvement of metabolic control and even ED, is debated (Seftel *et al.*, 2004; Lewis *et al.*, 2010; Glina *et al.*, 2013). Indeed, two recent randomized, double-blind, parallel, placebo-controlled trials have not confirmed those encouraging results (Gianatti *et al.*, 2014a,b) and a recent meta-analysis conducted by Corona *et al.* (2014a) has pointed out that the efficacy of T supplementation for ED treatment is uncertain in those subjects who are not affected by overt hypogonadism.

This being the case, we decided to retrospectively verify our data and especially whether in 'a real-life setting' our diabetic men who also suffered from overt hypogonadism and ED, subdivided into two groups according to the time in which the hypogonadism occurred (G1 = adult onset form and G2 = prepubertal onset), could benefit from receiving either combined T supplementation and Met or, in case of failure of reaching glycaemic target, the addition of L to the previous treatment regimen (TRT + Met) in order to obtain a better glycaemic control, to reduce weight and to improve ED eventually.

The baseline data of the two groups were comparable with those that have been reported in the literature so far (Romero *et al.*, 2000; Wesslle *et al.*, 2011). Indeed, the G1 patients who were older and affected by diabetes for a longer time when compared to the G2 men, showed a poor metabolic control and a worse IIEF score. Conversely, circulating T and its calculated active fractions (FT and BioT) in our participants did not turn out to be in the normal range for young adult men (T1) (Tables 1 and 4) (Wang *et al.*, 2008; Bhasin *et al.*, 2010). Hence, we started giving TU therapy since it allows to achieve stable levels of T in the range of young healthy men (Giagulli *et al.*, 2011a; Wang *et al.*, 2011; Corona *et al.*, 2014b).

In both groups of hypogonadal and diabetic men receiving TU and Met at T2 there was a significant improvement of the clinical characteristics (weight, BMI, WC, SBP), of the metabolic parameters (Gly, BbA1c, TC, TG, LDL) and of the IIEF score (Saad *et al.*, 2013; Zitzmann *et al.*, 2013). Indeed, although these results might be better attributed to T supplementation rather to Met therapy since T proves to be effective both in significantly reducing weight and in improving

glucose and lipid metabolism and ED (Isidori *et al.*, 2005; Corona *et al.*, 2011; Jones *et al.*, 2011; Hackett *et al.*, 2013,2014), while Met, beside its universally well-known anti-glycaemic role, is generally considered as a drug with weight-neutral effect (Handelsman *et al.*, 2015; Inzucchi *et al.*, 2015), and as not having an intrinsic positive effect on the lipid profile (Wulffelè *et al.*, 2004), it is necessary to point out that we were not able to identify each drug (T or Met) and/or lifestyle changes (or all together those factors) as predominantly having caused those clinical and metabolic improvements. However, in our experience, although that treatment regimen and serum T and its bioavailable fractions (FT and BioT) were in the normal range for adult men (Wang *et al.*, 2008; Bhasin *et al.*, 2010), neither clinical signs (BMI), metabolic parameter (HbA1c, TC and LDL) nor the IIEF score reached those values that were considered as appropriate targets for the treatment in young adult obese subjects affected by T2DM without severe complications (Apovian *et al.*, 2015; Handelsman *et al.*, 2015; Inzucchi *et al.*, 2015; Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, Cheng AY, 2013; Rosen *et al.*, 2002) (Tables 1 and 4). Consequently, for all those participants belonging to either groups of diabetic men both with adult (G1) and pre-pubertal (G2) onset hypogonadism who did not achieve the glycaemic target [HbA1c ≤7% (≤53 nmol/mol)], L (1.2 μg/day) was added at bed time to the previous therapeutic regimen for a further 12 months period (Tables 2 and 5) (Robinson *et al.*, 2013; International Diabetes Federation Guideline Development Group, 2014; Handelsman *et al.*, 2015; Inzucchi *et al.*, 2015). Conversely, those subjects who reached the glycaemic target were not added other drugs but exclusively observed for another year (T3) (Table 3). That choice arose from the well-known properties of GLP-1 agonists of reducing weight, blood pressure and TC in obese patients with and without diabetes (Visboll *et al.*, 2012; Robinson *et al.*, 2013) which would be expected to have a beneficial effect both on metabolic targets (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, Cheng AY, 2013; International Diabetes Federation Guideline Development Group, 2014; Handelsman *et al.*, 2015; Inzucchi *et al.*, 2015) and on the vascular system (and hence on ED) (Seftel *et al.*, 2004; Lewis *et al.*, 2010; Glina *et al.*, 2013). Indeed, from a pathophysiologic point of view, we speculated that the L co-administration could have potentiated the positive effect of T supplementation on metabolic

Table 4 Clinical characteristics, metabolic and hormonal parameters in pre-pubertal onset hypogonadal subjects (G2) at the start time point (T1) of the observation and after 12 months (T2) of therapy with Met and TU (1000 mg/12 week)

G2 (n = 13)	T1	T2	p
Age (years)	50.6 ± 4.3	–	–
Duration of diabetes (years)	3.0 ± 0.8	–	–
Height (cm)	172.4 ± 2.4	–	–
Weight (kg)	103.4 ± 9.7	100.1 ± 7.8	**<0.05**
BMI (kg/m^2)	34.7 ± 2.3	33.5 ± 1.9	**<0.05**
WC (cm)	105.1 ± 10.3	102.1 ± 9.4	**<0.01**
SBP (mmHg)	149.6 ± 9.0	145.6 ± 8.5	**<0.05**
DBP (mmHg)	88.5 ± 4.7	84.5 ± 3.1	<0.06
Gly (mg/dL)	184.4 ± 30.8	162.1 ± 20.4	**<0.001**
HbA1c (%)	8.6 ± 0.4	7.9 ± 0.4	**<0.01**
TC (mg/dL)	193.7 ± 14.5	81.1 ± 24.7	**<0.001**
TG (mg/dL)	289.4 ± 143.8	249.5 ± 99.2	**<0.001**
HDL (mg/dL)	36.8 ± 3.8	38.1 ± 3.2	<0.14
LDL (mg/dL)	111.0 ± 15.9	99.5 ± 20.0	**<0.02**
T (ng/dL)	309.4 ± 29.7	412.3 ± 47.5	**<0.001**
SHBG (nmol/L)	36.6 ± 2.7	37.6 ± 2.4	**<0.05**
FT (ng/dL)	5.8 ± 0.7	7.7 ± 1.2	**<0.001**
BioT (ng/dL)	135.9 ± 15.8	181.8 ± 27.1	**<0.001**
IIEF (score)	14.0 ± 2.0	16.3 ± 3.2	**<0.01**

Results were expressed as mean ± SD. Statistically significant p values are given in bold. Met, metformin; TU, testosterone undecanoate; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; Gly, glycaemia; HbA1c, glycosylated haemoglobin; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; T, testosterone; SHBG, sex hormone binding globulin; FT, free T; BioT, bioavailable T; IIEF, International Index of Erectile Function.

Table 5 Clinical characteristics and metabolic and hormonal parameters of the poor responders among patients belonging to the prepubertal onset hypogonadal group (G2N; n = 10) at T1, after 1 year of Met and TU therapy (T2) and after the addition of L (1.2 μg/day) for further 12 months (T3)

G2 N (n = 10)	T1	T2	p1 (p)	T3	p2 (p)
Age (years)	50.0 ± 4.7	–	–	–	–
Duration of diabetes (years)	3.2 ± 0.8	–	–	–	–
Height (cm)	172.9 ± 2.5	–	–	–	–
Weight (kg)	105.3 ± 9.8	102.5 ± 7.1	**<0.05**	98.0 ± 8.3	**<0.01**
BMI (kg/m^2)	34.8 ± 2.6	33.5 ± 2.0	**<0.05**	32.5 ± 2.3	**<0.05**
WC (cm)	106.8 ± 11.0	104.5 ± 9.9	**<0.05**	99.2 ± 9.8	**<0.001**
SBP (mmHg)	151.5 ± 8.8	147.5 ± 8.3	**<0.05**	142.0 ± 8.6	**<0.01**
DBP (mmHg)	89.5 ± 4.4	87.7 ± 2.6	<0.06	85.9 ± 4.1	**<0.05**
Gly (mg/dL)	211.4 ± 25.7	169.9 ± 15.3	**<0.01**	133.9 ± 12.8	**<0.01**
HbA1c (%)	8.8 ± 0.8	8.2 ± 0.3	**<0.04**	7.4 ± 0.6	**<0.01**
TC (mg/dL)	189.9 ± 12.4	176.5 ± 25.7	**<0.01**	175.8 ± 19.0	<0.15
TG (mg/dL)	319.1 ± 152.7	270.3 ± 104.9	**<0.01**	226.0 ± 48.7	**<0.001**
HDL (mg/dL)	35.7 ± 3.7	37.1 ± 3.0	<0.34	37.9 ± 3.5	<0.12
LDL (mg/dL)	105.6 ± 12.4	94.3 ± 19.5	**<0.05**	92.4 ± 18.9	**<0.05**
T (ng/dL)	304.8 ± 30.4	395.5 ± 40.0	**<0.01**	420.0 ± 27.5	**<0.01**
SHBG (nmol/L)	37.1 ± 2.6	38.4 ± 2.0	**<0.02**	40.8 ± 1.5	**<0.02**
FT (ng/dL)	5.6 ± 0.7	7.2 ± 0.8	**<0.01**	7.6 ± 0.6	<0.16
BioT (ng/dL)	132.8 ± 16.2	170.7 ± 18.0	**<0.01**	178.4 ± 13.6	<0.18
IIEF (score)	14.2 ± 1.8	16.5 ± 1.6	**<0.01**	19.9 ± 1.1	**<0.001**

Statistically significant p values are in bold. p1, significance T2 vs. T1; p2, significance T3 vs. T2; Met, metformin; TU, testosterone undecanoate; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; Gly, glycaemia; HbA1c, glycosylated haemoglobin; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; T, testosterone; SHBG, sex hormone binding globulin; FT, free T; BioT, bioavailable T; IIEF, International Index of Erectile Function.

parameters and on vascular function in those patients. As a matter of fact, increasing serum T by letrozole (aromatase inhibitor) therapy during a weekly hyperinsulinemic–euglycaemic clamp, was shown to bring about a rise in serum levels of glucose-dependent insulinotropic polypeptide and in insulin sensitivity in a group of healthy young men (Lapauw *et al.*, 2010). Similarly, L treatment led to greater weight loss and improved insulin sensitivity in overweight and/or obese individuals (Kim *et al.*, 2013). Moreover, several lines of evidence argue that T treatment might decrease insulin resistance by means of promoting the commitment of pluripotent stem cells into the myogenic lineage and inhibiting their differentiation into mature adipocytes as well as regulating their metabolism in ways that lowers insulin resistance (Singh *et al.*, 2003), while in an in vitro study, GLP-1 was able to raise lipolysis in adipocytes in a dose-dependent manner (Vendrell *et al.*, 2011; Russo *et al.,* 2015). In a meta-analysis Isidori *et al.* (2005) demonstrated that TRT can reduce serum TC especially in men with lower T levels and, in the same manner, a meta-analysis study proved that GLP-1 treatment reduced circulating TC in overweight and/or obesity subjects either diabetics or not (Visboll *et al.*, 2012). Finally, in the vascular system and in endothelial cells, in particular, GLP-1 and T showed, respectively, multiple protective effects that can sum up as follows: (i) GLP-1 and its agonist (L) increased eNOS phosphorylation and NO production via a 5′AMP-activated protein kinase-dependent pathway in human umbilical vein endothelial cells cultures (Hattori *et al.*, 2010) and Akt and eNOS phosphorylation, and subsequent NO production in human coronary artery endothelial cells (Erdogdu *et al.*, 2010); (ii) L prevented the rise in plasminogen activator inhibitor type-1 and vascular cell adhesion molecule-1 in response to tumour necrosis factor-α or hyperglycaemia in the human umbilical vein endothelial cell line (Liu *et al.*, 2009); (iii) different studies have demonstrated vascular relaxing effects of T on vascular smooth muscle, probably via inhibition of L-type calcium channels (Montano *et al.*, 2008); (iv) T can affect the penile structure and its vessels co-regulating erection mechanisms by up-regulating the type 5 phosphodiesterase gene and its protein (Morelli *et al.*, 2004), also reducing the vasoconstrictor effect of RhoA/Rho-kinase pathway (Wingard *et al.*, 2003).

In fact, the addition of L to combined therapeutic regimen consisting of TU plus Met (T3) allowed to reach the glycaemic and lipid targets (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, Cheng AY, 2013; International Diabetes Federation Guideline Development Group, 2014; Handelsman *et al.*, 2015; Inzucchi *et al.*, 2015) and to improve both clinical characteristics (weight, WC and SBP) and, as a consequence, nearly completely restored a normal IIEF score in G1N and G2N subjects (Tables 2 and 5), whereas G1Y men and three patients belonging to G2 who were not given the L therapy as the glycaemic target had been reached after the first year of combined therapy with TU and Met (G2Y), showed the initial clinical signs and metabolic parameters of anti-glycaemic therapy failure (Table 3). Interestingly, circulating SHBG significantly rose at T2 (but not at T3) in G1Y as well as at T2 and T3 in G1N and G2N whenever the metabolic control and especially the glycaemic target have been reached (Tables 2, 3 and 5). This result might be due to the improvement of insulin resistance by means of both the weight loss (Corona *et al.*, 2013) and the achievement of metabolic goals (Tong *et al.*, 2014). Similarly, the sustained progressive rise in plasma T levels obtained in those participants who had the better metabolic control, might be a consequence both of an increase in serum SHBG and of a drop in T clearance due to its volume redistribution that might result from the weight loss and the achievement of the glycaemic target (Kitabchi *et al.*, 2001).

Our result should be interpreted cautiously given the retrospective observational nature of the study, to the fact that it is based on a small number of participants and to the effect of L treatment which cannot be separated from the one originating from the selection of patients in the absence of an untreated control group. However, it has some main points that should be pointed out: it was conducted in a 'real-life setting' for a long observational period and the participants had poor metabolic control at the start point of observational period, while the previous randomized, placebo-controlled studies were shorter and often performed in well-controlled patients (Corona *et al.*, 2014a; International Diabetes Federation Guideline Development Group, 2014).

In conclusion, this retrospective study showed that in men suffering from T2DM and different forms of hypogonadism, the duration of the metabolic disease seems to affect the clinical and biochemical characteristics more than the time of onset of T deficit (pre- or post-pubertal onset). In addition, in men complaining of ED with the coexistence of hypogonadism and T2DM, TRT along with weight-reducing anti-hyperglycaemic compounds (in particular GLP-1 agonists) should be taken into consideration not only to maintain serum T levels stable in the normal range for young healthy men, but also to lower hyperglycaemia and hyperlipidaemia until reaching their treatment targets and to reduce weight in order to considerably improve ED. However, we need large, randomized, placebo-controlled studies to further evaluate the effectiveness of TRT on both the metabolic parameters and ED in obese, diabetic men with T deficit and ED.

CONFLICT OF INTEREST

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