Testosterone undecanoate improves lipid profile in patients with type 1 diabetes and hypogonadotrophic hypogonadism

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Abstract. Testosterone deficiency (Td) has been associated with the metabolic syndrome. Few studies have evaluated this condition in type 1 diabetes (T1D). The primary aim of this study was to evaluate the effectiveness of testosterone undecanoate (TU) on insulin sensitivity, glycemic control, anthropometric parameters, blood pressure and lipid profile in patients with Td and T1D. We performed a randomized placebo-controlled multicenter study. Inclusion criteria: a) age ≥ 18 years; b) autoimmune diabetes; c) Td (total testosterone <10 nmol/L or calculated free testosterone <225 pmol/L and low/normal LH; d) ability to sign informed consent; e) comply with the study protocol. Exclusion criteria: a) pituitary tumor, empty sella, hyperprolactinemia, panhypopituitarism or secondary hypogonadism; b) contraindications for treatment with testosterone undecanoate (TU); c) patients who did not agree to sign their informed consent. Six patients were randomly assigned to testosterone undecanoate (TU) treatment and 7 to placebo with the following dosing schedule: baseline, 6 weeks and 16 weeks. Blood test, anthropometric parameters, blood pressure and insulin sensitivity were determined at baseline, 6, 16 and 22 weeks. No differences were observed regarding insulin sensitivity, HbA1c or basal glucose, anthropometric parameters or blood pressure. At 22 weeks, the decrease in total cholesterol was 37.4 ± 27.5 mg/dL in the TU group compared with an increase of 13.2 ± 17.8 mg/dL in the placebo group (P<0.005), and LDL cholesterol concentration decreased 30.2 ± 22.1 mg/dL, compared with an increase of 10.5 ± 13.4 mg/dL in the placebo group (P=0.004). We conclude that treatment with TU in patients with T1D and Td improves lipid profile, with no effects on metabolic control or anthropometric parameters.

Key words: Type 1 diabetes, Metabolic syndrome, Lipid profile, Testosterone deficiency

Abbreviations : CUN-BAE, Clinica Universitaria de Navarra-Body Adiposity Estimator; eGDR, estimated glucose disposal rate; HDL-c, high-density lipoprotein cholesterol; IIEF-5, International Index of Erectile Function; LDL-c, low-density lipoprotein cholesterol; LH, luteinizing hormone; MRI, magnetic resonance imaging; T1D, type 1 diabetes; T2D, type 2 diabetes; Td, testosterone deficiency; TU, testosterone undecanoate

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In patients with T1D, the initial cause of abnormal carbohydrate metabolism is autoimmune and not due to excess adipose tissue, as occurs in T2D. However, in recent years the rising prevalence of overweight and obesity in patients with T1D has been highlighted, reaching over 50% in some studies [15], and has been particularly associated with a higher percentage of metabolic syndrome [16] and microvascular complications [16, 17].

Given the relationship among testosterone deficiency, metabolic syndrome and type 2 diabetes, the increased frequency of ‘double diabetes’ and the lack of studies in patients with T1D, we conducted a randomized clinical trial in male T1D patients with hypogonadotropic hypogonadism, the primary aim of which was to evaluate the effectiveness of testosterone replacement therapy on insulin sensitivity, glycemic control, anthropometric parameters, blood pressure and lipid profile. The secondary objective was to analyze changes in the International Index of Erectile Function (IIEF-5) after testosterone undecanoate (TU) therapy in these patients.

**Materials and Methods**

A randomized placebo-controlled multicenter study, which enrolled T1D patients with hypogonadotropic hypogonadism treated at diabetes units of 3 urban hospitals in Barcelona between July 2013 and December 2014, was conducted. A single 1:1 randomization list was generated for the three centers and patients were centrally assigned to one of the two arms, consecutively. Both, researchers and patients, were blinded. Inclusion criteria were: a) age ≥ 18 years; b) diagnosis of autoimmune diabetes; c) hypogonadotropic hypogonadism (total testosterone levels <10 nmol/L (< 2.99 ng/mL) or calculated free testosterone levels < 225 pmol/L (< 65 pg/mL), all associated with low or normal gonadotropin levels (luteinizing hormone [LH] < 8.6 mIU/mL) on two separate early-morning days at least 1 week apart); d) ability to sign their informed consent; and e) comply with the study protocol. The study protocol was approved by the local Ethics Committee and the Spanish Agency for Medicines and Health Products (AEMPS), and it was registered in the European Clinical Trials database (EUDRA-CTe number: 2012-000291-42).

Magnetic resonance imaging (MRI) focused on the pituitary was performed in all candidate patients. Exclusion criteria were: a) presence of pituitary tumor, empty sella, hyperprolactinemia, panhypopituitarism or secondary hypogonadism; b) patients with contraindications for treatment with TU; and c) failure to agree to sign the informed consent form.

**Measurements**

Patients who met the inclusion criteria were randomized to receive TU 1,000 mg or placebo at baseline, 6 weeks and 16 weeks. At each visit and 6 weeks after the last TU administration, extractions were made for analysis and anthropometric parameters, blood pressure and insulin sensitivity were determined. Prior to randomization and at week 22, the International Index of Erectile Function (IIEF-5), a self-administered questionnaire consisting of 5 items with five response options each and a score of 5-25, was completed [18].

Weight, height, waist-hip circumference and blood pressure were measured by standard methods. Diabetes complications were assessed by a specialist in diabetology. Fasting glucose, HbA1c, lipid profile, albumin, microalbumin/creatinine ratio, total testosterone, LH, follicle-stimulating hormone and sex hormone-binding globulin concentrations were measured. Free serum testosterone level was calculated using the Vermeulen formula.

Insulin resistance was calculated using the estimated glucose disposal rate (eGDR) according to the following equation: \[24.31 - (12.22 \times \text{waist to hip ratio}) - (3.29 \times \text{hypertension}) - (0.57 \times \text{HbA1c})\] units are expressed in milligrams per kilogram −1 per minute −1. Hypertension was = 1 if blood pressure was ≥ 140/90 mm Hg (or on medications) and HbA1c the percentage of HbA1c [19]. The metabolic syndrome was diagnosed in accordance with the modified criteria of the National Cholesterol Education Program-Adult Treatment Panel III, and the percentage of body fat was calculated using the CUN-BAE formula (body fat (%) = \(-44.988 + (0.503 \times \text{age}) + (10.689 \times \text{gender}) + (3.172 \times \text{BMI}) - (0.026 \times \text{BMI}^2) + (0.181 \times \text{BMI} \times \text{gender}) - (0.02 \times \text{BMI} \times \text{age}) - (0.005 \times \text{BMI}^2 \times \text{gender}) + (0.00021 \times \text{BMI}^2 \times \text{age}), \) where male = 0; BMI was expressed as kg/m^2 and age in years), validated in the Spanish population [20].

**Statistical analysis**

Sample size calculation: Accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, 6 sub-
Screening was performed in 202 T1D patients of whom 21 had hypogonadotropic hypogonadism, constituting a prevalence of 10.4% (95% CI: 6.2-14.6%). Six patients were excluded owing to contraindications for TU treatment (5 benign prostatic hyperplasia, 1 polyglobulia), one had an empty sella on MRI and one refused to give his consent. Of the total number of patients, one in each group left the study due to causes unrelated to the treatment. The data analysis was made on an intention-to-treat basis.

Mean patient age was 46.3 ± 9.8 years and mean duration of T1D 21.2 ± 12.4 years. Eighty-four percent of patients with hypogonadism had the metabolic syndrome and 92.3% were overweight or obese. No differences were found in baseline characteristics between groups (Table 1).

As expected, at 22 weeks, total and free testosterone were higher in TU group than in the placebo group (15.5 ± 5.7 nmol/L vs. 9.3 ± 5.1 nmol/L and 450.3 ± 157.7 pmol/L vs. 182.8 ± 87.2 pmol/L, respectively). No differences were found in SHBG, insulin sensitivity, HbA1c or basal glucose, anthropometric parameters, blood pressure or daily insulin requirements (Table 2). Regarding lipid profile, the TU group showed a reduction in total and LDL cholesterol concentrations from the second treatment dose. At 22 weeks, the drop in total cholesterol was 37.4 ± 27.5 mg/dL in the TU group compared with an increase of 13.2 ± 17.8 mg/dL in the placebo group (P<0.005). In the same week, LDL cholesterol concentration decreased 30.2 ± 22.1 mg/dL, compared with an increase of 10.5 ± 13.4 mg/dL in the placebo group (P=0.004). A greater reduction in total cholesterol/HDL cholesterol ratio, and triglycerides was observed in the TU group versus the placebo group (Table 2). Interestingly, a greater reduction in triglycerides/HDL cholesterol ratio, a known surrogate marker of insulin resistance [21], was also observed in the TU group (Table 2). All patients except one in each group received statin therapy, without dose modifications during the study.

With respect to the secondary endpoint, a trend towards an IIEF-5 score improvement was observed in the TU group (± 5.0 ± 7.5) compared to the placebo group (± 0.5 ± 2.1), without statistical significance. No differences in prostate-specific-antigen (PSA) were observed during the treatment period (Table 2). One patient in the placebo group reported pain at the injection site, which resolved with conventional analgesia in less than 48 hours. The group treated with TU showed an increase in hematocrit and hemoglobin levels (Table 2); however, withdrawal of the medication was not necessary in any case, and no complications occurred.

Discussion

The main findings of the present study were the neutral effect on insulin sensitivity and anthropometric parameters with TU therapy, although an improvement in lipid profile was observed.

In patients with type 2 diabetes, reductions in total cholesterol of 15-17 mg/dL with 3-6 months of testosterone administration, associated with a decrease in weight and waist circumference have been observed [6, 11]. In the present study, differences from placebo at 22 weeks were 50.6 mg/dL in total cholesterol and 40.7 mg/dL in LDL cholesterol. This important fall in LDL cholesterol levels without changes in metabolic and anthropometric parameters suggest that testosterone has an important role in regulating serum cholesterol metabolism, although, to date, the potential molecular mechanisms whereby testosterone deficiency affects cholesterol metabolism are unclear. Experimental studies have shown that testosterone deficiency induces PCSK9 expression and reduces LDLR expression in the livers of castrated male pigs.
| Characteristic                        | Overall (n=13) | Testosterone undecanoate (n=6) | Placebo (n=7) | \( P \) |
|--------------------------------------|----------------|--------------------------------|---------------|--------|
| Age (years ± SD)                     | 46.3 ± 9.8     | 47.9 ± 7.3                     | 45.2 ± 11.7   | 0.653  |
| T1DM duration (years ± SD)          | 21.2 ± 12.4    | 21.7 ± 13.0                    | 20.9 ± 12.9   | 0.922  |
| BMI (kg/m\(^2\) ± SD)               | 32.7 ± 7.0     | 30.2 ± 3.3                     | 34.5 ± 8.5    | 0.312  |
| 20-24 kg/m\(^2\) (n (%))            | 1 (7.7)        | 0 (0)                          | 1 (14.3)      | 0.462  |
| 25-30 kg/m\(^2\) (n (%))            | 3 (23.1)       | 3 (50)                         | 1 (14.3)      |        |
| > 30 kg/m\(^2\) (n (%))             | 9 (69.2)       | 3 (50)                         | 5 (74.4)      |        |
| Metabolic syndrome, n (%)           | 11 (84)        | 5 (83.3)                       | 6 (85.7)      | 0.217  |
| HbA\(_1c\) (% ± SD)                 | 8.18 ± 1.4     | 7.60 ± 0.5                     | 8.6 ± 1.7     | 0.246  |
| HbA\(_1c\) (mmol/mol)               | 65.8 ± 15.3    | 55.2 ± 4.8                     | 74.0 ± 21.7   |        |
| eGDR (mg/kg\(^{-1}\)·min\(^{-1}\) ± SD) | 5.42 ± 1.7     | 6.12 ± 2.2                     | 4.9 ± 1.3     | 0.959  |
| Insulin requirements (IU/kg/d ± SD)  | 0.86 ± 0.1     | 0.91 ± 0.1                     | 0.82 ± 0.2    | 0.386  |
| Hip circumference (cm ± SD)          | 112.7 ± 16.0   | 105.8 ± 4.9                    | 117.7 ± 19.5  | 0.216  |
| Waist circumference (cm ± SD)        | 108.4 ± 15.8   | 103.0 ± 10.7                   | 112.3 ± 18.4  | 0.340  |
| Waist-to-hip ratio (ratio ± SD)      | 0.96 ± 0.1     | 0.97 ± 0.1                     | 0.95 ± 0.1    | 0.643  |
| Fat mass (% ± SD)                    | 33.6 ± 8.5     | 31.2 ± 4.2                     | 35.3 ± 10.6   | 0.443  |
| Systolic BP (mm Hg ± SD)             | 141.9 ± 14.1   | 144.8 ± 12.9                   | 139.9 ± 15.6  | 0.550  |
| Diastolic BP (mm Hg ± SD)            | 82.6 ± 9.9     | 81.4 ± 1.1                     | 83.4 ± 13.3   | 0.979  |
| Total cholesterol (mg/dL ± SD)       | 188.1 ± 88.5   | 178.2 ± 18.5                   | 195.1 ± 118.3 | 0.455  |
| LDL cholesterol (mg/dL ± SD)         | 116.9 ± 64.6   | 114.2 ± 16.1                   | 118.9 ± 86.5  | 0.569  |
| HDL cholesterol (mg/dL ± SD)         | 41.1 ± 7.2     | 39.8 ± 9.1                     | 42.0 ± 6.1    | 0.956  |
| Total cholesterol/HDL cholesterol ratio (mean ± SD) | 4.72 ± 2.3 | 4.75 ± 1.6 | 4.71 ± 2.8 | 0.976 |
| Triglycerides (mg/dL; median (range))| 105 (61-700)   | 105 (71-150)                   | 105 (61-700)  | 1.0    |
| Triglycerides/HDL cholesterol ratio (median (range)) | 2.58 (1.42-16.7) | 2.39 (1.51-5.98) | 2.77 (1.42-16.7) | 1.0 |
| Total testosterone (nmol/L ± SD)     | 10.9 ± 4.1     | 12.4 ± 3.5                     | 9.9 ± 4.5     | 0.350  |
| Free testosterone (pmol/L ± SD)      | 191.6 ± 35.2   | 200 ± 17.6                     | 185.6 ± 44.3  | 0.510  |
| SHBG (nmol/L ± SD)                   | 23.6 ± 12.1    | 26.2 ± 3.4                     | 22.4 ± 15.2   | 0.755  |
| Neuropathy, n (%)                    | 3 (23.0)       | 0 (0)                          | 3 (42.8)      | 0.091  |

Nephropathy 0.345

No, n (%) 10 (76.9) 5 (83.3) 6 (85.7)

Microalbuminuria, n (%) 2 (15.4) 1 (17.7) 0 (0)

Macroalbuminuria, n (%) 1 (7.7) 0 (0) 1 (14.3)

Retinopathy, n (%) 5 (38.5) 2 (33.3) 3 (42.3) 0.921

Stroke, n (%) 1 (7.7) 0 (0) 1 (14.3) 0.377

BP, blood pressure; eGDR, estimated glucose disposal rate; HbA\(_1c\), glycosylated hemoglobin; HDL, high-density lipoproteins; LDL, low-density lipoproteins; T1DM, type 1 diabetes mellitus.
Table 2  Observed changes compared with baseline in TEST-T1D patients

| Outcome                          | Testosterone undecanoate (n=6) | Placebo (n=7) | P       |
|----------------------------------|---------------------------------|---------------|---------|
| eGDR (mg·Kg$^{-1}$·min$^{-1}$ ± SD) |                                 |               |         |
| 6 weeks                          | +0.24 ± 0.6                     | +0.29 ± 1.1   | 0.921   |
| 16 weeks                         | +0.46 ± 0.8                     | +0.46 ± 0.8   | 0.573   |
| 22 weeks                         | +0.30 ± 0.7                     | −1.15 ± 0.6   | 0.276   |
| HbA1c (%)                        |                                 |               |         |
| 6 weeks                          | −0.5 ± 0.4                      | −0.4 ± 0.6    | 0.638   |
| 16 weeks                         | −0.4 ± 0.6                      | −0.7 ± 0.7    | 0.506   |
| 22 weeks                         | −0.4 ± 1.0                      | −0.4 ± 0.9    | 0.977   |
| FPG (mg/dL ± SD)                 |                                 |               |         |
| 6 weeks                          | −26.2 ± 48.6                    | +48.8 ± 75.7  | 0.089   |
| 16 weeks                         | −37.4 ± 85.1                    | +32.7 ± 70.3  | 0.161   |
| 22 weeks                         | −0.6 ± 93.1                     | −11.7 ± 87.9  | 0.844   |
| BMI (Kg·m$^{-2}$ ± SD)           |                                 |               |         |
| 6 weeks                          | +0.02 ± 1.0                     | −0.00 ± 0.6   | 0.959   |
| 16 weeks                         | +1.46 ± 3.2                     | +0.16 ± 0.4   | 0.352   |
| 22 weeks                         | +0.13 ± 1.1                     | +0.28 ± 0.3   | 0.753   |
| Waist circumference (cm ± SD)    |                                 |               |         |
| 6 weeks                          | −2.8 ± 6.8                      | −1.0 ± 7.4    | 0.686   |
| 16 weeks                         | −5.4 ± 5.8                      | −2.1 ± 6.0    | 0.065   |
| 22 weeks                         | −5.4 ± 5.1                      | +1.5 ± 6.3    | 0.081   |
| Hip circumference (cm ± SD)      |                                 |               |         |
| 6 weeks                          | −3.2 ± 3.4                      | −0.2 ± 5.5    | 0.311   |
| 16 weeks                         | −3.6 ± 4.6                      | −0.7 ± 7.9    | 0.483   |
| 22 weeks                         | −4.6 ± 4.2                      | −2.3 ± 4.3    | 0.401   |
| Fat mass (%)                     |                                 |               |         |
| 6 weeks                          | +0.03 ± 1.3                     | +0.2 ± 0.5    | 0.987   |
| 16 weeks                         | +1.6 ± 3.5                      | +0.2 ± 0.4    | 0.347   |
| 22 weeks                         | +0.2 ± 1.4                      | +0.3 ± 0.3    | 0.862   |
| Systolic BP (mm Hg ± SD)         |                                 |               |         |
| 6 weeks                          | −10.6 ± 6.2                     | −5.0 ± 19.1   | 0.548   |
| 16 weeks                         | −5.0 ± 4.4                      | −1.3 ± 16.9   | 0.651   |
| 22 weeks                         | −9.6 ± 11.9                     | +8.8 ± 22.8   | 0.138   |
| Diastolic BP (mm Hg ± SD)        |                                 |               |         |
| 6 weeks                          | −5.0 ± 6.4                      | +2.8 ± 14.4   | 0.291   |
| 16 weeks                         | +0.0 ± 6.6                      | −0.2 ± 10.3   | 0.976   |
| 22 weeks                         | +1.0 ± 4.5                      | −1.2 ± 19.9   | 0.819   |
| Total cholesterol (mg/dL ± SD)   |                                 |               |         |
| 6 weeks                          | −20.0 ± 27.3                    | +8.0 ± 30.1   | 0.144   |
| 16 weeks                         | −33.4 ± 19.4                    | +0.5 ± 12.7   | 0.007   |
| 22 weeks                         | −37.4 ± 27.5                    | +13.2 ± 17.8  | 0.005   |

Table 2  Cont.

| Outcome                          | Testosterone undecanoate (n=6) | Placebo (n=7) | P       |
|----------------------------------|---------------------------------|---------------|---------|
| LDL cholesterol (mg/dL ± SD)     |                                 |               |         |
| 6 weeks                          | −17.2 ± 21.3                    | −0.5 ± 15.7   | 0.169   |
| 16 weeks                         | −29.6 ± 13.2                    | −0.0 ± 8.0    | 0.001   |
| 22 weeks                         | −30.2 ± 22.1                    | +10.5 ± 13.4  | 0.004   |
| HDL cholesterol (mg/dL ± SD)     |                                 |               |         |
| 6 weeks                          | +6.6 ± 6.5                      | +0.5 ± 6.7    | 0.165   |
| 16 weeks                         | +2.4 ± 7.6                      | −1.0 ± 2.1    | 0.321   |
| 22 weeks                         | +2.2 ± 4.3                      | +0.9 ± 3.1    | 0.567   |
| Total cholesterol/HDL cholesterol ratio |                                 |               |         |
| 6 weeks                          | −1.2 ± 1.5                      | +0.1 ± 0.5    | 0.073   |
| 16 weeks                         | −1.2 ± 1.4                      | +0.1 ± 0.4    | 0.066   |
| 22 weeks                         | −1.3 ± 1.2                      | +0.2 ± 0.3    | 0.016   |
| Triglycerides (mg/dL ± SD)       |                                 |               |         |
| 6 weeks                          | −29.7 ± 40.6                    | +46.8 ± 85.3  | 0.101   |
| 16 weeks                         | −16.9 ± 58.1                    | +22.2 ± 58.4  | 0.297   |
| 22 weeks                         | −39.7 ± 34.6                    | +20.2 ± 33.0  | 0.017   |
| Triglycerides/HDL cholesterol ratio |                                 |               |         |
| 6 weeks                          | −1.06 ± 1.6                     | +1.00 ± 1.8   | 0.079   |
| 16 weeks                         | −0.52 ± 2.5                     | +0.49 ± 1.3   | 0.409   |
| 22 weeks                         | −1.38 ± 1.7                     | +0.49 ± 0.92  | 0.042   |
| IIEF-5 (points ± SD)             |                                 |               |         |
| 22 weeks                         | +5.0 ± 7.5                      | +0.5 ± 2.1    | 0.800   |
| IIEF-5                           |                                 |               | 0.558   |
| No changes n (%)                 | 3 (60.0)                        | 4 (66.7)      |         |
| Improvement n (%)                | 2 (40.0)                        | 1 (16.7)      |         |
| Worsening n (%)                  | 0 (0)                           | 1 (16.7)      |         |
| PSA (ng/mL)                      |                                 |               |         |
| 6 weeks                          | +0.20 ± 0.2                      | +0.10 ± 0.1   | 0.435   |
| 16 weeks                         | +0.24 ± 0.3                      | +0.10 ± 0.0   | 0.228   |
| 22 weeks                         | +0.33 ± 0.3                      | +0.10 ± 0.0   | 0.128   |
| Hemoglobin (g/dL)                |                                 |               |         |
| 6 weeks                          | +0.50 ± 0.5                      | −1.30 ± 1.0   | 0.025   |
| 16 weeks                         | +1.05 ± 0.5                      | −0.50 ± 0.7   | 0.014   |
| 22 weeks                         | +1.63 ± 0.9                      | −0.30 ± 0.9   | 0.019   |
| Hematocrit (%)                   |                                 |               |         |
| 6 weeks                          | +1.40 ± 1.5                      | −3.00 ± 2.6   | 0.082   |
| 16 weeks                         | +3.43 ± 1.7                      | −1.67 ± 2.3   | 0.036   |
| 22 weeks                         | +4.10 ± 2.7                      | −0.56 ± 2.6   | 0.039   |

BMI, body mass index; BP, blood pressure; eGDR, estimated glucose disposal rate; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin; HDL, high-density lipoproteins; IIEF, international index of erectile function; LDL, low-density lipoproteins.
following a high fat and cholesterol diet [22]. These findings suggest that increased serum cholesterol levels associated with testosterone deficiency may be attributed to impaired LDL cholesterol clearance. Furthermore, a study in testosterone deficient-LDLr-/-mice suggests a functional modulator role of LDLR in processes associated with hypogonadism-induced metabolic alterations [23].

With respect to triglycerides, in type 2 diabetic patients with hypogonadism, TU therapy produced a decrease of 62 mg/dL at 12 months [7], and up to 138 mg/dL at 8 years [9]. In the present study, the drop observed at 22 weeks versus placebo was 59.9 mg/dL, a figure similar to that found in type 2 diabetes.

However, we were unable to confirm the improvement in insulin resistance, glycemic control or anthropometric changes observed in T2D patients. A trend towards an improvement in IIEF-5 was observed, without reaching statistical significance.

With respect to the adverse effects of treatment, no differences were found in PSA, although there was a rise in hematocrit in the TU treatment group. Hemoglobin levels did not exceed 17.0 g/dL in any case, or were associated with any serious adverse event.

The small sample size in the present study could have limited its power to detect significant differences in anthropometric and metabolic parameters. Furthermore, insulin sensitivity and body fat percentage were calculated based on validated formulas, with a good correlation with the hyperinsulinemic euglycemic clamp and bioimpedanciometry, respectively, but were not measured directly, which signifies that the results may not be completely accurate.

In conclusion, treatment with TU in patients with T1D and hypogonadotropic hypogonadism seems to produce a greater improvement in lipid profile than that observed in patients with type 2 diabetes or the metabolic syndrome, with no significant effects on metabolic control or anthropometric parameters. Further studies with a larger sample size and follow-up are needed to confirm these data and to clarify the pathophysiologic mechanisms.

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References

1. Blouin K, Després JP, Couillard C, Tremblay A, Prud’homme D, et al. (2005) Contribution of age and declining androgen levels to features of the metabolic syndrome in men. *Obesity* 13: 1034-1040.

2. Simon D, Charles MA, Nahoul K, Orssaud G, Kremski J, et al. (1997) Association between plasma testosterone and cardiovascular risk factors in healthy adult men: the Telecom Study. *J Clin Endocrinol Metab* 82: 682-685.

3. Anderson SG, Heald A, Younger N, Bujawanska S, Narayanan RP, et al. (2012) Screening for hypogonadism in diabetes 2008/9: results from the Cheshire Primary Care cohort. *Prim Care Diabetes* 6: 143-148.

4. Chillarón JJ, Fernández-Miró M, Albareda M, Vila L, Colom C, et al. (2015) Age, insulin requirements, waist circumference and triglycerides predict hypogonadotropic hypogonadism in patients with type 1 diabetes. *J Sex Med* 12: 76-82.

5. Holt SK, Lopushnyan N, Hotaling J, Sarma AV, Dunn RL, et al. (2014) Prevalence of low testosterone and predisposing risk factors in men with type 1 diabetes mellitus: findings from the DCCT/EDIC. *J Clin Endocrinol Metab* 99: E1655-E1660.

6. Kapoor D, Goodwin E, Channer KS, Jones TH (2006) Testosterone replacement therapy improves insulin resistance, glycaemic control, visceral adiposity and hypercholesterolaemia in hypogonadal men with type 2 diabetes. *Eur J Endocrinol* 154: 899-906.

7. Heufelder AE, Saad F, Bunc Mc, Gooren L (2009) Fifty-two-week treatment with diet and exercise plus transdermal testosterone reverses the metabolic syndrome and improves glycaemic control in men with newly diagnosed type 2 diabetes and subnormal plasma testosterone. *J Androl* 30: 726-733.

8. Jones TH, Arver S, Behre HM, Buvat J, Meuleman E, et al. (2011) Testosterone replacement in hypogonadal men with type 2 diabetes and/or metabolic syndrome (the TIMES2 study). *Diabetes Care* 34: 828-837.

9. Saad F, Yassin A, Doros G, Haider A (2016) Effects of long-term treatment with testosterone on weight and waist size in 411 hypogonadal men with obesity classes I-III: observational data from two registry studies. *Int J Obes (Lond)* 40: 162-170.

10. Traish AM, Haider A, Doros G, Saad F (2014) Long-term testosterone therapy in hypogonadal men ameliorates elements of metabolic syndrome: an observational, long-term registry study. *Int J Clin Pract* 68: 314-329.

11. Cornoldi A, Caminiti G, Marazzi G, Vitale C, Patrizi R, et al. (2010) Effects of chronic testosterone administration on myocardial ischemia, lipid metabolism and insulin resistance in elderly male diabetic patients with coronary artery disease. *Int J Cardiol* 142: 50-55.

12. Bagatell CJ, Heiman JR, Matsumoto AM, Rivier JE, Bremner WJ (1994) Metabolic and behavioral effects of high-dose, exogenous testosterone in healthy men. *J Clin Endocrinol Metab* 79: 516-517.

13. Malkin CJ, Pugh PJ, Jones RD, Kapoor D, Channer KS, et al. (2004) The Effect of testosterone replacement on endogenous inflammatory cytokines and lipid profiles in hypogonadal men. *J Clin Endocrinol Metab* 89: 3313-3318.

14. Senmaru T, Fukui M, Okada H, Mineoka Y, Yamazaki M, et al. (2013) Testosterone deficiency induces markedly decreased serum triglycerides, increased small dense LDL, and hepatic steatosis mediated by dysregulation of lipid assembly and secretion in mice fed a high-fat diet. *Metabolism* 62: 851-860.

15. Chillarón JJ, Goday A, Flores-Le-Roux JA, Benaiges D, Carrera MJ, et al. (2009) Estimated glucose disposal rate in assessment of the metabolic syndrome and microvascular complications in patients with type 1 diabetes. *J Clin Endocrinol Metab* 94: 3530-3534.

16. Thorn LM, Forsblom C, Fagerudd J, Thomas MC, Pettersson-Fernholm K, et al. (2005) Metabolic syndrome in type 1 diabetes: association with diabetic nephropathy and glycemic control (the FinnDiane study). *Diabetes Care* 28: 2019-2024.

17. Chillarón JJ, Flores-Le-Roux JA, Benaiges D, Pedro-Botet J (2014) Type 1 diabetes, metabolic syndrome and cardiovascular risk. *Metabolism* 63: 181-187.

18. Rosen RC, Riley A, Wagner G, Osterloh IH, Kirkpatrick J, et al. (1997) The international index of erectile function (IIEF): a multidimensional scale for assessment of erectile dysfunction. *Urology* 49: 822-830.

19. Williams KV, Erbev JR, Becker D, Arslanian S, Orchard T (2000) Can clinical factors estimate insulin resistance in type 1 diabetes? *Diabetes* 49: 626-632.

20. Gómez-Ambrosi J, Silva C, Catalán V, Rodriguez A, Galofré JC, et al. (2012) Clinical usefulness of a new equation for estimating body fat. *Diabetes Care* 35: 383-388.

21. Fukuda Y, Hashimoto Y, Hamaguchi M, Fukuda T, Nakamura N, et al. (2016) Triglycerides to high-density lipoprotein cholesterol ratio is an independent predictor of incident fatty liver; a population-based cohort study. *Liver Int* 36: 713-720.

22. Cai Z, Xi H, Pan Y, Jiang X, Chen L, et al. (2015) Effect of testosterone deficiency on cholesterol metabolism in pigs fed a high-fat and high-cholesterol diet. *Lipids Health Dis* 14: 18.

23. Constantinou C, Mpatsoulis D, Natsos A, Petropoulou PI, Zvintzou E, et al. (2014) The low density lipoprotein receptor modulates the effects of hypogonadism on diet-induced obesity and related metabolic perturbations. *J Lipid Res* 55: 1434-1447.