# Testosterone therapy does not affect coagulation in male hypogonadism: a longitudinal study based on thrombin generation

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The authors have nothing to disclose.

## Abstract

**Context**. Testosterone therapy has been variably associated with increased thrombotic risk but investigations of global coagulation in this setting are lacking.

**Objective**. To compare global coagulation of hypogonadal men before (T0) and 6 months after (T1) starting testosterone replacement therapy (TRT), and healthy controls.

**Design**. Observational prospective cohort study.

**Setting**. Two tertiary endocrinological ambulatory care centers.

**Patients**. Thirty-eight men with hypogonadism (mean age 55, SD 13) and 38 age-matched healthy controls.

**Interventions**. Thrombin generation assay (TGA) was performed at T0 and T1 in hypogonadal men and in controls. TGA is an *in vitro* procedure based on the continuous registration of thrombin generation and decay under conditions mimicking the process that occurs *in vivo*.

**Main Outcome Measures**. The following TGA parameters were recorded: lag-time; thrombin-peak concentration; time-to-reach the peak, velocity index and endogenous thrombin potential (ETP), the latter representing the total amount of thrombin generated under the driving forces of procoagulants opposed by the anticoagulants. PC, antithrombin, factor (F)VIII, and fibrinogen were assessed.

**Results**. No changes of TGA parameters were observed between T0 and T1. Hypogonadal men displayed significantly higher ETP, fibrinogen, and significantly lower antithrombin levels both at T0 and T1 compared to controls. Thrombin-peak of hypogonadal men was significantly higher than controls at T0 but not at T1. ETP and antithrombin were correlated with testosterone levels.

**Conclusions**. Hypogonadal men display a procoagulant imbalance detected by increased thrombin generation. Short-term TRT does not worsen global coagulation, suggesting that the treatment can be safely prescribed to men diagnosed with hypogonadism.

## Introduction

Venous thromboembolism (VTE) is a multifactorial disease resulting from hemodynamic changes such as reduction of blood flow or turbulence, endothelial injury or dysfunction, and blood hypercoagulability (1). Circumstantial risk factors that may influence VTE risk are recent surgery, cancer and prolonged immobilization, with sex hormones advocated as additional pathogenetic factors (2).

An increased risk of thromboembolic events has been reported in association with oral contraceptive pills and hormonal replacement therapy in women (3–5). However, the role of endogenous testosterone and testosterone replacement therapy (TRT) in men remains controversial.

Testosterone, at physiological concentrations, has a beneficial influence on the haemostatic system as measured *in vitro*. In fact, both testosterone and its 5 alpha-reduced derivative dihydrotestosterone exert an inhibitory effect on primary haemostasis, by preventing ADP-mediated platelet aggregation (6–8). Moreover, testosterone has an inhibitory effect on coagulation and promotes fibrinolysis (9). Consistently, low testosterone levels have been correlated with increased platelet activity and a procoagulant profile (10–13). Current evidence, however, failed to provide association between endogenous testosterone levels in the lower quartile of normal range and new incident cases of VTE as compared to subjects with testosterone levels in the middle or upper quartiles (14,15).

It is well known that TRT leads to an increase of hematocrit, blood viscosity (16) and estrogen circulating levels (17), all factors that may potentially influence the VTE risk. Four population studies reported association between testosterone therapy and thrombotic risk (18–21). Interestingly, studies by Martinez et al and Walker et al showed that this association reached a peak within six months since the start of treatment (18,19). Nevertheless, three metanalyses of randomized controlled trials did not find an increased risk of VTE associated with TRT compared to placebo (22–24).

With this gap of knowledge, we aimed to investigate the variation in the global coagulation profile of hypogonadal men before and 6 months after starting TRT. Secondary aims were: to compare the global coagulation profile of hypogonadal men with healthy controls, and to assess the association of coagulation with hormonal and metabolic variables. Coagulation was assessed by thrombin generation assay (TGA), an *in vitro* procedure based on the continuous registration of thrombin generation (mediated by procoagulants) and decay (mediated by anticoagulants).

Compared to traditional coagulation tests such as the prothrombin time, activated partial thromboplastin time or viscoelastometry, that hardly reflect the complex and integrated mechanisms of coagulation, TGA can be considered as the closest approximation to the process occurring *in vivo* (25). In fact, coagulation in TGA is activated by much smaller amounts of tissue factor and phospholipids than those used in other coagulation tests (26). Furthermore, the formulations of traditional tests do not include thrombomodulin, the physiological protein C activator; hence protein C in those tests cannot be optimally activated to represent what occurs *in vivo*. A growing body of evidence has demonstrated the ability of TGA to predict the risk of first and recurrent thrombotic events (27–29).

## Materials and methods

### Study design and procedures

We conducted a multicentre, observational prospective cohort study to assess the effects of short-term TRT on the global coagulation profile of hypogonadal men.

Patients were selected among those followed up at two tertiary endocrinological units in Milan, Northern Italy (Fondazione IRCCS Ca'Granda Ospedale Maggiore Policlinico and IRCCS Istituto Auxologico Italiano). Male hypogonadism was defined by reduced levels of total and/or calculated free testosterone (<12.0 nmol/L and <220 pmol/L, respectively) in men with sexual dysfunctions (i.e., erectile dysfunction, decreased libido, reduced nocturnal/morning erections), according to

current guidelines (30). Clinical history was collected and radiological investigations and further hormonal assessments (in particular, luteinizing hormone, LH, and follicle stimulating hormones, FSH) were carried out as appropriate to differentiate between functional hypogonadism, organic primary and secondary hypogonadism (30). Adult patients (18 years old or older) with newly diagnosed hypogonadism and who had not received treatment with testosterone or gonadotrophins, were included. Patients with known hereditary coagulation disorders, Klinefelter syndrome (1), or on anticoagulation (parenteral or oral) treatment, were excluded. Among patients with secondary hypogonadism, only those without pituitary hormones' excess, and without uncompensated pituitary hormones' deficiencies, were selected.

Patients' evaluations were scheduled before and six months after starting TRT (T0 and T1, respectively). Blood samples were collected for assessment of the coagulation profile.

Patients received transdermal testosterone 2% gel on a daily basis, or long-acting injectable testosterone undecanoate, which was administered at baseline, after 6 weeks (loading dose), and then every 12 weeks.

Information on smoking habit, arterial hypertension, dyslipidemia, diabetes mellitus, thromboembolic events, body mass index (BMI), fasting plasma glucose, total cholesterol, triglycerides, low-density-lipoprotein (LDL) cholesterol, total testosterone, complete blood count and prostate specific antigen (PSA) were extracted from hospital records. Diagnostic delay was arbitrarily estimated as the time period between self-reported sexual symptoms' onset and start of TRT.

Healthy controls were recruited among male medical students and hospital staff. They were matched by age (±5 years) to the patient population and were free from current and past thrombotic events, anticoagulant drugs, or coagulation disorders known to affect TGA.

All study procedures were in accordance with the principles set out in the Declaration of Helsinki. The study was approved by the Milan Area 2 ethics committee (approval ID 396). Written informed consent was obtained from all individuals included in the study.

## Blood sampling and plasma preparation

Blood was collected from an antecubital vein into vacuum tubes containing 1/10 volume of trisodium citrate 109 mM (Becton Dickinson). For hypogonadal patients receiving TRT, blood samples were taken 2 hours after application of testosterone transdermal gel, or at the end of the dosing interval in case of injectable long-acting testosterone undecanoate (31). Little variability in plasma testosterone concentrations is expected during treatment with either formulation (32,33). Nevertheless, this sampling schedule was established in accordance with current guidelines on testosterone treatment monitoring (31).

Citrated whole blood was centrifuged for 20 minutes (controlled room temperature) at 2880*g* to prepare platelet poor plasma that was aliquoted in plastic-capped tubes, quickly frozen by immersion in liquid nitrogen, and stored at –70 °C until testing. To limit between-assay variability, an equal number of samples from patients and controls were tested in the same run. All the experimental procedures were conducted at the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Ospedale Maggiore Policlinico, Milan, Italy.

## Thrombin generation assay (TGA)

TGA was assessed according to Hemker et al (34) with a homemade method as described (35). Testing was based on the activation of coagulation after addition to plasma of small amounts of human recombinant relipidated tissue factor (rTF, 1 pM) (Recombiplastin 2G, Werfen) and synthetic phospholipids (PL, 1.0 μM) (Avanti Polar) as coagulation triggers. Testing was performed with addition of soluble rabbit thrombomodulin (Haematologic Technologies) (2 nM). Thrombomodulin is the physiological activator of protein C and is located on endothelial cells (25). Registration of thrombin generation was obtained with a fluorogenic substrate (Z-GlyGly-Arg-AMC HCl, Bachem) (617 μM) by means of a dedicated fluorometer (Fluoroskan Ascent, Thermo Labsystems). The readings were recorded and analyzed with dedicated software (Thrombinoscope,

Thrombinoscope BV), which displays the curve of thrombin concentration as a function of time and calculates the following parameters: the time (minutes) between the addition of the triggers and the initiation of thrombin generation (lag time); the thrombin peak (nM); the time (minutes) needed to reach the peak (time to peak); the area under the curve, defined as endogenous thrombin potential (ETP) and expressed as nM × min; and the velocity index, defined as [peak/(TT peak – lag time)] and expressed as nM/min.

**Other coagulation parameters**

Protein C (PC) and antithrombin were measured as chromogenic activity by means of commercial kits (Hemosil antithrombin and Hemosil PC; Werfen). Factor (F)VIII, and fibrinogen were measured as described (36). FVIII results were reported as percentage activity relative to pooled normal plasma with an (arbitrary) activity of 100%.

**Hormonal assay**

Circulating total testosterone concentrations were assessed by an Elecsys Testosterone II (Calibrator reference: 05200067190) test marketed by Roche Diagnostics (RRID:AB_2783736). This method is standardized via isotope dilution–gas chromatography/mass spectrometry. The assay has a lower limit of detection of 0.087 nmol/L, a functional sensitivity of 0.4 nmol/L, and interassay or intra-assay coefficients of variation of less than 5%.

**Endpoints**

The primary endpoint was the variation in the global coagulation profile of hypogonadal men from baseline to six-month-TRT, as defined by TGA and other coagulation parameters.

Secondary endpoints were the comparison of the coagulation profile of hypogonadal men with healthy controls and the association of coagulation parameters with hormonal and metabolic variables.

## Statistical analysis

Distribution of quantitative variables was assessed by Shapiro-Wilk test. Normally distributed quantitative variables were expressed as mean and standard deviation (SD), whereas variables with a skewed distribution were reported as median and interquartile range (IQR) or min-max range; qualitative variables were represented as absolute frequencies. Paired or unpaired t test was performed to compare means of normally distributed variables. Alternatively, the nonparametric Mann-Whitney and Wilcoxon tests were used. Comparisons among 3 groups were performed by Kruskal-Wallis test. Fisher's exact test or Chi square were employed to compare frequencies of qualitative variables between 2 or more groups. Bonferroni's correction for multiple comparisons was applied where indicated. Univariate association analysis was performed by Pearson correlation test or Spearman rank correlation test as appropriate. Correction for covariates was performed by Analysis Of Covariance (ANCOVA).

A 2-sided P value was considered statistically significant when less than .05. Analysis was performed with GraphPad Prism (version 10) and IBM SPSS Statistics (version 29).

## Power analysis

For power analysis, we used ETP as reference parameter.

Given that no data about TGA in subject affected with hypogonadism are available, we considered our previously published data on TGA in a group of normal subjects compared to patients with Cushing's syndrome, a condition proven to be related to hypercoagulability (37). In that study, normal subjects showed a mean (SD) ETP value of 902 (222) nM/min, while in CS patients ETP was 1284 (353) nM/min (mean difference: 302 (439) nM/min). Since current evidence on coagulative derangements in male hypogonadism is inconsistent, we assumed hypogonadal men at baseline to be similar to normal subjects.

For the primary outcome, using a t-test for dependent samples, a sample size of 25 subjects is required to reject the null hypothesis that the mean ETP is not different before and after treatment, with a power of 90% and a probability of type I error of 5%.

For the secondary outcome a 1:1 ratio between the 2 groups (hypogonadal patients and controls) was set. Assuming a mean ETP of 982 (222) nM/min in the control group and of 1284 (353) in the experimental group, and using a t-test for independent samples, a sample size of 22 subjects per group is needed to reject the null hypothesis that the mean levels of ETP are equal in the 2 populations, with a power of 90% and a probability of type I error of 5%.

**Sensitivity analysis**

Different treatment formulations may provide different amounts of testosterone throughout the dosing interval (38). For this reason, a sensitivity analysis was conducted by grouping participants according to the prescribed testosterone formulation (transdermal gel or long acting intramuscular injection).

**Results**

Thirty-eight hypogonadal patients and 38 controls were enrolled between November 2017 and July 2020. All subjects completed the study.

Table 1 summarizes baseline characteristics of hypogonadal patients and controls. Median BMI was significantly higher in the hypogonadal group compared to controls. Ten patients presented primary hypogonadism, 20 secondary hypogonadism and 8 functional hypogonadism. Except for a lower prevalence of arterial hypertension in patients with primary hypogonadism, no significant differences at baseline were observed among these three subgroups (Table 2).

Two patients were treated with long-acting injectable testosterone undecanoate, while the remaining 36 received transdermal testosterone 2% gel (median daily dose 30 mg, IQR 20.75-40 mg).

Mean/median testosterone concentrations, haemoglobin, haematocrit and PSA significantly increased from T0 to T1 (Tables 3 and 4). Testosterone increased from baseline in all patients and it was above the lower limit of normal (12 nmol/L) in 29/38 patients at T1. These parameters remained within safety limits (30) in all patients except 2: in one patient PSA increased by more than 1.4 ng/mL (PSA 3.5 ng/mL at T1), while in another patient haematocrit raised over 54%.

**Global coagulation profile in hypogonadal men**

No VTE event occurred during the six-month observation period.

Baseline TGA parameters were not different among primary, secondary or functional hypogonadism (Table 2).

No changes were observed in the whole cohort of hypogonadal men from T0 to T1 in ETP [1502 (536) vs. 1485 (512) nM x min, p=0.77], lag time (p=0.81), thrombin peak (p=0.31), time to peak (p=0.59), and velocity index (p=0.21) (Figure 1).

The activity of FVIII, PC, antithrombin and fibrinogen were comparable between the two time points (Figure 2).

**Sensitivity analysis**

Patients treated with long-acting injectable testosterone were (non-significantly) younger than those treated with testosterone gel. With the limitations of small samples' size (n=2 and n=36 respectively), no other significant difference in baseline characteristics was observed (Table 4).

ETP and thrombin peak were comparable between the two subgroups at both time-points. TGA parameters did not show significant changes from T0 to T1 in either subset of patients (Figure 3), although, among injectable testosterone-treated subjects, (non-significantly) lower ETP, thrombin peak and velocity index were observed at T1 compared to T0.

**Comparison of the global coagulation profile between hypogonadal men and controls**

Hypogonadal men at baseline displayed significantly increased ETP compared to controls [1502 (536) vs. 1201 (438.1) nM x min, p=0.009] (Fig. 1). ETP in patients persisted higher than controls six months after starting TRT (p=0.01).

The velocity index and thrombin peak showed a significant increase at baseline, but the difference was no longer significant after six months, compared to controls (Fig. 1). Lag time and time to peak were comparable to controls (Fig. 1).

Since hypogonadal men had significantly higher BMI than controls, analysis was repeated including BMI as a covariate. A significant difference in ETP (p=0.009), thrombin peak (p=0.02) and velocity index (p=0.02) was confirmed after correcting by BMI.

FVIII was lower in hypogonadal men (both at T0 and at T1) than controls (Fig. 2). Antithrombin was lower and fibrinogen was higher in hypogonadal patients than in controls (Fig. 2). The differences in FVIII, antithrombin and fibrinogen were no longer statistically significant after controlling by BMI.

**Correlations**

No correlation was observed for ETP, antithrombin and fibrinogen at T0, with: age; BMI; diagnostic delay; total cholesterol; triglycerides; arterial hypertension.

Testosterone levels at baseline displayed a significant correlation with ETP (r=-0.49, p=0.002) and antithrombin (r=0.45, p=0.005) (Figure 4), but not with fibrinogen.

## Discussion

An increased risk of VTE is observed for women of reproductive age or after menopause on oral contraceptive pills and hormonal replacement therapy (3–5). Whether this risk is increased in hypogonadal men treated with TRT has not been well established (23).

In women, the use of combined hormonal contraception and hormonal replacement therapy has been linked with alterations in plasma levels of nearly all proteins involved in fibrinolysis and coagulation (i.e. rises in coagulation factors II, V, VII-XII and decreases in Tissue Factor Pathway Inhibitor and antithrombin levels) (39–41). Therefore, oral contraceptives users have a VTE relative risk ranging from 1.3 to 5.6 depending on the dose of the estrogenic component, estroprogestinic formulation (3,42,43) and treatment duration (44), while women on hormonal replacement therapy have a different VTE risk depending on the route of administration of the estrogenic component, oral (OR 2.5, 95% CI, 1.9-3.4) or transdermal (OR 1.2, 95% CI, 0.9-1.7) (5).

Herein we report that short term TRT does not affect the coagulation profile of hypogonadal men as measured with a global coagulation procedure that mimics much more than any other coagulation test the process that occurs *in vivo*. Hypogonadal men display a procoagulant imbalance compared to age-matched controls, which is correlated with testosterone deficiency but not with hypogonadism subtype (primary, secondary or functional) or with testosterone formulation administered.

Testosterone replacement therapy could affect VTE risk leading to a raise of hematocrit, blood viscosity (16) and plasma estrogen concentration (17). In 2015 the US Food and Drug Administration (FDA) reported a surge in TRT prescriptions, of which 28–40% were provided without a biochemical diagnosis of hypogonadism (45). Moreover, based on post marketing surveillance data, in June 2014 FDA and Health Canada issued a labeling change in the product information of all approved TRT formulations regarding the risk of VTE (23). Since then, several clinical trials and metanalyses have investigated the relationship between TRT and VTE risk, reporting conflicting results (18–24,46–48).

Three metanalyses of randomized controlled trials documented no association between TRT and VTE risk compared to placebo (22–24). Similarly, three observational studies did not detect a significant link between testosterone therapy and risk of VTE (including deep vein thrombosis or

pulmonary embolism) in adult men with low testosterone levels compared to hypogonadal men not receiving TRT (46–48).

However, four observational studies found an increased VTE risk among patients using testosterone (18–21). Martinez et al and Walker et al demonstrated that this association was most significant within six months since therapy start (18,19).

However, in the study by Martinez et al most patients received TRT following the diagnosis of "non pathological" hypogonadism based only on clinical data, and in the study by Walker et al only 7.8% of the study population receiving TRT had a biochemical diagnosis of hypogonadism. Furthermore, Kavoussi et al. reported an increased risk of deep vein thrombosis only in those hypogonadal patients, diagnosed on the basis of Endocrine Society guidelines, receiving TRT and presenting other potential etiologies for deep venous thrombosis (i.e. venous stasis, trauma, genetic disorders). After exclusion of these cases, the overall incidence of thrombosis was similar to general population (20).

In the present study, we show that the global coagulation profile of hypogonadal men was not affected by six month-TRT, and that a procoagulant imbalance is present in this category of patients compared to age-matched controls. Indeed, some TGA parameters, i.e. thrombin peak and velocity index, are significantly higher in hypogonadal men compared to controls at baseline, but no longer six months after starting TRT. This observation may suggest a trend towards the improvement of the procoagulant imbalance following TRT, but a longer time may be needed to achieve normalization. The need for a longer time to attain parameters' normalization has already been observed in other endocrine disorders characterized by an increased thrombotic risk like Cushing's syndrome (37). However, the effects of a longer duration therapy are still to be determined.

We also observed paradoxical reduction in FVIII levels in the hypogonadal group, which could be regarded as a compensatory mechanism. Testosterone deficiency appears (at least in part) to contribute to this condition, since testosterone levels are inversely correlated with ETP and directly correlated with antithrombin.

Our results are consistent with previously published observations (6–9,11,13–15). Low testosterone levels have been correlated with increased platelet activity and a procoagulant profile (increased factor V, VII, X and fibrinogen and reduced antithrombin) (11,13). Conversely, both testosterone and dihydrotestosterone have an inhibitory effect on primary haemostasis as measured by *in vitro* tests, by preventing ADP-mediated platelet aggregation (6). This effect is obtained directly by the activation of a receptor on platelets membrane, and indirectly through the antiaggregatory effect of nitric oxide produced by the stimulation of endothelial lining cells *via* the androgen receptor (7,8). Moreover, testosterone increases the expression of tissue factor pathway inhibitor and tissue plasminogen activator and reduces the secretion of plasminogen activator inhibitor-1, thus inhibiting the coagulation cascade and promoting fibrinolysis (9).

Taken together, these studies suggest that testosterone, at physiological concentrations, has a beneficial influence on haemostasis, thus reducing the risk of VTE. However, two population studies have documented no association between endogenous testosterone levels in the lower quartile and new incident cases of VTE as compared to subjects with testosterone levels in the mid or upper quartile in age-adjusted analysis (14,15). These results have been corroborated by a mendelian randomization study which failed to find association between testosterone concentrations and VTE risk (OR 1.02, 0.74-1.4, p = 0.92, for each 0.1 nmol/L increase in calculated free testosterone) (49). In conclusion, current evidence regarding the association between male hypogonadism and VTE is still scarce and controversial, but suggests that low testosterone levels may actually have a negative impact on the haemostatic system.

It is worth of note that several studies have reported a higher prevalence of thrombophilic disorders (like Factor V Leiden or Lupus Anticoagulant) among patients receiving TRT and experiencing VTE, compared with both subjects with VTE not on TRT (17,50), and subjects treated with TRT and no thrombotic event (51). In our cohort no patient experienced VTE during the first six months of therapy, and subjects with previously known thrombophilia were excluded. In this way, our study may have missed to investigate a subset of subjects with the highest thrombotic risk. Further studies

are needed to explore the effects of testosterone therapy on thrombin generation in the presence of pre-existing prothrombotic conditions.

Strengths of this study are the investigation of coagulation of a relatively large and well characterized group of patients by means of a global assay that takes into consideration much more than any other coagulation test the process that occurs *in vivo*. There are some limitations. First, hypogonadal patients had a considerably higher median BMI compared to controls, a condition which may be regarded as a potential confounding factor (52–54). Overweight and obesity are considered as comorbidities commonly associated to male hypogonadism and have a higher prevalence in this population (55). On the other hand, components of the metabolic syndrome, including visceral adipose tissue, can negatively affect thromboembolic risk (54). Nevertheless, it has been shown that male hypogonadism has a negative impact on hemostasis *per se*, independently from the association with metabolic syndrome (11–13). Additionally, TGA parameters in our study were still significantly increased in the hypogonadal group compared to the control group after correcting for BMI, and BMI values showed no correlation with ETP, fibrinogen and antithrombin. Conversely, testosterone levels had a significant association with ETP and antithrombin. Overall, our results may support a direct role of testosterone deficiency regardless of overweight/obesity. Yet, further studies should compare the coagulation profile in overweight/obese men with and without hypogonadism.

Second, other coagulation factors which influence thrombin generation (e.g. tissue factor pathway inhibitor, factor V, protein S and factor X) (56), platelet activity, endothelial function and fibrinolysis have not been evaluated in our study. Third, Martinez et al. (18) and Glueck et al. (50) reported that the incidence of VTE events peaked at 3 months since TRT start. Since in our study intermediate assessments were not performed, any TGA change occurring between 0 and 6 months may have been missed.

In conclusion, this study shows that the procoagulant imbalance observed in hypogonadal men does not worsen following short term TRT,although robust longitudinal clinical data on the incidence of

VTE are lacking, As in the general population, antithrombotic prophylaxis should be warranted in hypogonadal men in case of exposure to other risk factors for venous thrombosis. Further studies are needed to evaluate whether longer term TRT is able to normalize the procoagulant profile of men with androgen deficiency.

## Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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**Tables and figures legends.**

**Table 1. Characteristics of hypogonadal patients at baseline (Hypo) and of healthy controls (HC).**

Results are presented as mean (SD) or median [IQR] for quantitative variables, and as absolute frequencies for categorical variables. LDL, low-density-lipoprotein. PSA, prostate specific antigen. N/A, not available. N.A., not applicable. ns, not significant.

*BMI was available in 35 patients and 28 controls.

**Table 2. Baseline characteristics and coagulation parameters assessed before starting testosterone replacement therapy (T0) in patients with primary, secondary and functional hypogonadism.**

Median and interquartile range are reported for continuous variables. Absolute frequencies are reported for categorical variables.

**Table 3. Changes in testosterone concentrations, complete blood count and prostate specific antigen (PSA) in hypogonadal men before (T0) and six months after starting testosterone replacement therapy (T1).**

Results are reported as mean (SD) or median [IQR].

**Table 4. Baseline characteristics and coagulation parameters assessed before (T0) and six months after starting testosterone treatment (T1), according to testosterone formulation.**

Median and min-max range are reported.

**Figure 1. Comparison of thrombin generation assay parameters in hypogonadal men before (T0) and six months after starting testosterone replacement therapy (T1), and age-matched**

**healthy controls (HC).** Panel (A): Endogenous Thrombin Potential, which accounts for the total amount of thrombin generated in the assay (area under the curve). Panel (B): thrombin peak. Panel (C): Velocity index, which results from the following formula: [peak/(time to peak – lag time)]. Panel (D): Time to peak, i.e. the time (minutes) needed to reach the thrombin peak. Panel (E): Lag Time, i.e. the time (minutes) between the addition of the triggers and the initiation of thrombin generation.

ns, not significant, * $p<0.05$, ** $p<0.01$.

**Figure 2. Comparison of other coagulation parameters in hypogonadal men before (T0) and six months after starting testosterone replacement therapy (T1), and age-matched healthy controls (HC)**. Panel (A): antithrombin (AT). Panel (B): fibrinogen. Panel (C): Factor VIII (FVIII). Panel (D): Protein C (PC).

ns, not significant, * $p<0.05$, ** $p<0.01$.

**Figure 3. Comparison of coagulation parameters at baseline (T0) and six months after starting testosterone treatment (T1), according to testosterone formulation.**

ETP: Endogenous thrombin potential; ns, not significant.

**Figure 4. Correlation of basal testosterone concentrations in hypogonadal men with endogenous thrombin potential (ETP, left panel) and antithrombin (right panel).**

r, Spearman rank correlation's coefficient.

**Table 1.**

| | **Hypo (N=38)** | **HC (N=38)** | **p-value** |
|---|---|---|---|
| Age (years) | 55 (13) | 55 (13) | ns |
| *Body Mass Index (Kg/m$^2$) | 28 [26.9-31] | 25 [22.9-26.5] | <0.0001 |
| *BMI classes<br><18.5 Kg/m$^2$<br>18.5-24.9 Kg/m$^2$<br>25-29.9 Kg/m$^2$<br>30-34.9 Kg/m$^2$<br>35-39.9 Kg/m$^2$<br>>40 Kg/m$^2$ | <br>0<br>1<br>20<br>10<br>2<br>2 | <br>0<br>14<br>12<br>1<br>1<br>0 | <br><0.001 |
| Diabetes mellitus (n) | 3 | 4 | ns |
| Arterial hypertension (n) | 12 | 14 | ns |
| Dyslipidaemia (n) | 18 | 11 | ns |
| Smoke (n) | 4 | 6 | ns |
| Thromboembolic events (n) | 0 | 0 | ns |
| Diagnostic delay (months) | 12 [6-25] | N.A. | - |
| Fasting plasma glucose (mg/dL) | 95 [86-104] | N.A. | - |
| Total cholesterol (mg/dL) | 182 (36) | N.A. | - |
| Triglycerides (mg/dL) | 129 [90-206] | N.A. | - |
| LDL cholesterol | 108 (31) | N.A. | - |

**Table 2.**

| | Primary Hypogonadism (N=10) | Secondary hypogonadism (N=20) | Functional hypogonadism (N=8) | p-value |
|---|---|---|---|---|
| Age (years) | 48 (42-71) | 57 (46-66) | 62 (41-68) | 0.95 |
| Body Mass Index ($Kg/m^2$) | 28.4 (27.0-30.7) | 28.0 (26.8-32.0) | 29.0 (26.2-31.7) | 0.99 |
| Diabetes mellitus (n) | 0 | 2 | 1 | 0.55 |
| Arterial hypertension (n) | 0 | 8 | 4 | 0.04 |
| Dyslipidaemia (n) | 5 | 8 | 5 | 0.55 |
| Smoke (n) | 0 | 4 | 0 | 0.13 |
| Diagnostic delay (months) | 18 (5-84) | 7 (6-24) | 15 (12-24) | 0.63 |
| Endogenous thrombin potential T0 (nM x min) | 1353 (988-1812) | 1849 (1282-1984) | 1481 (1068-1724) | 0.13 |
| Thrombin peak T0 (nM) | 278 (172-377) | 376 (262-422) | 304 (227-409) | 0.48 |
| Velocity Index T0 (nM/min) | 111 (57-175) | 156 (103-181) | 126 (91-196) | 0.56 |
| Lag Time T0 (min) | 9.2 (8.5-9.5) | 10.0 (9.0-11.1) | 9.8 (8.5-10.5) | 0.15 |
| Time to peak T0 (min) | 11.8 (10.6-12.3) | 12.3 (11.4-13.6) | 12.0 (10.9-12.8) | 0.30 |

**Table 3**.

| | **T0** | **T1** | **Absolute difference (median)** | **p-value** |
|---|---|---|---|---|
| Testosterone (nmol/L) | 6.4 [2.6-8.7] | 16.0 [12.5-27.0] | 9.9 | <0.0001 |
| Haemoglobin (g/dL) | 14.1 (1.1) | 15.1 (1.2) | 1.1 | <0.0001 |
| Haematocrit (%) | 41.7 (3.1) | 44.6 (3.3) | 2.9 | <0.0001 |
| PSA (ng/mL) | 0.55 [0.33-1.03] | 0.66 [0.50-1.27] | 0.17 | <0.0001 |

**Table 4**.

| | Transdermal testosterone 2% gel | Long-acting injectable testosterone undecanoate | p-value |
|---|---|---|---|
| N | 36 | 2 | - |
| Age (years) | 58 (27-80) | 41 (34-48) | 0.12 |
| Body Mass Index ($Kg/m^2$) | 28 (22-42) | 28 (28-28) | 0.92 |
| Testosterone T0 (nmol/L) | 6.4 (0.1-12.5) | 8.0 (5.5-10.4) | 0.62 |
| Testosterone T1 (nmol/L) | 16.5 (4.3-85.3) | 14.1 (12.8-15.5) | 0.49 |
| Haemoglobin T0 (g/dL) | 14.0 (11.7-17.7) | 14.9 (14.6-15.2) | 0.19 |
| Haemoglobin T1 (g/dL) | 14.8 (12.7-18.1) | 16.6 (15.7-17.5) | 0.11 |
| Haematocrit T0 (%) | 41.2 (36.1-51.4) | 43.2 (41.8-44.7) | 0.39 |
| Haematocrit T1 (%) | 44.0 (38.9-54.6) | 47.9 (46.2-49.6) | 0.10 |
| Prostate Specific Antigen T0 (ng/mL) | 0.65 (0.00-2.18) | 0.47 (0.39-0.55) | 0.61 |
| Prostate Specific Antigen T1 (ng/mL) | 0.76 (0.13-3.49) | 0.56 (0.56-0.56) | 0.65 |
| ETP T0 (nMxmin) | 1579 (131-2373) | 1857 (1624-2090) | 0.31 |
| ETP T1 (nMxmin) | 1551 (169-2368) | 1438 (1125-1751) | 0.90 |
| Thrombin peak T0 (nM) | 331 (17-535) | 369 (313-426) | 0.70 |
| Thrombin peak T1(nM) | 292 (24-542) | 279 (201-357) | 0.67 |
| Velocity index T0 (nM/min) | 137 (3-294) | 170 (157-183) | 0.33 |
| Velocity index T1 (nM/min) | 111 (4-298) | 110 (79-140) | 0.74 |
| Lag Time T0 (min) | 9.5 (7.3-13.8) | 7.7 (7.3-8.2) | 0.03 |
| Lag time T1(min) | 9.5 (7.5-13.5) | 8.0 (7.9-8.1) | 0.07 |
| Time to peak T0 (min) | 12.2 (10.0-17.3) | 9.9 (9.7-10.2) | 0.02 |
| Time to peak T1 (min) | 12.2 (10.2-17.7) | 10.5 (10.4-10.7) | 0.08 |

**Figure 1.**

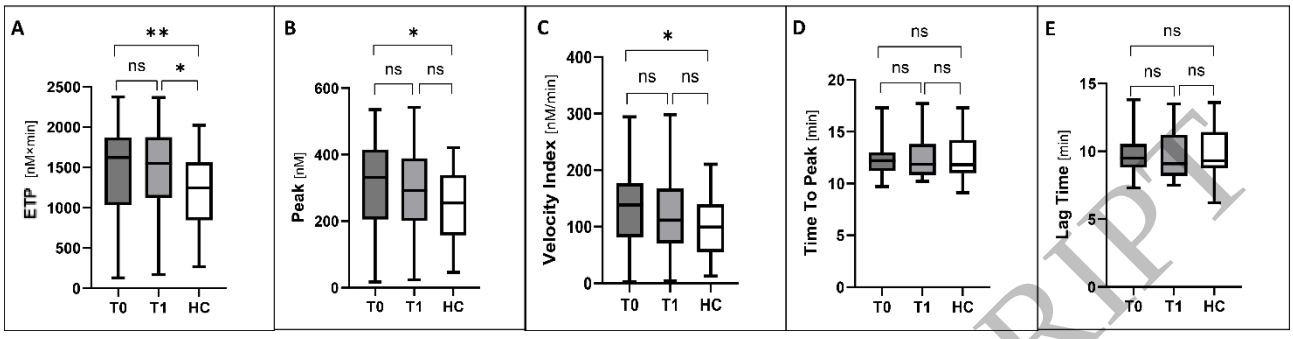


*Figure 1*
*322x119 mm ( x DPI)*

**Figure 2.**

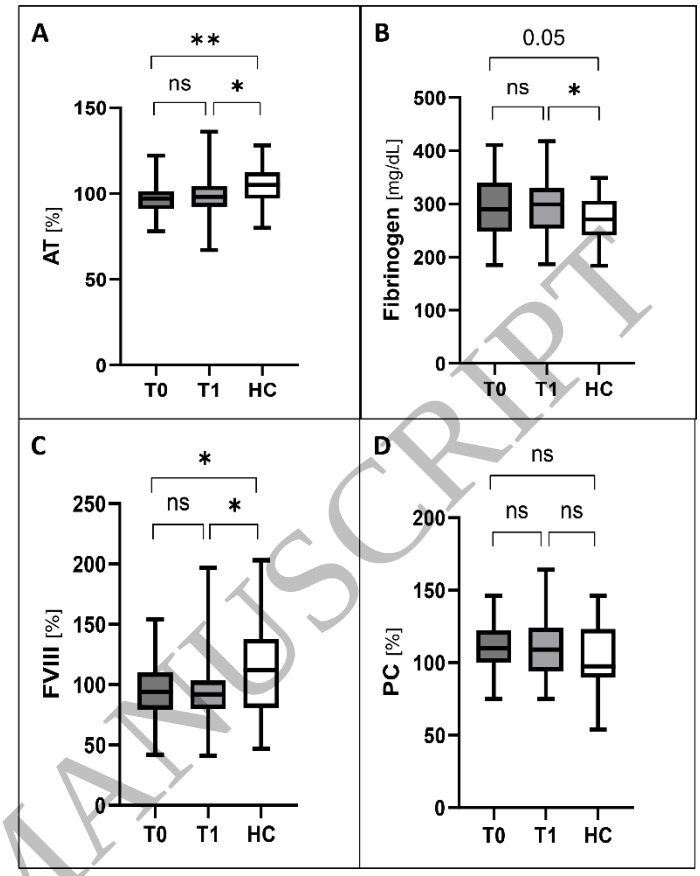


*Figure 2*
*246x171 mm ( x DPI)*

**Figure 3**

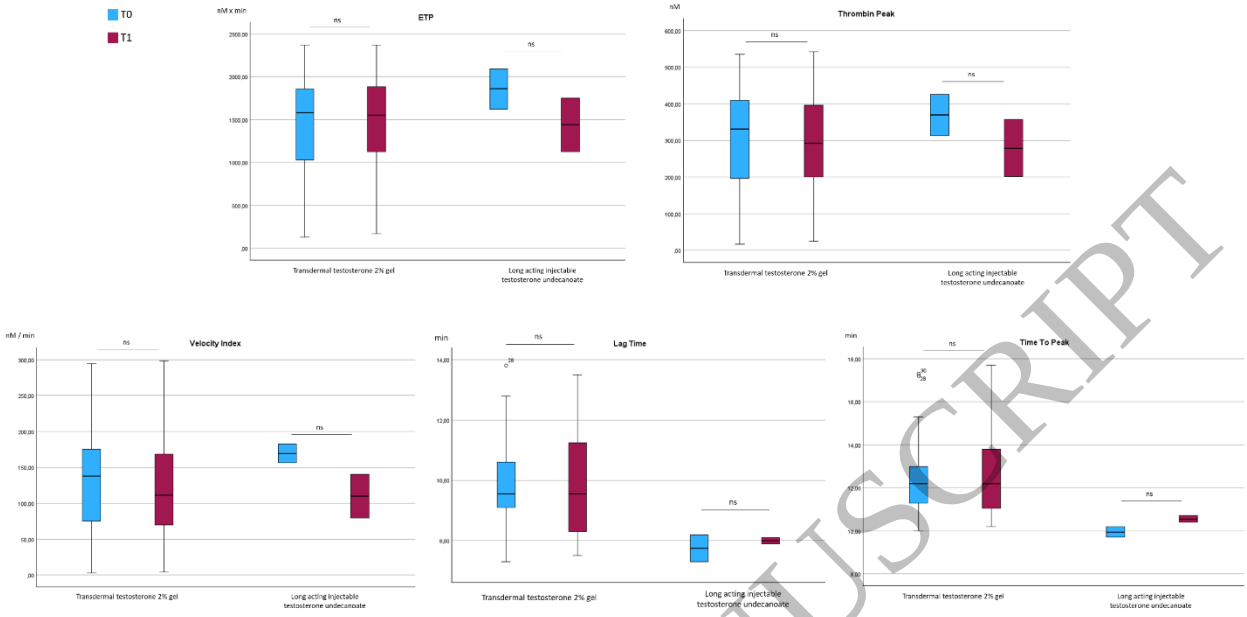


*Figure 3*
*339x190 mm ( x DPI)*

**Figure 4**

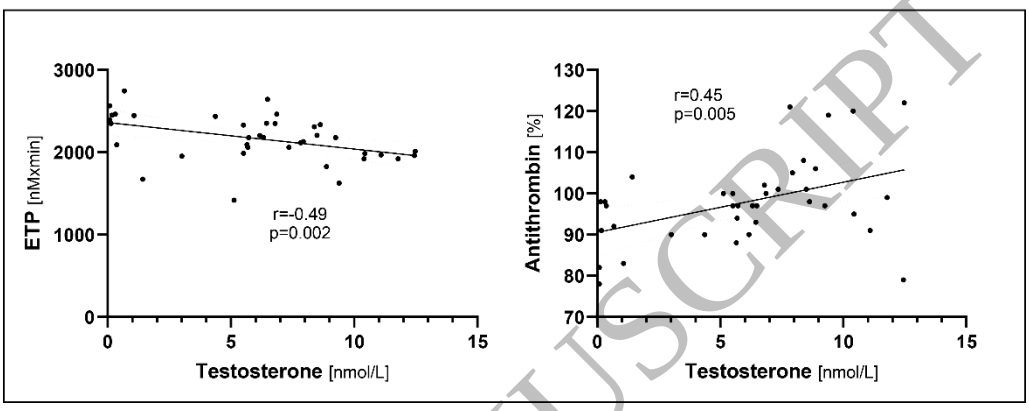


*Figure 4*
*264x140 mm ( x DPI)*