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Long-term testosterone therapy in hypogonadal men ameliorates elements of the metabolic syndrome: an observational, long-term registry study

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SUMMARY

Aim: The goal of this study was to determine if long-term testosterone (T) therapy in men with hypogonadism, henceforth referred to as testosterone deficiency (TD), ameliorates or improves metabolic syndrome (MetS) components. **Methods:** We performed a cumulative registry study of 255 men, aged between 33 and 69 years (mean 58.02 ± 6.30) with subnormal plasma total T levels (mean: 9.93 ± 1.38; range: 5.89–12.13 nmol/l) as well as at least mild symptoms of TD assessed by the Aging Males' symptoms scale. All men received treatment with parenteral T undecanoate 1000 mg (Nebido®, Bayer Pharma, Berlin, Germany), administered at baseline and 6 weeks and thereafter every 12 weeks for up to 60 months. Lipids, glucose, liver enzymes and haemoglobin A_{1c} analyses were carried out in a commercial laboratory. Anthropometric measurements were also made throughout the study period. **Results:** Testosterone therapy restored physiological T levels and resulted in reductions in total cholesterol (TC) [7.29 ± 1.03 to 4.87 ± 0.29 mmol/l (281.58 ± 39.8 to 188.12 ± 11.31 mg/dl)], low-density lipoprotein cholesterol [4.24 ± 1.07 to 2.84 ± 0.92 mmol/l (163.79 ± 41.44 to 109.84 ± 35.41 mg/dl)], triglycerides [3.14 ± 0.58 to 2.16 ± 0.13 mmol/l (276.16 ± 51.32 to 189.78 ± 11.33 mg/dl)] and increased high-density lipoprotein levels [1.45 ± 0.46 to 1.52 ± 0.45 mmol/l (56.17 ± 17.79 to 58.85 ± 17.51 mg/dl)] ($p < 0.0001$ for all). There were marked reductions in systolic and diastolic blood pressure, blood glucose, haemoglobin A_{1c}, C-reactive protein (6.29 ± 7.96 to 1.03 ± 1.87 U/l), alanine aminotransferase and aspartate aminotransferase ($p < 0.0001$ for all). **Conclusions:** Long-term T therapy, at physiological levels, ameliorates MetS components. These findings strongly suggest that T therapy in hypogonadal men may prove useful in reducing the risk of cardiometabolic diseases.

What's known
Metabolic syndrome (MetS) is associated with increased risk for cardiovascular disease and diabetes mellitus. There is a strong association between MetS and testosterone (T) deficiency. Clinical interventions with diet, exercise and behavioural therapy (lifestyle changes) and use of statins and antidiabetic agents to normalise lipid profiles, control hypertension, improve insulin sensitivity and reduce abdominal obesity are among the steps taken to ameliorate this disorder and reduce cardio-metabolic risk.

What's new
Testosterone therapy significantly reduced total cholesterol, low-density lipoprotein cholesterol, triglycerides and increased HDL cholesterol levels. Furthermore, T treatment significantly reduced blood glucose and HbA_{1c} levels and improved systolic and diastolic blood pressure. These findings strongly suggest that T therapy ameliorates MetS components and this may prove useful in reducing the risk of cardio-metabolic diseases.

Introduction

It has been recognised for quite some time that testosterone (T) is a metabolic and vascular hormone and plays a key role in regulation of functional metabolism and is a relevant therapeutic hormone in hypogonadism (1,2). T exerts a wide range of beneficial physiological effects critical to men's health (3–6). T deficiency (TD; hypogonadism) is known to alter functional metabolism and significantly contributes to changes in body anthropometric parameters and body composition [reviewed in (7)]. TD also increases vascular disease risk factors, such as type 2 diabetes mellitus (T2DM), MetS and obesity, thus contributing to cardiovascular risk (3,8–24). It has been suggested that changes in functional metabolism lead to development and/or progression of MetS, a disorder characterised by a cluster of cardiovascular risk factors including increased abdominal obesity, elevated triglycerides (TGs), reduced high-density lipoprotein (HDL), high blood pressure, increased fasting glucose and hyperinsulinaemia (4–7,9–11,25–34).

Testosterone regulates body composition (fat and muscle mass), and androgen deficiency produces impaired glucose metabolism and higher levels of TGs and cholesterol concomitant with reduced HDL cholesterol (4–7,13,34). Brand et al. (25) evaluated a number of studies investigating the relationship between T and MetS and concluded that reduced T levels (TD) are associated with MetS. Similarly, Li et al. (35) showed that the prevalence of MetS in 1226 men is associated with reduced levels of total T,

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free T and bioavailable T and sex hormone-binding globulin (SHBG). Corona et al. (36) demonstrated a higher prevalence of MetS, waist circumference (WC) and TGs as a function of reduced T and age, suggesting that a strong association exists between TD and MetS. Corona also reported increased components of MetS such as elevated BP, hyperglycaemia, WC, high TGs and low HDL cholesterol in 1491 men attending an andrology unit for sexual dysfunction (36). In the SHIP study, Haring et al. (33) suggested that the components of the MetS at baseline predicted low total T in all age groups investigated. We have previously pointed out that the relationship between TD and MetS is bidirectional (9–11), and we believe that restoring physiological T ameliorates the components of MetS and appropriate clinical management of MetS may normalise or increase T levels. This premise has recently been investigated and support for this contention is presented in recent studies (22,37).

Metabolic syndrome and TD are closely linked (38). Epidemiological studies suggested that TD is associated with obesity, insulin resistance (IR) and an adverse lipid profile in men. Conversely, men with MetS and type 2 diabetes have a high prevalence of TD. MetS and TD are both independently associated with increased all-cause and cardiovascular mortality. Observational and experimental data suggest that physiological replacement of T produces improvement in IR, obesity, dyslipidaemia and sexual dysfunction along with improved quality of life. However, there are no prospective long-term interventional studies to assess the effect of T replacement on mortality in men with low T levels.

As MetS is a risk factor for cardiovascular disease (CVD), it is likely that TD contributes to the onset and/or progression of CVD by altering endothelial function, lipid profiles, inflammatory responses, vascular smooth muscle reactivity and other critical cellular signalling pathways in the vascular beds (4–6,9–11). T therapy has been shown to ameliorate IR and improve glycaemic control (23,39–44) with objective measures of reduced body fat mass and increased lean muscle mass (7,34). T therapy is also associated with reduced cholesterol and TGs levels (9–11). A number of studies investigated the effects of T on body composition, muscle mass and lipid profiles, and a strong positive association was demonstrated between T therapy and improved insulin sensitivity, increased muscle mass and reduced fat mass and potential amelioration of some of the MetS elements (7,45–48). However, many of these studies had small sample size and/or were of short duration and therefore were subjected to criticism that the long-term effects of T therapy remained unknown. Using a registry (49), we have examined the effects of T therapy in 255 hypogonadal men over a 5-year period on the functional metabolic profiles as it relates to MetS. Here, we report that long-term T therapy ameliorates elements of the MetS.

Methods and procedures

We performed a cumulative registry study of 255 mainly elderly men, aged between 33 and 69 years (mean 58.02 ± 6.30). All subjects had sought urological consultation in a single urologist's office for various medical conditions, e.g. erectile dysfunction, decreased libido, questions about their T status or a variety of urological complaints. A number of subjects, for instance, men with osteoporosis, had been referred by other specialists with a suspicion of TD. Upon clinical and laboratory investigation, the subjects were found to have subnormal plasma total T levels (mean: 9.93 ± 1.37; range: 5.89–12.13 nmol/l) as well as at least mild symptoms of hypogonadism assessed by the Aging Males' symptoms scale. All men received treatment with parenteral T undecanoate 1000 mg (Nebido®, Bayer Pharma, Berlin, Germany), administered at baseline and 6 weeks and thereafter every 12 weeks for up to 60 months.

Although there is no international consensus as to the normal range of T, clinical data suggest that the normal range of T in adult men is between 12 and 40 nmol/l (50). A threshold of 12.1 nmol/l was recently confirmed by Bhasin et al. in an analysis of a number of well-known studies such as Framingham Heart Study generations 2 and 3, European Male Aging Study and the Osteoporotic Fractures in Men Study (51).

Measurements of anthropometric parameters were performed at baseline (height, weight, WC) and at each visit (weight, WC) and blood samples drawn at each visit, prior to the next injection of T. Therefore, T levels were trough levels at the end of an injection interval. WC was measured midway between the last rib and the uppermost border of the right iliac crest. T was measured by standard laboratory measurement as described previously (49). Because of the cumulative registry design of the study, the number of subjects decreased over time. New subjects are entered into the database once they have received 1 year of treatment with T. All 255 subjects were followed up for at least 1 year, 215 for at least 2 years, 182 for 3 years, 148 for 4 years and 116 for 5 years. The declining number of patients reflects duration of treatment but not drop-out rates. On the contrary, adherence to treatment was excellent, and T was only discontinued in three men.

Total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, HDL cholesterol, plasma TGs,

fasting glucose, haemoglobin A_{1c}, C-reactive protein (CRP) and liver function tests were carried out by a commercial laboratory using standard test methods. Measurements of systolic and diastolic blood pressure were performed via a sphygmomanometer.

Statistical analyses

For continuous variables, the mean, median, standard deviation, range, minimum, maximum, and sample size for the overall sample and various groups were reported at each time point. For categorical variables, the frequency distribution was reported. We tested the hypotheses regarding change in outcome scores across the study period by fitting a linear mixed effects model to the data. Time (to indicate follow-up interviews) was included as fixed effect in the model. A random effect was included in the model for the intercept. Estimation and test of change in scores were determined by computing the differences in least square means at baseline vs. the score at each follow-up interview. For the correlation study, Pearson correlation was calculated between baseline changes in outcomes at various time points. The significance of each correlation was tested using Fisher's exact test.

Results

A host of comorbidities was reported in the patients in this registry (Table 1). We have considered a number of specific concerns that pertains to MetS such as use of antihypertensive medications, which may not be associated with the respective diagnosis. Also, we examined blood pressure measured at the baseline visit as well as use of statins as lipid-lowering medications. We further recorded the use of antidiabetic medication in these men. The distribution of MetS components at baseline was as follows:

244 (96%) men had WC $\geq$ 94 cm and 174 men (68%) had WC $\geq$ 102 cm. 237 men (93%) had hypertension (diastolic BP $\geq$ 130 and/or systolic BP $\geq$ 85) and 255 men (100%) had dyslipidaemia (TGs $\geq$ 150 mg/dl and/or HDL $\leq$ 40 mg/dl). 127 men (50%) had IR (T2D, antidiabetic medication or fasting glucose $\geq$ 100 mg/dl). Only 11 patients did not fulfil three or more MetS criteria as described in the 'reconciled' definition by the International Diabetes Federation and the American Heart Association/National Heart, Lung and Blood Institute (52).

Total plasma T levels in men with TD treated with TU for 5 years

Total T levels increased significantly from 9.9 nmol/l at the beginning of therapy to about 18 nmol/l within the first 12 months of therapy ($p < 0.0001$), then reached a plateau at physiological levels and remained constant at this level throughout the course of treatment approaching 5 years (Figure 1).

Effects of T therapy on obesity

The effects of T on anthropometric parameters have been reported elsewhere (49). In short, we observed reductions of WC by 8.5 $\pm$ 0.17 cm and body weight by 15.35 $\pm$ 0.43 kg ($p < 0.0001$ for both) (49).

Effects of T therapy on MetS components

Metabolic syndrome is a cluster of cardiovascular risk factors including increased central abdominal obesity, elevated TGs, reduced HDL, elevated blood

Table 1 Baseline characteristics of patients (mean age in years: 58.02 $\pm$ 6.30) in the registry

Comorbidities relevant to metabolic syndrome at baseline				
	Patient-reported		Investigator-assessed	
	n	Proportion (%)	n	Proportion (%)
Elevated waist circumference			244	96
Hypertension	101	40	237*	93
Dyslipidaemia	47	18		
Elevated triglycerides			255*	100
Reduced HDL cholesterol			57*	22
Type 2 diabetes	80	31	81	32
Elevated fasting glucose			46*	18
Coronary artery disease	40	16		
Postmyocardial infarction	39	15		
Erectile dysfunction	145	57	173†	68

*Fulfilling metabolic syndrome criteria according to criteria discussed in reference (52).
†At least mild ED according to IIEF-EF (questions 1–15 plus 15).

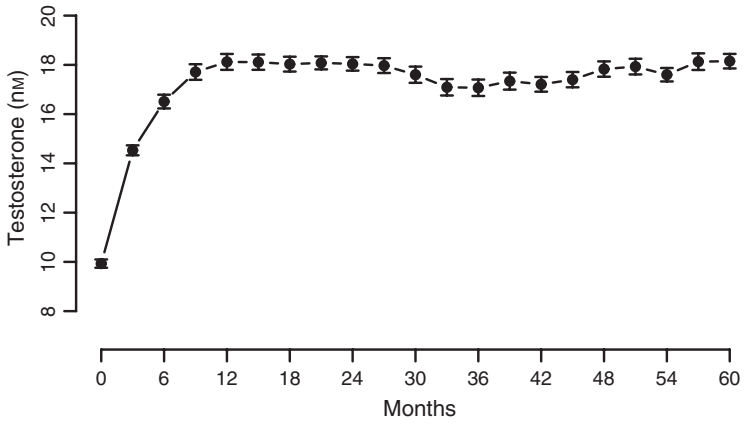

Figure 1 Mean total plasma testosterone levels in men with TD undergoing T therapy for a period of 5 years

pressure and elevated fasting glucose or accordant treatment [reviewed in (9–11)]. To determine if T therapy ameliorates the components of the MetS, we analysed TC, LDL cholesterol, HDL cholesterol, TGs, systolic and diastolic blood pressure, glucose, haemoglobin A_{1c} and CRP.

Effects of T therapy on lipid profiles

Testosterone treatment in hypogonadal men resulted in gradual and consistent decrease in TC levels. The decrease was statistically significant as early as 12 months ($p < 0.0001$) and reached a plateau at 24 months ($p < 0.0001$ vs. 12 months, thereafter non-significant). The level of cholesterol at baseline was approximately 7.3 mmol/l (282 mg/dl). This concentration was reduced to about 4.9 mmol/l (188 mg/dl) and remained low throughout the 5-year period of the study (Figure 2A). Similarly, T treatment resulted in marked and significant gradual and consistent decrease in LDL cholesterol levels from approximately 4.2 mmol/l (164 mg/dl) to approximately 2.8 mmol/l (110 mg/dl). The reduction in LDL levels was significant within the first year of treatment ($p < 0.0001$), significant at 24 months ($p < 0.0001$ vs. 12 months) and stable thereafter (Figure 2B). Remarkably, the LDL cholesterol levels remained low over the course of 5-year period of T treatment. HDL cholesterol levels slightly but significantly increased and remained elevated over the 5-year period of treatment (Figure 3A). The increase was gradual and significant within the first year of treatment ($p < 0.0001$). The TC/HDL ratio is thought to predict the risk of CVD, in particular, ischaemic heart disease. The ratio of TC/HDL in these treated patients improved considerably from 5.44 ± 1.61 to 3.49 ± 1.09 ($p < 0.0001$) (Figure 3B), suggesting a favourable change in the lipid profile and a potential reduction in CVD risk.

With T treatment, we also observed gradual and consistent decrease in TG levels from approximately 3.1 mmol/l (276 mg/dl) to 2.2 mmol/l (190 mg/dl) and they remained low throughout the 5-year period of the study (Figure 4). The decrease was statistically significant within the first year ($p < 0.0001$), again significant at 24 months vs. 12 months ($p < 0.0001$) and thereafter remained low over the entire treatment period.

Effects of T treatment on systolic and diastolic blood pressure

Testosterone treatment of men with TD produced marked and sustained gradual decrease in systolic blood pressure (SBP) from 153.55 ± 17.6 to 137.72 ± 10.9 mmHg ($p < 0.0001$) (Figure 5A). The decrease was significant and gradual over the first 2 years and remained low over the entire course of the 5 years of treatment. Similar results were recorded with the diastolic blood pressure, which decreased from 93.49 ± 11.32 to 79.59 ± 7.36 mmHg ($p < 0.0001$) (Figure 5B), in that a marked and rapid decrease was noted over the first 2 years of treatment and then remained low over the entire 5 years of treatment.

Effects of T treatment on blood glucose and haemoglobin A1c levels

Testosterone treatment of men with TD resulted in a significant gradual decrease in fasting blood glucose from 5.74 ± 0.8 mmol/l (103.35 ± 14.42 mg/dl) to 5.41 ± 0.8 mmol/l (97.56 ± 2.35 mg/dl) (Figure 6A). The decrease was significant after 12 months ($p < 0.0001$), further declined after 24 months ($p = 0.012$ vs. 12 months) and then reached a plateau. The decrease in fasting blood glucose was paralleled by a marked decrease in haemoglobin A_{1c} from $7.06 \pm 1.54\%$ to $6.16 \pm 1.35\%$. In contrast to fasting glucose, the decrease in HbA_{1c} was statistically significant after 12 months ($p < 0.0001$), between 24 and 12 months ($p < 0.0001$), between 36 and 24 months ($p = 0.0036$),

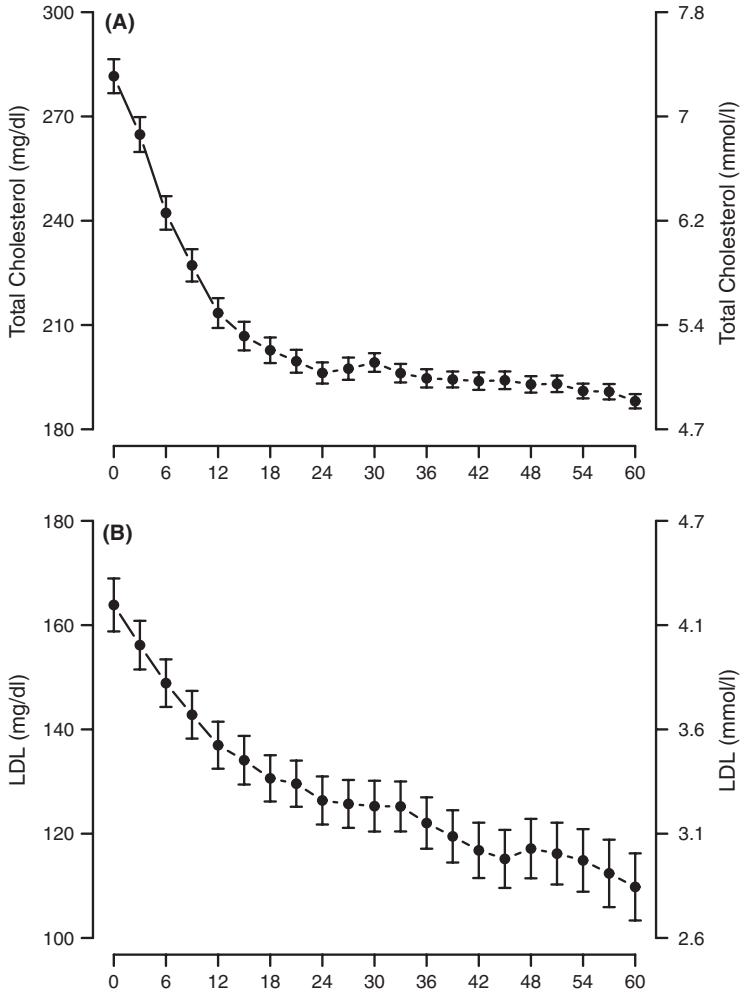

Figure 2 Total cholesterol (A) and LDL cholesterol levels (B) in men with TD undergoing T therapy for 5 years

between 48 and 36 months ($p = 0.0049$), and between 60 and 48 months ($p = 0.0149$) (Figure 6B).

Effects of T treatment on CRP levels and markers of liver function

We have noted a marked and significant decrease in the levels of CRP (from 6.29 ± 7.96 to 1.03 ± 1.89 U/l) ($p < 0.0001$ with a plateau after 36 months), aspartate transaminase (AST) from 43.05 ± 17.29 to 20.18 ± 3.22 U/L) ($p < 0.0001$ with a plateau after 24 months) and alanine transaminase (ALT) from 43.89 ± 18.11 to 20.55 ± 3.92 U/l ($p < 0.0001$ with a plateau after 36 months) suggesting a reduced inflammatory response and improvement in liver function. (Figures 7, 8A,B).

Testosterone therapy and prostate safety

In this observational study, mean prostate volume increased from 28.51 ± 11.2 ml to 30.04 ± 12.35 ml ($p < 0.0001$), reaching a plateau after 3 years. Mean prostate specific antigen increased from 1.77 ± 0.97 to 1.83 ± 0.95 ng/ml ($p < 0.0001$) with a plateau after 2 years. There were no occurrences of urinary retention or other problems related to benign prostatic hyperplasia (BPH). In addition, few subjects had increased haematocrit values > 52%, which were all resolved without intervention. With regard to prostate cancer (PCa), only three patients were diagnosed with PCa. This represents an incidence of 1.2% [95% CI (0.24–3.4%)] and an incidence of 30.3 [95% CI (0.9783–9.4052)] per 10,000 person-years, as previously reported (49). In the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial in which 38,345 men ages 55–74 years in the control arm were followed up to 13 years, Andriole et al., (53) showed that 3815 men were diagnosed with PCa representing an incidence of 97.1 per 10,000 person-years. In the European Randomized Study of Screening for Prostate Cancer Patients, Schröder et al. (54) reported on 72,891 patients, with mean ages 55–69 year and a follow up of 11 years. The authors demonstrated that 6963 patients were diag-

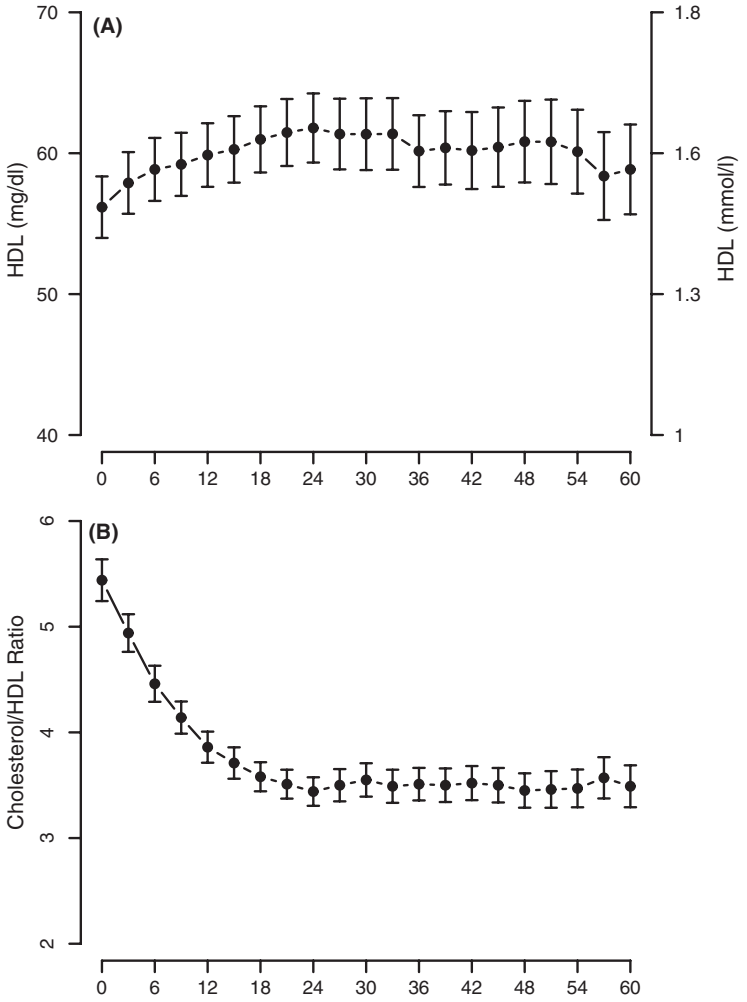

Figure 3 HDL cholesterol (A) and total cholesterol/HDL-C ratio (B) in men with TD undergoing therapy for 5 years

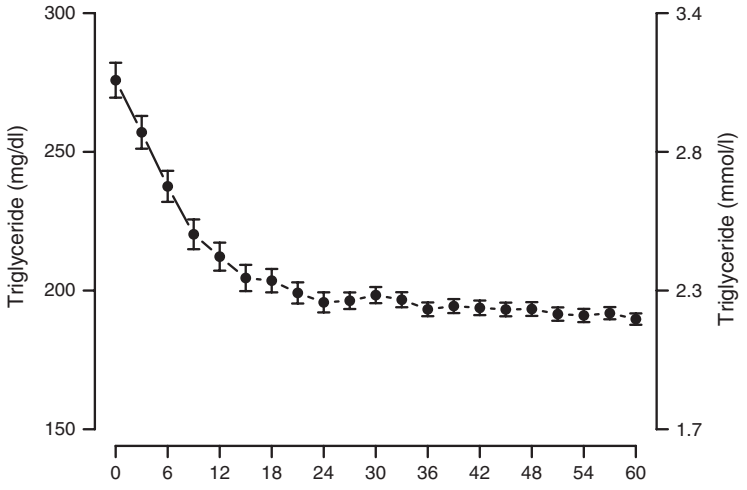

Figure 4 Triglyceride levels in men with TD undergoing T therapy for 5 years

nosed with PCa (9.6%) and an incidence of 96.6 per 10,000 person-years (54). If one carefully examines the data from such extensive screening trials, it becomes clear that the incidence of PCa reported in our cohort remained far lower than expected. As discussed by Saad et al. (49) in their recent report on

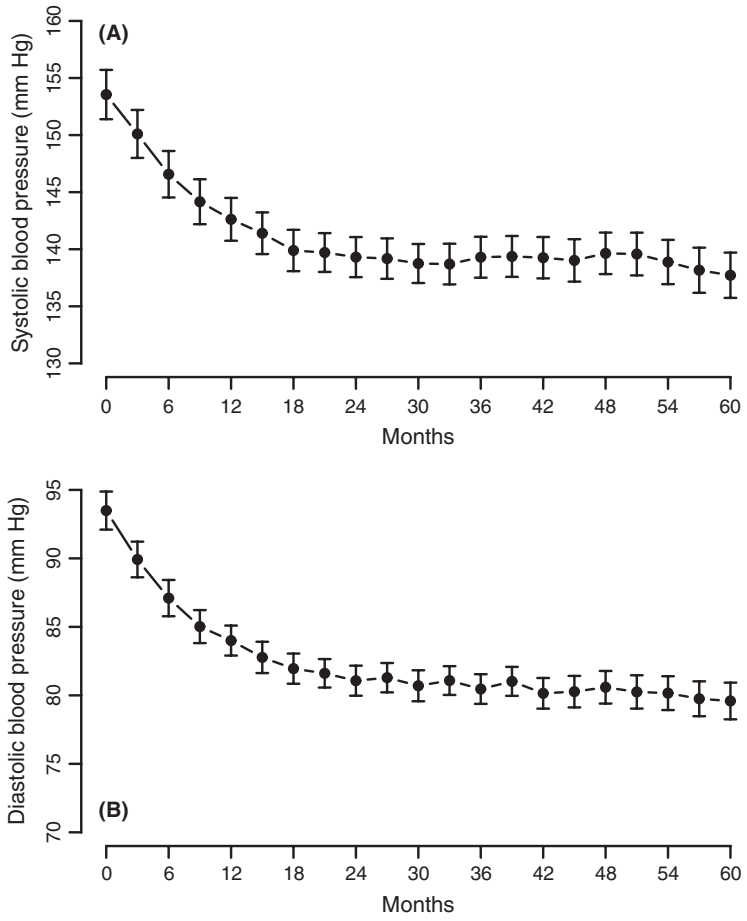

Figure 5 Systolic blood pressure (A) and diastolic blood pressure (B) in men with TD undergoing T therapy for 5 years

long-term T treatment, this incidence is far lower than reported in previous studies. To date, there is no compelling evidence that T is the driving factor in the development or progression of PCa (55). Recent guidelines developed for monitoring T treatment need be adhered to in order to ensure a safe therapy in men without suspicion of PCa. Recent reports have placed fears regarding T therapy and on PCa in a more rational perspective (55–57).

Discussion

In this long-term observational registry study, we investigated the effects of T therapy in 255 men with TD on MetS components. T therapy restored physiological T levels within the first 12 months and these levels were maintained with T therapy throughout the entire 5-year period.

One of the key findings of this study is that T therapy markedly and significantly reduced TC levels. This reduction in TC was pronounced and sustained over the entire treatment period. Interestingly, the reduction in TC was in a magnitude of 25–40% of TC at baseline, suggesting that T therapy influences both synthesis and disposal of TC. This confirms observations reported in previous studies (58–60). To our knowledge, this is the first study to report on 5-year long-term T therapy on cholesterol levels in men with TD.

Our study demonstrates that long-term T therapy progressively and significantly reduced LDL and this reduction was sustained throughout the treatment cycle. Remarkably, total LDL levels significantly reduced over the entire course of treatment period. This finding is of clinical importance since reduction in LDL is thought to correlate with reduced CVD risk. Furthermore, the reduction in LDL suggests that T therapy plays an important role in improving overall lipid profiles in men with TD and represents an important metabolic function in attenuating CVD risk and may ameliorate this pathology in men with TD. Our data confirm previous observations in which LDL reduction in response to T therapy was reported (13,58–60). In cross-sectional and prospective observational studies, TD was found to be associated with increased LDL and reduced HDL levels

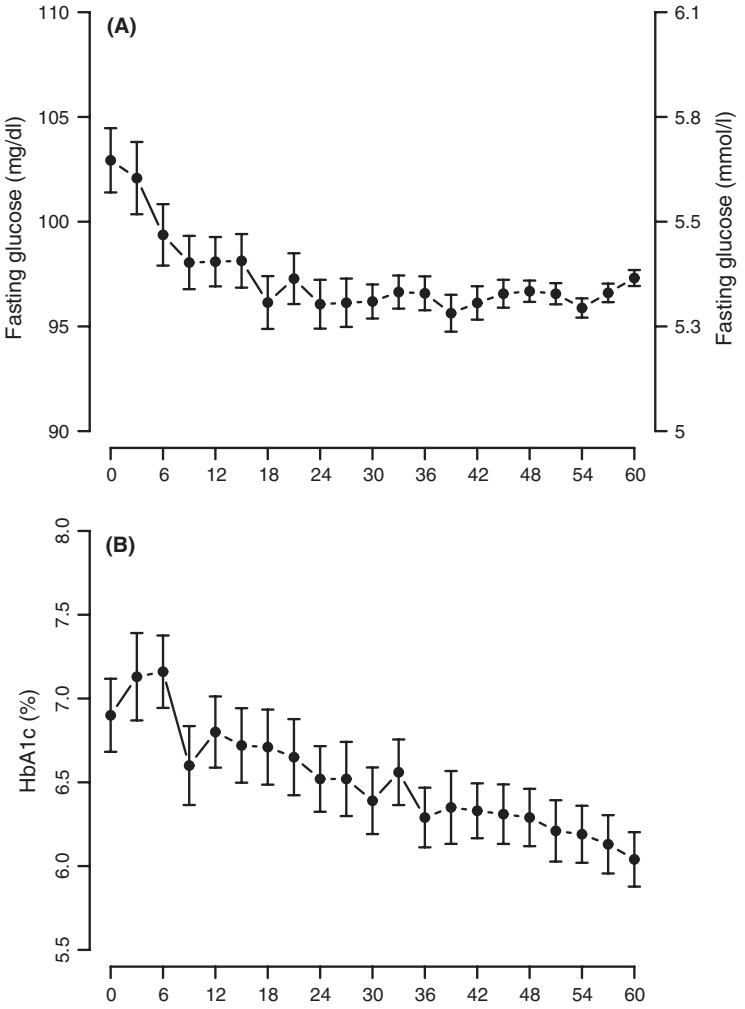

Figure 6 Glucose concentration (Panel A) and HbA1c levels (Panel B) in men with TD undergoing T therapy for 5 years

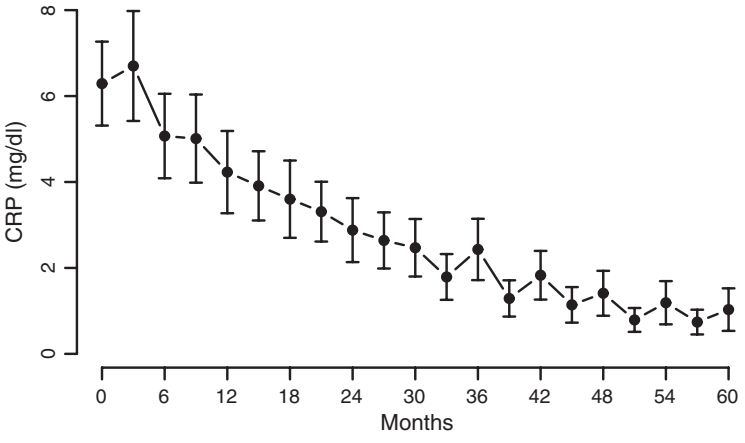

Figure 7 C-reactive protein (CRP) levels in men with TD undergoing T therapy for 5 years

(61). Furthermore, T therapy in men with TD is associated with reduced levels of LDL and TC coupled with beneficial increase in HDL (13). Thus, we believe that the effects of T contribute to reduced CVD risk (62) and increased benefits, such as improved lipid profile (61).

It is worthy to note that we observed a marked and significant increase in HDL in response to T

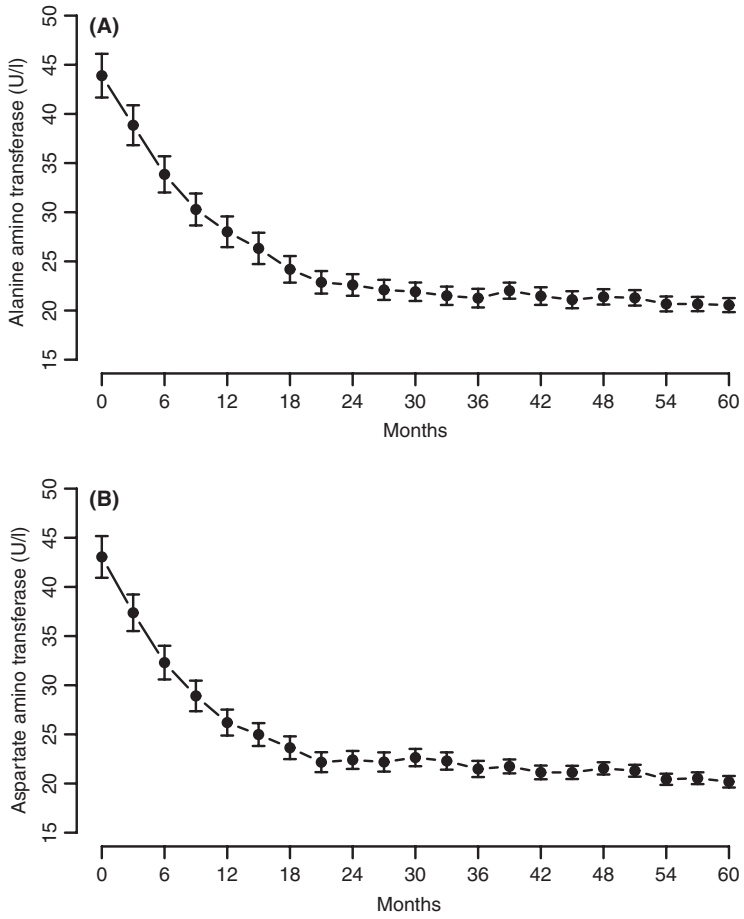

Figure 8 Alanine aminotransferase (A) and aspartate aminotransferase (B) in men with TD undergoing T therapy for 5 years

therapy over the entire course of 5 years of follow up. This is of clinical importance, as HDL is thought to play a critical role in reducing CVD risk. Furthermore, these data suggest that the increase in HDL levels may be attributed to restoration of physiological T levels. It should be noted that other studies have shown increase (58), decrease (63) or no changes (48) in HDL levels in men with TD treated with T [reviewed in (13)]. We suggest that the discrepancies in the various studies may relate to use of varying formulations of T, dosage administered, duration of the studies, methods of assessment of the various end-points and age and comorbidities of subjects enrolled in the various studies. Isidori et al. (64) noted that the decrease in HDL in response to T therapy was seen mostly in studies with supraphysiological levels of T.

It should be noted that HDL is not a single particle but rather a composite of heterogeneous particles differing in size and apolipoprotein composition between individuals (65). It was shown that nondenaturing 2D electrophoresis analysis of HDL revealed the presence of distinct HDL subpopulations from the same individual, suggesting that alterations in HDL subpopulations and their distribution may correlate with CHD (65). T therapy has been shown to reduce HDL3-C subfraction and LpA-I:A-II particles but not the more anti-atherogenic HDL2-c and LpA-I particles (66). Rubinow et al. showed that androgen deprivation increased cholesterol efflux from macrophages and demonstrated that sex steroid manipulation modified the HDL proteome (67). Furthermore, T therapy in older hypogonadal men altered HDL protein and lipid composition but did not significantly change serum HDL-mediated cholesterol efflux (68).

The TC/HDL ratio is thought to predict the risk of CVD because it may indicate a cluster of abnormalities (69). It should be noted that variations in TC/HDL-C ratio may be associated with changes in metabolic function and this ratio may be predictive of IR and ischaemic heart disease risk (69). Modification in lipid metabolism by pharmacotherapy to lower the TC/HDL ratio is believed to reduce the

risk of CVD. It is thought that the TC/HDL ratio is a better lipid predictor of CVD in T2DM patients (70).

Recent studies have also suggested that the non-HDL-C/HDL-C ratio is a better marker than the apoB/apoA1 ratio for identifying IR and MetS in Koreans (71). A recent study had demonstrated that patients with peripheral artery disease treated with atorvastatin showed improvement in endothelial function and this was associated with decreased TC/HDL ratio, suggesting that this ratio may relate to endothelial damage (72).

Another critical finding of this study was the marked and significant reduction in total TGs levels in response to T therapy in men with TD. It is important to note that the observed reduction represented normalisation of lipid profiles in men with TD in response to T therapy. As visceral fat storage is dependent on accumulation of TGs, these data are congruent with our previous report that T therapy in men with TD, who are overweight or obese, resulted in marked reduction in body weight, WC and body mass index (BMI) (49). Also, these results are in concordance with a number of studies demonstrating increased lean body mass and reduced fat mass in response to T therapy (7,34,49). Agledahl et al. (73) reported that a linear increase in serum TGs levels was found in men with total T levels below the 50th percentile, while serum TGs levels did not change in men with T levels above the 50th percentile. Total T and SHBG were inversely and independently associated with TGs, and positively and independently associated with HDL. Men with an unfavourable lipid profile had significantly lower levels of total T and SHBG in age- and BMI-adjusted analyses, compared with men with a normal lipid profile. In a high-fat diet-induced rabbit model of MetS, Maneschi et al. (74) showed that T treatment of these animals ameliorated the metabolic profile and reduced visceral adipose tissue.

The relationship between T and lipids was assessed in a large group of men (75). The data showed that (i) TG levels were negatively associated with quartile levels of T and the magnitudes of associations were greater for postprandial TGs than for fasting TGs; (ii) HDL cholesterol (HDL-C) levels were positively related to quartile levels of T, but the associations became insignificant after further control of TGs; and (iii) the calculated LDL cholesterol (LDL-C) levels were positively associated with quartile levels of T. The favourable association of T with HDL-C counterbalances the unfavourable association of it with LDL-C, while the net influence of T on plasma lipids for cardiovascular system was still in the beneficial direction because of its negative association with postprandial plasma TG levels (75). Data from clinical trials have shown that the efficacy of statins in reducing TC and LDL cholesterol ranges from 17% to 53% (76,77). Interestingly, T therapy produced marked and significant reductions in TC and LDL cholesterol. These observations suggest that T therapy modifies lipid metabolism resulting in favourable lipid profiles. This is an important finding and needs be further investigated.

Men with TD may be at a high risk of altered lipid profiles and MetS. T therapy may normalise lipid profiles and reduce the risk of CVD (62). Some studies suggested that T therapy does not reduce LDL cholesterol levels while reducing TC levels (64). One must consider the number of confounding factors that may have contributed to data in the various reported studies, such as T formulation, dosages and duration of T therapy. A marked reduction in TC and LDL cholesterol was reported in response to T treatment (78). Similar findings were reported in patients with T2DM and MetS treated with T (43,79,80).

Although TC and LDL cholesterol are not considered in the definitions of MetS, the reduced levels of HDL cholesterol and increased levels of TGs are considered among the key components of MetS. Clearly, T therapy has been shown to improve lipid profiles; however, a number of studies produced discrepant levels of HDL cholesterol in response to T treatment [reviewed in (13)]. It is not surprising that endogenous T levels were shown to inversely correlate with TC and LDL cholesterol (81–87) but positively with HDL cholesterol (88).

We have previously reviewed the relationship between T and lipid profiles and have reported that the relationship between T and HDL levels is controversial (13) with a number of studies suggesting that HDL cholesterol levels increased in men in response to T therapy (13,88,89). The discrepancies in many of these studies are attributed to differences in age of men in the studies, route of administration and the preparation, as well as dose and duration of treatment. It is also possible that in studies where supraphysiological levels of T were achieved, HDL levels were thought to show a decrease (64) but without taking into account the various changes in HDL subfractions.

Testosterone deficiency is associated with higher TGs levels (39,40,73,90). A marked decrease in TG levels was reported in men treated with T (91), but this was not verified in other studies (73,90). A number of studies have shown that T treatment ameliorated IR, reduced HOMA index and improved glycemic control (23–37,39–46,80,92,93). In the Tri-US Registry with approximately 37% of men with

TD having MetS at baseline (94), after 12 month of T treatment (n = 849), a marked decrease in fasting glucose, WC and blood pressure was noted in the men with MetS (94). In a recent study (IPASS) (n = 1438), men with TD were treated with T and followed up for up to 12 months. In a subgroup of 60 men with poorly controlled diabetes (mean HbA_{1c} 7.9%), treatment with T resulted in a mean decrease in HbA_{1c} of 1.1% after 12 months (91).

Of considerable interest is the marked reduction in systolic and diastolic blood pressure values noted in our study of men with TD in response to T therapy. It has been suggested that T therapy improved hypertension in patients with TD, but the data provided were limited. This 5-year long-term T therapy study provided important and consistent data on these parameters. However, the exact mechanism of how T therapy modulates blood pressure is incompletely understood. Potential mechanisms on the role of T as a vascular hormone have been postulated recently by Jones et al. (4–6). Testosterone modulates arterial blood pressure, through a host of mechanisms, such as direct effects on the heart, the kidney and the vessels, as well as the endothelium (95,96). In elderly men with isolated systolic hypertension, it was shown that the plasma T levels were lower than those of the normotensive subjects and a strong inverse relationship appears to exist between T levels and SBP, suggesting that low T contributes to the increased arterial stiffness (97). Svartberg et al. (98) proposed that lower endogenous T levels are associated with higher blood pressure. The authors reported that in 1548 men aged 25–84 years, total T was inversely associated with SBP. Men with categorical hypertension had lower levels of total and free T before and after adjusting for BMI. Furthermore, data from TRiUS (Testim®, Auxilium Pharmaceuticals, Malvern, PA, USA) Registry study showed that MetS patients demonstrated significant decreases in WC, fasting blood glucose levels and blood pressure in response to T therapy after 12 months (94). Also, in a case–control study, hypogonadism was shown to be associated with higher SBP (82). Furthermore, in obese, hypogonadal and diabetic men treated with oral T, blood pressure was shown to decrease favourably (92,99). In contrast, men treated with androgen deprivation therapy for PCa have been shown to have increased arterial stiffness (100,101).

Among the key components of MetS is IR and hyperglycaemia with concomitant increase in the surrogate marker haemoglobin A_{1c} (HbA_{1c}). As noted in this study, T therapy of men with TD demonstrated progressive and sustained reduction in blood glucose and the fraction of measurable HbA_{1c}, suggesting that T therapy improved glucose utilisation and increased insulin sensitivity, thus ameliorating this MetS component. The mechanisms by which T restores physiological glucose transport are discussed in recent reviews by Jones et al. (4–6).

Low circulating levels of TT, FT and SHBG were shown to be independently associated with an increase in HbA_{1c} in middle-aged and older men, even across its normal range. Clarifying the nature of this relationship may provide new insights into determinants of glucose and sex hormone metabolism and how these may contribute to risk of CVD (102) Interestingly, statins have been shown to increase HDL-C, but did not show any differential effect on glucose metabolism (103). In contrast, T therapy had improved HDL-C and also reduced glucose levels and reduced HbA_{1c}.

It is important to note that the levels of CRP, a non-specific marker of inflammation, were markedly reduced over the course of T therapy in men with TD. We have previously reported that T therapy results in significant and sustained weight loss (WL) (49). As WL significantly reduces plasma CRP concentrations (104), it is likely that the reduced weight re-establishes a new equilibrium with reduced inflammatory responses and reduced CRP levels. Interestingly, in the Jackson Heart Study, it was reported that CRP concentration was significantly and directly associated with change in SBP and WC but inversely associated with HDL cholesterol. The authors concluded that higher circulating CRP concentrations predict incident MetS (105). Furthermore, in longitudinal studies, elevated levels of hs-CRP predict future MetS independently of age, sex and smoking in apparently healthy Koreans (106). Ridker et al. (107) proposed that hs-CRP should be added to clinical criteria for MetS.

In addition, we noted a reduction in the activities of several liver enzymes, used as markers of liver function, suggesting that T therapy attenuates the inflammatory response and improves various physiological functions. Liver ALT levels are commonly used as markers in screening for liver disease with the upper-normal limit of 40 IU/l (108,109). The incidence of non-alcoholic fatty liver disease (NAFLD), a phenotype of MetS of the liver, is increasing (110,111). Epidemiological studies suggested that increased activities of liver enzymes such as ALT and AST may be associated with MetS and CVD and increased levels of ALT are associated with long-term development of multiple metabolic disorders (112). Even after age- and gender-adjusted analyses, increased ALT levels were significantly associated with an increased risk of T2DM and CVD. These findings are also supported by the Western Australian Health Department data linkage system, in

which a strong association was demonstrated between levels of ALT and MetS, independent of IR (113). Hoyos et al. were first to demonstrate a significant reduction in liver fat content in response to short-term T therapy (114). Taken together, these findings strongly suggest that normalising T levels in men with TD ameliorates a host of MetS components and reduces inflammation, which may include NAFLD, thus reducing the risk of cardiometabolic diseases.

A number of studies reported a strong association of increased cardiovascular and metabolic disorders in men with low T (22,62,115,116). Increased all-cause and CVD mortality in men with TD has recently been reported (117). T therapy in men with TD reduced CVD risk factors with concomitant improvement in insulin sensitivity and glycaemic control, even in studies of shorter duration (45,46). Furthermore, a marked decrease in visceral fat mass and circulating inflammatory cytokines concomitant with improvement in endothelial cell function have been reported (43,69).

Testosterone is well known to regulate a host of metabolic functions in liver, adipose tissue, muscles, coronary arteries and the heart. Thus, it is not surprising that T therapy reduces the risk of CVD. An inverse relationship exists between T and obesity and TD is associated with dyslipidaemia, atherosclerosis, CVDs, MetS and diabetes. T therapy of men with TD improves lipid profile and lowers cholesterol, blood sugar and IR (118). Conditions such as androgen deficiency or androgen deprivation are recognised to promote adipogenesis, which contributes to MetS and obesity. Androgens strongly inhibit differentiation of pluripotent cells into adipocytes and promote myogenesis (119,120). In MetS, the increase in circulating glucocorticoids may inhibit the androgen function, via increased activity of aldo-keto reductase 1C enzymes, which contribute to androgen inactivation and increased adipogenesis (121,122). Visceral fat accumulation and weight gain has been linked with metabolic alterations, and the modulation of body fat distribution is likely a significant implication of TD in men. Clinically, the relevance of T therapy is the improvement in body composition/fat distribution, reduced inflammation and amelioration of the MetS components (7,34). In a recent randomised, double-blind, placebo-controlled and parallel group trial, T therapy, Hoyos et al. (114) demonstrated increased insulin sensitivity, reduced liver fat and increased muscle mass to a greater extent than placebo. T treatment decreased arterial stiffness and decreased the respiratory quotient compared with placebo. It was suggested that in obese men, T therapy improved cardiometabolic parameters but did not differentially reduce overall weight or the MetS and suggested that longer term studies are required (114). In a recent meta-analysis, Corona et al. (22) showed that WL is associated with an increase in both bound and unbound T levels. The normalisation of sex hormones induced by body WL is a possible mechanism contributing to the beneficial effects of surgery in morbid obesity. This finding is supported by data published recently on body weight fluctuations and sex hormones in two epidemiological studies (32,115). Androgen deprivation therapy in men with PCa produced accumulation of visceral and subcutaneous abdominal fat and increased visceral fat area appears more closely linked to T than estradiol deficiency. This was also associated with increased IR (123).

Recently, it has been shown that reduced levels of TT, free T and SHBG are associated with adverse plasma levels of TGs, insulin and HDL cholesterol in a young male population. T and SHBG are also correlated inversely with SBP. The effect of T and SHBG on CVD risk factors in men was attributed in part to the higher risk for type 2 diabetes, MetS and CVD (124). TD is associated with a pro-atherogenic lipid profile as observed in relation to the MetS. Studies using depot injections of intramuscular T undecanoate have shown reductions in TG, TC and LDL cholesterol and increases in HDL cholesterol (89).

The link between the various comorbidities underlying the MetS such as overweight, obesity, T2DM, hypertension and unfavourable lipid profile is commensurate with cardiometabolic dysfunction and CVD. Appropriate management and treatments of T2DM, hypertension and dyslipidaemia represent a challenging paradigm, and to date, there has been no single pharmaco-therapeutic agent that could ameliorate or improve most of these conditions. A host of agents is needed to treat such comorbidities (125). T therapy appears to improve many of such comorbidities and this is attributed to common mechanisms of action linking the pathophysiology of such conditions.

We have recently discussed the potential adverse side effects of T therapy in patients enrolled in this registry (49). There were no occurrences of urinary retention or other problems related to BPH. In addition, few subjects had increased haematocrit values > 52%, which all resolved without intervention. Our data are congruent with a recent study showing that T supplementation was well tolerated and there were fewer cardiovascular events in the T-treated groups compared with placebo (126).

A limitation of the study is its observational nature. The study was not designed to specifically investigate the effects of T on the MetS, and patients were not selected for specific comorbidities. On the

contrary, patients represented a cohort in a real-life setting with various symptoms, comorbidities and conditions. Another limitation of this study was that a number of plasma hormones such as estradiol and gonadotrophins were not measured in all patients, in part because of financial constraints.

In this study, we demonstrate that long-term treatment with T to restore physiological levels ameliorates a number of the components of the MetS. T treatment significantly reduced TC, LDL cholesterol, TGs and increased HDL cholesterol levels. Furthermore, T treatment significantly reduced blood glucose and HbA_{1c} levels and improved systolic and diastolic blood pressure. These findings strongly suggest that T therapy ameliorates MetS components and this may prove useful in reducing the risk of CVD. These findings suggest that long-term treatment of men with TD restoring physiological levels of T produces important clinical benefits. This study differs from previous studies in that it followed men with TD for a period of 5 years, which is the longest reported duration of treatment to date.

Author contributions

Dr Traish was involved in the concept and design of the study, as well as data analysis and interpretation and drafting of the manuscript. Dr Haider was responsible for data collection and also study concept and design. Dr Doros was responsible for performing all the statistical analyses and participated in data analyses and interpretations. Dr Saad was responsible for concept and design of the study as well as data analysis and interpretation and had a critical role in the revision of the manuscript. All authors contributed to the analysis and interpretation of study data and critically reviewed and approved the manuscript before submission.

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