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ORIGINAL ARTICLE

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Testosterone therapy reduces insulin resistance in men with adult-onset testosterone deficiency and metabolic syndrome. Results from the Moscow Study, a randomized controlled trial with an open-label phase

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Abstract

Aims: To describe changes in homeostasis model assessment of insulin resistance index (HOMA-IR) following testosterone therapy in men with hypogonadism and metabolic syndrome (MetS).

Materials and Methods: A randomized, placebo-controlled, double-blind randomized controlled trial (RCT) comprising 184 men with MetS and hypogonadism (testosterone undecanoate [TU]: 113 men, placebo: 71 men) was conducted. This was followed by an open-label phase in which all men were given TU. We focused on men who were not receiving antiglycaemic agents (TU: 81 men; placebo: 54 men) as these could affect HOMA-IR. Inter-group comparison of HOMA-IR was restricted to the RCT (30 weeks), whilst intra-group comparison was carried out on men provided TU during the RCT and open-label phases (study cohort) and men given placebo during the RCT and then switched to TU during the open-label phase (confirmatory cohort).

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Regression analysis was performed to identify factors associated with change in HOMA-IR (ΔHOMA-IR).

Results: The median HOMA-IR was significantly reduced at almost every time point (after 18 weeks) compared to baseline in men receiving TU in both the study and confirmatory cohorts. There was a significant decrease in median values of fasting glucose (30 weeks: −2.1%; 138 weeks: −4.9%) and insulin (30 weeks: −10.5%; 138 weeks: −35.5%) after TU treatment. Placebo was not associated with significant ΔHOMA-IR. The only consistent predictor of HOMA-IR decrease following TU treatment was baseline HOMA-IR ($r^2 \geq 0.64$).

Conclusions: Baseline HOMA-IR predicted ΔHOMA-IR, with a greater percentage change in insulin than in fasting glucose. In men with MetS/type 2 diabetes (T2DM) not on antiglycaemic therapy, improvements in HOMA-IR may be greater than suggested by change in fasting glucose. Our results suggest that hypogonadism screening be included in the management of men with MetS/T2DM.

KEYWORDS
adult-onset hypogonadism, insulin resistance, metabolic syndrome, testosterone therapy, waist circumference

1 | INTRODUCTION

Metabolic syndrome (MetS) is described as a cluster of associated metabolic factors that individually are predictors of cardiovascular disease.[1] In 2009, a consensus was reached on key variables to provide the basis for diagnosing MetS, mirroring those of the International Diabetes Federation (IDF) classification. These variables are central obesity (ethnic-specific waist circumference [WC]) and presence of at least two of the following: (1) triglycerides ≥1.7 mmol/L (2) high-density lipoprotein <1.03 mmol/L in males and <1.29 mmol/L in females; (3) blood pressure ≥135/85; and (4) fasting plasma glucose ≥5.6 mmol/L.[2] Central obesity, a surrogate maker for visceral adiposity, and insulin resistance (IR) are considered drivers of MetS.[1]

IR, defined as impaired insulin action on tissues (principally skeletal muscle, adipose tissue and liver), is a key predictor of type 2 diabetes mellitus (T2DM) development/progression.[3] Visceral fat accumulation appears related to IR via increased secretion of proinflammatory markers and reduced adiponectin secretion, an anti-atherosclerotic adipokine.[3–6]

Adult-onset testosterone deficiency, characterized by low serum testosterone with related symptoms/signs, has a prevalence of 0.6%–12% in men aged over 50 years, is associated with MetS and present in 40% of men with T2DM.[7–9] Low testosterone levels are associated with increased all-cause mortality,[10–13] whilst restoring hormone levels using testosterone therapy (TTh) improves the symptoms/signs associated with testosterone deficiency and reduces mortality in men with T2DM.[7,11–14]

Some studies have suggested that low serum testosterone mediates IR.[15] Ottarsdottir et al.[15] assessed 1400 men between 2002 and 2005, with 657 of the men followed up in 2012–2014 (mean follow-up 9.7 years). Low baseline testosterone levels appeared to be associated with high homeostasis model assessment of IR index (HOMA-IR), after adjusting for confounders and baseline HOMA-IR.[15] Interestingly, high baseline HOMA-IR did not predict low testosterone at the end of follow-up, hinting that low testosterone levels could be a driver of IR pathogenesis.[15] This observation is reinforced by studies showing that acute androgen withdrawal resulted in deterioration of IR markers.[16–18]

Several studies have assessed the impact of TTh on IR in men with MetS/T2DM. The TIMES2 study, a randomized controlled trial (RCT), evaluated changes in HOMA-IR (mean baseline ± SD = 5.4 ± 3.6) over 12 months in 220 hypogonadal men with T2DM and/or MetS following transdermal testosterone gel (2%).[19] TTh (vs. placebo) led to a significant reduction in HOMA-IR: 15.2% ($p = 0.018$) after 6 months and 16.4% ($p = 0.006$) after 12 months.[19] Similarly, Groti et al.[20] carried out an RCT measuring changes in HOMA-IR following TTh in 55 men (28 men on testosterone undecanoate [TU], 27 men on placebo, administered 10-weekly), with T2DM, obesity and hypogonadism, and on oral antiglycaemic therapy. In the men on TU, after 12 months, HOMA-IR (mean baseline ± SD) decreased significantly (11.45 ± 7.34 to 6.81 ± 4.18; $p < 0.001$), whilst no significant change was observed in the men on placebo.[20] However, a 40-week RCT (TU vs. placebo) carried out by Gianatti et al.[21] in 88 men (45 men on TU, 43 men on placebo) with T2DM and serum total testosterone (TT) ≤12.0 nmol/L did not demonstrate a significant change in HOMA-IR compared to placebo. The baseline median (interquartile range [IQR]) HOMA-IR values were 2.11 (1.69–2.94) and 2.78 (1.76–3.93) in the TU and placebo arms, respectively.[21]

The Moscow Study was a 30-week RCT in 184 men with MetS (113 men [32 with T2DM] on TU, 71 men [24 with T2DM] on

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placebo) with low serum TT (<12 nmol/L) and/or low calculated free testosterone (cFT; <0.225 nmol/L).[22] HOMA-IR decreased significantly ($p = 0.04$) in the men on TU (mean baseline HOMA-IR: 5.4, 95% confidence interval [CI] 4.7–6.1; change in HOMA-IR [ΔHOMA-IR]: −1.49) compared to men on placebo (mean baseline HOMA-IR: 4.8, 95% CI 4.0–5.7; ΔHOMA-IR: 0.20).[22] Despite significant reductions in weight, WC and body mass index in the men on TU, no significant change in serum glucose levels were observed.[22] More recently, the T4DM Study investigated 1007 men with prediabetes and basal serum TT levels <14 nmol/L and showed a 40% reduction in T2DM progression.[23] Although fasting glucose significantly decreased in men on TU, improvement in glycated haemoglobin (HbA1c) was not evident.[23]

From the outset, the Moscow Study was scheduled as an open-label phase (not previously published) post RCT, in which men in the treatment arm continued TU, whilst those on placebo switched to TU. In this further analysis of the Moscow Study, we aimed to evaluate the impact of TU on HOMA-IR in a subgroup of men not on antiglycaemic agents during the RCT and open-label phases. We then determined whether baseline HOMA-IR, after adjusting for confounders (age, serum TT, sex hormone-binding globulin [SHBG], cFT, oestradiol [E2] and WC) was independently associated with ΔHOMA-IR.[24–28]

2 | MATERIALS AND METHODS

The Moscow Study (ClinicalTrials.gov identifier: NCT00696748—randomized controlled study/open-label phase—https://clinicaltrials.gov/ct2/show/NCT00696748) was carried out between 2005 and 2010 in accordance with the Declaration of Helsinki (2000) and approved by the institution's committee on scientific investigations in human subjects, the Federal Service of Control and Supervision in Public Health and Social Development, and the Russian Pharmacological Committee of the Ministry of Health.[21,29,30] All participants received written information on the study, potential benefits and risks, and participation requirements. Informed consent was obtained and signed and dated by the physician and participant. Details of the study are shown in Figure 1.

2.1 | RCT phase

The Moscow Study[22,29,30] was a 30-week randomized controlled double-blind study including 184 men aged 35–70 years with MetS (IDF criteria)[1,2] and low testosterone levels (serum TT <12 nmol/L [350 ng/dL] and/or cFT <0.225 nmol/L [6.5 ng/dL]). Of the total cohort, 113 and 71 men were randomized to the treatment (TU) and placebo arms, respectively (the study design included unequal randomization as only small changes in outcomes were anticipated in the men provided placebo).[22] Exclusion criteria included participation in another study, being unable to provide informed consent or to undergo measurements required in the study protocol, having serious organic or mental health disease based on history and/or examination, suspicion of prostate cancer (based on digital rectal examination or serum prostate-specific antigen >4 ng/mL), breast cancer, history of hepatic tumours, hepatic disease (serum alanine aminotransferase or aspartate aminotransferase four times the upper limit of reference range), serum creatinine value >150 μmol/L, haematocrit >0.55, a previous allergic reaction to testosterone preparations or other injections, and a judgement of possible non-compliance with study requirements. Medications (diabetes, lipid-lowering and antihypertensive agents) were not altered during the RCT. HOMA-IR data were available in 133 men (TU 79 men, placebo 54 men) at baseline (Visit 1) and at 18 weeks (Visit 3) and in 120 men (72 men on TU and 48 men on placebo) at the end of the RCT (Visit 4; Figure 1).

2.2 | Open-label phase

Following the RCT, TU was continued in the RCT treatment arm ('study cohort') in the open-label phase (30 weeks RCT phase/108 weeks open-label phase). The numbers of men on TU during the open-label phase decreased: 72 men at 30 weeks, 59 men at 66 weeks, 43 men at 102 weeks and 39 men at 138 weeks (Table 1). The men on placebo during the RCT phase were switched to TU for 102 weeks (data after 30 weeks on placebo [RCT phase] were considered the new 0 weeks baseline data in the open-label phase) and, once switched to TU, were termed the 'confirmatory cohort'. The number of men in this phase decreased during follow-up; 48 men at 0 weeks, 40 men at 30 weeks, 34 men at 66 weeks and 32 men at 102 weeks (Table 1).

2.3 | Administration of TU or placebo

All the men received written lifestyle recommendations (improving diet and increasing activity, such as walking for at least 30–40 min/day). Eligible participants were assigned to receive either parenteral TU 1000 mg or matching placebo. The study medication was prepared by Bayer Schering Pharma, Germany, with packages numbered. Only when the code was broken was it apparent which contained TU or placebo. After baseline measurements were obtained (Visit 1), patients received TU or placebo, and subsequent injections were given at 6 weeks (Visit 2), 18 weeks (Visit 3) and 30 weeks (Visit 4). From 30 weeks onwards, the study and confirmatory cohorts were administered TU (open-label phase; Table 1).

2.4 | Outcomes and measures

Primary outcome variables included body composition measurements (e.g., WC, waist-to-hip ratio) and serum lipids. Details of the outcome measures have been reported.[21,30] These included fasting glucose, insulin and HOMA-IR. HOMA-IR was chosen as the principal outcome measure, as opposed to the updated HOMA2-IR, as we wished to

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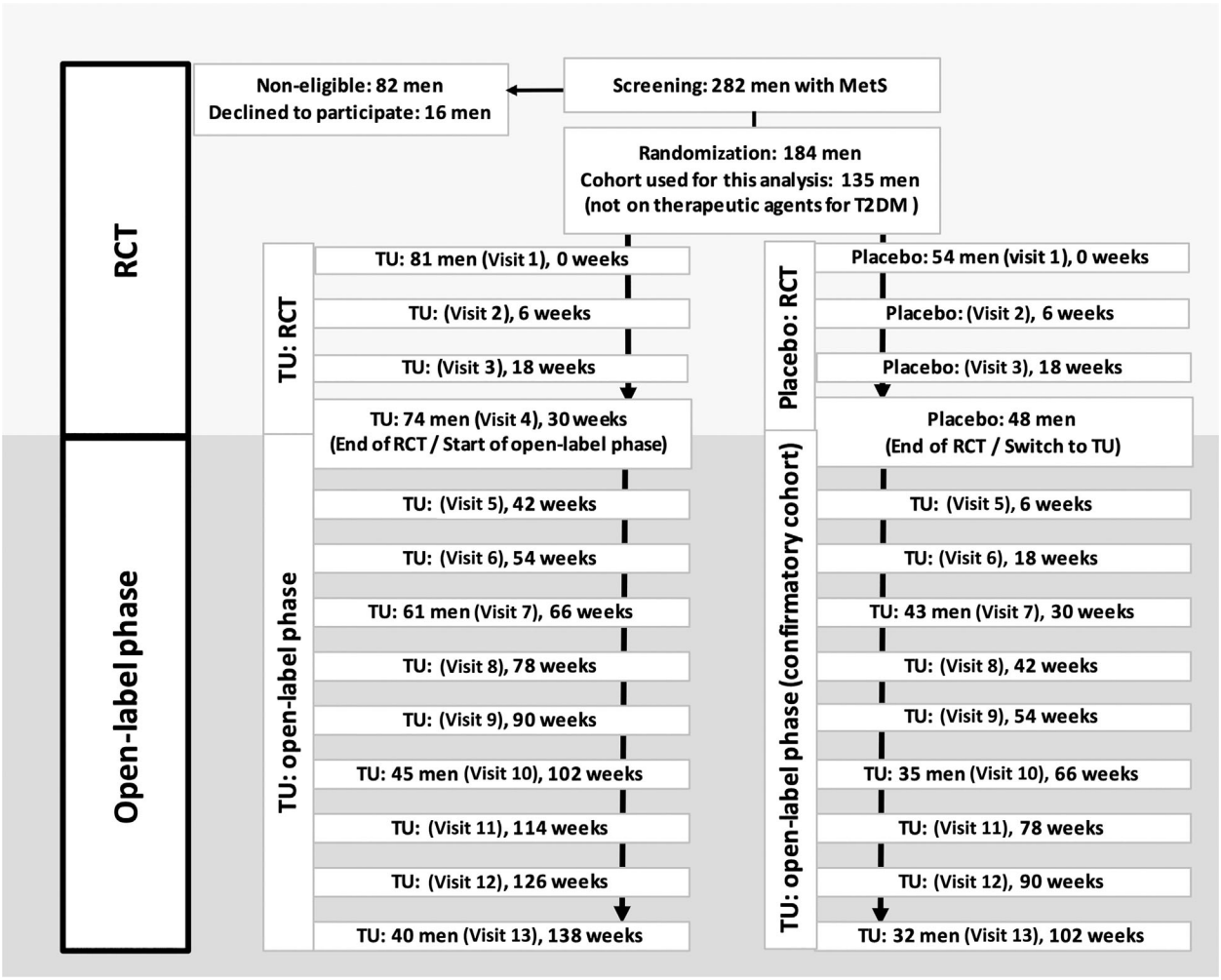

	Placebo	TU	
Patients (*n*)	54	81	
	Baseline values: median (IQR), *n*		*p* (rank-sum)
Age (years)	53 (46, 59)	49 (42, 56)	0.05
TT (nmol/l)	8.3 (6.5, 9.9)	7.6 (5.3, 9.7)	0.34
E2 (pmol/l)	99 (80, 122), *n*=52	89 (71, 128), *n*=79	0.44
SHBG (nmol/l)	35.5 (25.4, 47.7)	33.3 (26 , 43.7)	0.34
cFT (nmol/L)	0.15 (0.11, 0.20)	0.15 (0.11, 0.19)	0.85
WC (cm)	115 (107, 124)	115 (106, 124)	0.90
	n (%)		*p* (chi-sq)
MetS/T2DM (diet controlled)	9 (16.7%)	5(6.2%)	0.050
MetS (without T2DM)	45 (83.3%)	76 (93.8%)	

FIGURE 1 Recruitment process and protocol used for the Moscow Study (randomized controlled trial [RCT] and the open-label phase) and baseline values of the men not on antiglycaemic agents in the testosterone undecanoate (TU) and placebo arms. Study measurements: Visits 1 and 3–13: weight, waist circumference (WC), Ageing Male Symptoms Scale, International Index of Erectile Function-5, International Prostate Symptom Score, Beck depression inventory, haemoglobin, white blood cells, platelets, haematocrit (HCT), erythrocyte sedimentation rate. Visits 1, 3, 4, 7, 10, 13: total testosterone (TT), sex hormone-binding globulin (SHBG), oestradiol (E2), fasting glucose, fasting insulin, prostate-specific antigen (PSA), lipids, bilirubin. Visits 1, 3, 4, 7, 10: leptins. Visits 1, 4: interleukin (IL)-1B, IL-6, IL-10, tumour necrosis factor, C-reactive protein. Study outcomes: Patient deaths—two deaths in RCT phase; placebo (one patient after Visit 2—myocardial infarction, one patient after Visit 3—unknown cause), two deaths in the open-label phase; TU—two patients (one patient after Visit 6—unknown cause, one patient after Visit 10—heart failure). Patients not completing the study: RCT: TU group ($n = 7$)—protocol break ($n = 2$), non-compliance ($n = 2$), loss to follow-up ($n = 1$), moved ($n = 1$), decrease in testicular volume ($n = 1$). Placebo group ($n = 6$)—death ($n = 2$), non-compliance ($n = 1$), angina ($n = 1$), family planning ($n = 1$), gluteal abscess ($n = 1$); Open-label phase: TU ($n = 34$)—deaths ($n = 2$), non-compliance ($n = 10$), lost to follow-up ($n = 7$), moved ($n = 7$), allergic reaction ($n = 1$), adrenal tumour ($n = 1$), increased PSA level ($n = 2$), anaemia ($n = 1$), family planning ($n = 1$), increased HCT ($n = 1$), testicular pain ($n = 1$); Placebo group ($n = 16$)—non-compliance ($n = 6$), lost to follow-up ($n = 4$), moved ($n = 2$), increased PSA level ($n = 1$), gluteal abscess ($n = 1$), non-diabetes treatment changes affecting study outcomes ($n = 2$). cFT, calculated free testosterone; MetS, metabolic syndrome; T2DM, type 2 diabetes.

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TABLE 1 Changes in homeostasis model assessment-insulin resistance index, total testosterone, calculated free testosterone, oestradiol, sex hormone-binding globulin and waist circumference in the men on testosterone undecanoate and placebo during the randomized controlled trial and open-label phases of the Moscow Study.

A: Changes in HOMA-IR, TT, cFT, E2, SHBG and WC in the men over 138 weeks (30 weeks RCT phase, followed by 108 weeks open-label phase) in men who were initially randomized to TU therapy in the Moscow RCT.

		Median (IQR), *p* (sign-rank vs. baseline values), *n*					
TU		**HOMA-IR**	**Serum TT, nmol/L**	**cFT, nmol/L**	**Serum E2, pmol/L**	**SHBG, nmol/L**	**WC, cm**
RCT phase	Baseline	4.13 (2.11, 7.81), $n = 79$	7.6 (5.3, 9.7), $n = 81$	0.15 (0.11, 0.19), $n = 81$	89.0 (71.0, 128.0), $n = 79$	33.3 (26.0, 43.7), $n = 81$	115.0 (106.0, 124.0), $n = 81$
	18 weeks	2.94 (1.96, 4.93), $p < 0.0001$, $n = 77$	12.4 (9.3, 16.6), $p < 0.0001$, $n = 77$	0.26 (0.19, 0.33), $p < 0.0001$, $n = 77$	107.0 (80.8, 150.0), $p = 0.0040$, $n = 76$	30.3 (22.7, 40.5), $p = 0.044$, $n = 77$	113.0 (104.0, 120.0), $p < 0.0001$, $n = 78$
	30 weeks	3.43 (2.12, 5.60), $p = 0.026$, $n = 72$	12.8 (10.6, 17.8), $p < 0.0001$, $n = 74$	0.28 (0.21, 0.37), $p < 0.0001$, $n = 74$	103.0 (72.0, 153.0), $p = 0.067$, $n = 72$	30.4 (22.8, 39.0), $p = 0.0078$, $n = 74$	111.8 (102.0, 120.0), $p < 0.0001$, $n = 74$
Open-label phase	66 weeks	2.11 (1.42, 3.48), $p < 0.0001$, $n = 59$	15.2 (11.6, 21.5), $p < 0.0001$, $n = 60$	0.31 (0.25, 0.45), $p < 0.0001$, $n = 60$	107.0 (80.0, 147.0), $p = 0.63$, $n = 57$	30.9 (19.3, 40.7), $p = 0.0054$, $n = 60$	109.0 (100.0, 119.0), $p < 0.0001$, $n = 61$
	102 weeks	2.45 (1.48, 3.34), $p < 0.0001$, $n = 43$	16.7 (12.8, 21.5), $p < 0.0001$, $n = 45$	0.39 (0.32, 0.53), $p < 0.0001$, $n = 45$	99.0 (65.5, 152.0), $p = 0.35$, $n = 44$	27.7 (18.3, 35.0), $p = 0.0006$, $n = 44$	110.0 (100.0, 118.0), $p < 0.0001$, $n = 45$
	138 weeks	1.94 (1.48, 2.89), $p < 0.0001$, $n = 39$	17.2 (12.7, 22.1), $p < 0.0001$, $n = 40$	0.39 (0.26, 0.51), $p < 0.0001$, $n = 40$	95.5 (66.0, 126.0), $p = 0.97$, $n = 40$	26.6 (18.7, 37.1), $p = 0.0040$, $n = 40$	108.0 (97.5, 117.5), $p = 0.0001$, $n = 40$

B: Changes in HOMA-IR, TT, cFT, E2, SHBG and WC in the men who were randomized to placebo for 30 weeks (RCT phase).

		Median (IQR), *p* (sign-rank vs. baseline values), *n*					
Placebo		**HOMA-IR**	**Serum TT, nmol/L**	**cFT, nmol/L**	**Serum E2, pmol/L**	**SHBG, nmol/L**	**WC, cm**
RCT phase	Baseline	3.86 (2.72, 6.67), $n = 54$	8.3 (6.5, 9.9), $n = 54$	0.15 (0.11, 0.20), $n = 54$	99.0 (80.0, 122.0), $n = 52$	35.5 (25.4, 47.7), $n = 54$	115.0 (107.0, 124.0), $n = 54$
	18 weeks	5.23 (2.71, 6.95), $p = 0.41$, $n = 50$	8.5 (6.5, 11.8), $p = 0.41$, $n = 50$	0.15 (0.11, 0.21), $p = 0.18$, $n = 50$	107.0 (78.0, 118.0), $p = 0.77$, $n = 49$	35.0 (25.5, 44.2), $p = 0.20$, $n = 50$	113.0 (106.0, 123.0), $p = 0.0003$, $n = 50$
	30 weeks	4.65 (2.69, 7.10), $p = 0.23$, $n = 48$	7.7 (6.3, 11.2), $p = 0.74$, $n = 48$	0.14 (0.12, 0.21), $p = 0.42$, $n = 48$	101.0 (82.0, 114.0), $p = 0.23$, $n = 45$	35.3 (25.9, 43.0), $p = 0.073$, $n = 48$	113.0 (107.0, 124.5), $p = 0.072$, $n = 48$

C: Changes in HOMA-IR, TT, cFT, E2, SHBG and WC in the men switched to TU for 102 weeks (open-label phase – confirmatory cohort).

		Median (IQR), *p* (sign-rank vs. baseline values), *n*					
TU		**HOMA-IR**	**Serum TT, nmol/L**	**cFT, nmol/L**	**Serum E2, pmol/L**	**SHBG, nmol/L**	**WC, cm**
Open-label phase	Baseline	4.65 (2.69, 7.10), $p = 0.23$, $n = 48$	7.7 (6.3, 11.2), $p = 0.74$, $n = 48$	0.14 (0.12, 0.21), $p = 0.42$, $n = 48$	101.0 (82.0, 114.0), $p = 0.23$, $n = 45$	35.3 (25.9, 43.0), $p = 0.073$, $n = 48$	113.0 (107.0, 124.5), $p = 0.072$, $n = 48$
	30 weeks	2.84 (2.10, 4.26), $p = 0.0001$, $n = 40$	14.7 (12.2, 16.4), $p < 0.0001$, $n = 41$	0.32 (0.25, 0.40), $p < 0.0001$, $n = 41$	110.0 (83.0, 126.0), $p = 0.51$, $n = 38$	30.8 (22.8, 40.6), $p = 0.0078$, $n = 40$	113.0 (101.0, 119.0), $p = 0.0005$, $n = 43$
	66 weeks	2.66 (1.90, 4.00), $p < 0.0001$, $n = 34$	17.4 (12.4, 23.6), $p < 0.0001$, $n = 35$	0.37 (0.26, 0.53), $p < 0.0001$, $n = 35$	106.0 (78.0, 132.0), $p = 0.73$, $n = 31$	33.8 (20.9, 40.10, $p = 0.086$, $n = 34$	110.0 (100.0, 120.0), $p = 0.0002$, $n = 35$
	102 weeks	2.21 (1.59, 3.73), $p < 0.0001$, $n = 32$	18.0 (14.1, 22.5), $p < 0.0001$, $n = 32$	0.37 (0.28, 0.56), $p < 0.0001$, $n = 32$	100.5 (78.0, 138.0), $p = 0.43$, $n = 29$	30.5 (20.2, 38.7), $p = 0.013$, $n = 32$	108.0 (99.5, 120.0), $p = 0.0001$, $n = 32$

Abbreviations: cFT, calculated free testosterone; E2, oestradiol; HOMA-IR, homeostasis model assessment-insulin resistance; IQR, interquartile range; RCT, randomized controlled trial; SHBG, sex hormone-binding globulin; TT, total testosterone; TU, testosterone undecanoate; WC, waist circumference; Δ, change in.

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compare our results with previously carried out studies with varying findings.[19–21]

2.5 | Laboratory measurements

Blood was collected from the antecubital vein following an overnight fast using BD Vacutainer gel tubes (BD, Franklin Lakes, NJ, USA) and serum was obtained by centrifuging (2000 *g*, 15 min, 4°C). Glucose was measured on a Hitachi 912 autoanalyser (Roche Diagnostics GmbH) and TT, E2, insulin and SHBG were measured using a chemiluminescence immunoassay (Vitros 3600 system; Ortho-Clinical Diagnostics, Johnson & Johnson company, NJ, USA). HOMA-IR was calculated using the equation HOMA-IR = fasting insulin × fasting glucose/22.5.[31]

2.6 | Statistical analyses

As the study outcome was HOMA-IR, we included men not on antiglycaemic agents. Baseline HOMA-IR values in this cohort were not normally distributed, hence nonparametric analyses were performed to compare within (sign-rank) and between (rank-sum) group values. In within-group analyses (sign-rank analyses) the men who dropped out would be excluded. Baseline HOMA-IR did not vary (rank-sum analyses) between the men included in the study and those who dropped out at each visit (details in Figure 1; $p = 0.17$ [Visit 3], $p = 0.73$ [Visit 4], $p = 0.34$ [Visit 7], $p = 0.18$ [Visit 10], $p = 0.35$ [Visit 13]). In accordance with the publication by Schmidt and Finan,[32] linear/multiple regression analyses were used to identify predictors of ΔHOMA-IR during follow-up.

3 | RESULTS

Of the 184 men with MetS in the 30-week RCT, 113 and 71 men were commenced on TU and placebo, respectively. No significant between-group (TU vs. placebo) differences (rank-sum) were seen in age ($p = 0.39$), serum TT ($p = 0.15$), serum E2 ($p = 0.68$), serum SHBG ($p = 0.10$) or WC ($p = 0.45$) at baseline. Further, no significant between-group difference ($p = 0.51$, chi-squared) was observed in the proportion of men with T2DM: TU: 33 (29.20%), placebo: 24 (33.80%). Similarly, antiglycaemic therapy did not significantly vary ($p = 0.51$, chi-squared) between the groups: TU: 32 (28.32%), placebo: 17 (23.94%). We focused on the 135 men not on antiglycaemic agents (TU: 81 men; placebo: 54 men). Figure 1 shows that age ($p = 0.050$, rank-sum) and T2DM ($p = 0.050$, chi-squared) differed between men on TU and those on placebo; other parameters were not significantly different (Table 1).

Because there were between-group differences in baseline age and T2DM, we focused on within-group changes. Table 1 shows HOMA-IR, serum TT, serum E2, serum SHBG and WC values in the study cohort on TU (0–30 weeks of the RCT and 30–138 weeks of the open-label phase), the confirmatory cohort on TU (30–132 weeks, previously on placebo during the RCT) and the placebo group (0–30 weeks of the RCT).

3.1 | Changes in HOMA-IR and other variables following TU and lifestyle measures in the RCT phase

Table 1A shows the changes seen in the men on TU (study cohort) in the RCT. As expected, serum TT levels increased significantly following TU administration (18 and 30 weeks). HOMA-IR decreased significantly ($p < 0.0001$, sign-rank) after 18 weeks of TU. Interestingly, after 30 weeks of TU, HOMA-IR was significantly lower ($p = 0.026$, sign-rank) than the baseline value but higher than at 18 weeks (although this was not significant [$p = 0.20$, sign-rank]). Table 1 also presents the changes in E2, SHBG concentrations and WC measurements. E2 levels increased (vs. baseline) at 18 and 30 weeks, although statistical significance was only noted at the 18-week time point. SHBG and WC decreased significantly at 18 and 30 weeks.

Although we focused on men not on antiglycaemic agents, we analysed the complementary group on TU and antiglycaemic agents. The median (IQR) HOMA-IR at the various time points was as follows: 8.32 (4.91, 11.98) at baseline ($n = 32$), 5.65 (3.22, 14.20) at 18 weeks ($n = 31$) and 4.80 (2.89, 10.51) at 30 weeks ($n = 31$). The HOMA-IR values at 18 weeks ($p = 0.18$, sign-rank) and 30 weeks ($p = 0.068$, sign-rank) were not significantly different from baseline, although patient numbers were smaller than our study group not on antiglycaemic agents.

3.2 | Changes in HOMA-IR and other variables following placebo and lifestyle measures in the RCT phase

Table 1B shows that there were no significant changes in serum TT, E2 or SHBG levels. WC measurements decreased at 18 and 30 weeks, although statistical significance was only evident at 18 weeks. An increase in HOMA-IR was observed at both 18 and 30 weeks, although statistical significance was seen only at 18 weeks.

3.3 | Changes in HOMA-IR and other variables following TU and lifestyle measures in the open-label phase: study cohort

Table 1A shows HOMA-IR, serum TT, E2, SHBG and WC values after 66, 102 and 138 weeks of TU. Compared to baseline (0 weeks) HOMA-IR, SHBG and WC were significantly lower at all the above

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time points. Serum TT was significantly higher in all men on TU, however, no change in E2 was evident during the open-label phase.

3.4 | Changes in HOMA-IR and other variables following TU and lifestyle measures in the open-label phase: confirmatory TU cohort

Table 1C shows that the baseline values in men switched from placebo to TU (confirmatory cohort) were those measured at the final RCT visit (30 weeks). Serum TT increased significantly at all time points, whilst no significant change in serum E2 was observed. SHBG decreased, although significance was reached only at 30 and 102 weeks. WC decreased significantly at all time points. Table 1C shows that median (IQR) HOMA-IR values decreased significantly from 4.65 (2.69, 7.10) at baseline to 2.84 (2.10, 4.26) after 30 weeks of TU and continued to decrease after 66 and 102 weeks of treatment.

3.5 | Variables associated with ΔHOMA-IR during the initial 30 weeks of TU treatment

Table 2 shows predictors of ΔHOMA-IR over a 30-week period in men randomized to TU in the RCT (study cohort) and those switched to TU in the open-label phase (confirmatory cohort). Initially, univariate linear regression analyses were carried out with age, baseline HOMA-IR, and baseline and changes in serum TT, cFT, serum E2, serum SHBG and WC as independent variables and ΔHOMA-IR as the dependent variable. Baseline HOMA-IR was significantly associated with ΔHOMA-IR in both cohorts, whilst baseline WC (not ΔWC) was significantly associated only in the study cohort. When baseline HOMA-IR, baseline WC and ΔWC were entered in multivariate models with ΔHOMA-IR as the dependent variable, only baseline HOMA-IR achieved significance. The inclusion of age and T2DM in the multivariate regression model did not alter the association between baseline HOMA-IR and ΔHOMA-IR.

TABLE 2 Associations (univariate linear regression models followed by a multivariate model including factors that were significant in the univariate models) between change in homeostasis model assessment-insulin resistance index (HOMA-IR; outcome) and baseline HOMA-IR, age, baseline, and changes in total testosterone, calculated free testosterone, oestradiol, sex hormone-binding globulin and waist circumference over the first 30 weeks of testosterone undecanoate treatment.

Outcome: ΔHOMA-IR (30 weeks—baseline)	**TU (RCT phase)**			**TU (open-label phase)—Confirmatory cohort**		
Independent variables	**Coefficient (95% CI)**	***p***	**r^2 (*n*)**	**Coefficient (95% CI)**	***p***	**r^2 (*n*)**
Δ: (value at 30 weeks of TU treatment) − (value at baseline)						
0–30 weeks of TU (significant decrease ($p < 0.0001$, sign-rank) in HOMA-IR was evident in both groups—as seen in Table 2)						
Univariate models						
Age at baseline (years)	0.099 (−0.11, 0.31)	0.36	0.012 ($n = 72$)	−0.12 (−0.32, 0.83)	0.24	0.037 ($n = 40$)
Baseline HOMA-IR	**−0.92 (−1.09, −0.76)**	**<0.001**	**0.64 ($n = 72$)**	**−0.78 (−0.92, −0.64)**	**<0.001**	**0.77 ($n = 39$)**
Baseline TT (nmol/L)	−0.049 (−0.57, 0.47)	0.85	0.018 ($n = 72$)	0.36 (−0.094, 0.81)	0.12	0.089 ($n = 40$)
ΔTT (nmol/L)	−0.19 (−0.54, 0.16)	0.28		0.026 (−0.29, 0.34)	0.87	
Baseline cFT (nmol/L)	−15.40 (−49.22, 18.43)	0.38	0.066 ($n = 72$)	−16.80 (−44.70, 11.10)	0.23	0.0075 ($n = 40$)
ΔcFT (nmol/L)	−11.16 (−25.45, 3.13)	0.12		−2.21 (−16.59, 12.18)	0.76	
Baseline E2 (pmol/L)	−0.038 (−0.080, 0.0043)	0.078	0.051 ($n = 70$)	0.048 (−0.015, 0.11)	0.13	0.12 ($n = 37$)
ΔE2 (pmol/L)	−0.024 (−0.063, 0.015)	0.22		−0.037 (−0.094, 0.019)	0.19	
Baseline SHBG (nmol/L)	0.068 (−0.084, 0.22)	0.37	0.012 ($n = 72$)	−0.057 (−0.23, 0.12)	0.51	0.013 ($n = 40$)
ΔSHBG (nmol/L)	0.081 (−0.10, 0.27)	0.39		−0.025 (−0.17, 0.11)	0.72	
Baseline WC (cm)	**−0.19 (−0.35, −0.028)**	**0.022**	0.062 ($n = 72$)	−0.086 (−0.24, 0.068)	0.27	0.034 ($n = 40$)
ΔWC (cm)	−0.017 (−0.29, 0.26)	0.90		−0.075 (0.43, 0.28)	0.68	
Multivariate models						
Baseline HOMA-IR	**−0.93 (−1.10, −0.75)**	**<0.001**	0.65 ($n = 72$)	**−0.80 (−0.94, −0.65)**	**<0.001**	0.78 ($n = 40$)
Baseline WC	0.015 (−0.091, 0.12)	0.77		0.038 (−0.040, 0.12)	0.33	
ΔWC	0.085 (−0.087, 0.26)	0.33		0.12 (−0.078, 0.32)	0.69	

Note: Bolded indicates signifance values (*p* valves).

Abbreviations: cFT, calculated free testosterone; E2, oestradiol; HOMA-IR, homeostasis model assessment-insulin resistance; IQR, interquartile range; RCT, randomized controlled trial; SHBG, sex hormone-binding globulin; TT, total testosterone; TU, testosterone undecanoate; WC, waist circumference; Δ, change in.

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3.6 | Variables associated with ΔHOMA-IR in men on TU treatment for between 30 and 102 weeks

Table 3 shows the factors associated with ΔHOMA-IR between 30 and 102 weeks (post 30 weeks of TU [Table 2]) in the men randomized to TU in the RCT phase (study cohort) and those switched to TU in the open-label phase (confirmatory cohort). Initially, univariate linear regression analyses were carried out with age, baseline HOMA-IR and baseline and changes in serum TT, cFT, serum E2, serum SHBG and WC as independent variables and ΔHOMA-IR as the dependent variable. Only baseline (value after 30 weeks) HOMA-IR was significantly associated with ΔHOMA-IR in both cohorts.

3.7 | Change in fasting glucose in the RCT phase

Fasting glucose at baseline was significantly different ($p = 0.032$, rank-sum) between the study arms. Baseline median (IQR) glucose values were as follows: TU: 5.5 (5.1, 6.0) mmol/L ($n = 81$); placebo: 6.0 (5.3, 6.4) mmol/L ($n = 54$). After 18 weeks, TU was associated with a significant decrease in fasting glucose (median [IQR] fasting glucose: 5.4 [5.0, 5.9] mmol/L; $p = 0.026$) whilst the change was not significant in the men on placebo (median [IQR] fasting glucose: 5.8 [5.2, 6.4] mmol/L; $p = 0.93$). In contrast, after 30 weeks, the fasting glucose values were not significantly different (sign-rank) from baseline (median [IQR] fasting glucose: TU: 5.3 [4.9, 6.0] mmol/L, $p = 0.069$; placebo: 6.0 [5.6, 6.3] mmol/L, $p = 0.51$). After 138 weeks of TU (combined RCT and open-label phases) there was a modest although statistically significant decrease ($p = 0.0057$, sign-rank) in fasting glucose concentrations in the study cohort compared with baseline (median [IQR] fasting glucose: 5.2 [4.9, 5.6] mmol/L).

3.8 | Change in fasting insulin concentrations in the RCT phase

Fasting insulin values at baseline did not differ ($p = 0.64$, rank-sum) between the TU and placebo groups. Baseline median (IQR) fasting insulin values were: TU: 17.4 (9.5, 28.6) mIU/L ($n = 79$); placebo: 14.7 (10.6, 23.6) mIU/L ($n = 54$). After 18 weeks, TU was associated with a significant decrease (sign-rank) in fasting (median [IQR] fasting insulin: 12.2 [8.4, 20.6] mIU/L; $p < 0.0001$), whilst the change was not significant (sign-rank) in the men on placebo (median [IQR] fasting insulin: 18.85 [11.5, 26.4] mIU/L; $p = 0.24$). In contrast, after 30 weeks, the fasting insulin values were not significantly different (sign-rank) from baseline (median [IQR] fasting insulin: TU: 14.7 (9.5, 22.9) mIU/L, $p = 0.059$; placebo: 16.5 [11.1, 26.7] mIU/L, $p = 0.44$). After 138 weeks of TU (combined RCT and open-label phases), there was a statistically significant reduction ($p < 0.001$, sign-rank), with fasting insulin concentrations in the study cohort more than halving compared with baseline (median [IQR] fasting insulin: 8.35 [6.9, 12.6] mIU/L).

TABLE 3 Associations (univariate linear regression models) between change in homeostasis model assessment-insulin resistance index (HOMA-IR; outcome) and baseline HOMA-IR, age, baseline, and changes in total testosterone, calculated free testosterone, oestradiol, sex hormone-binding globulin and waist circumference between 30 and 102 weeks of testosterone undecanoate treatment.

Outcome: ΔHOMA-IR (102–30 weeks)	**TU (open-label phase)**			**TU (open-label phase)—Confirmatory cohort**		
Independent variables	**Coefficient (95% CI)**	***p***	**r^2 (*n*)**	**Coefficient (95% CI)**	***p***	**r^2 (*n*)**
Δ: (value at 102 weeks of TU treatment) − (value after 30 weeks of TU treatment)						
30–102 weeks of TU (significant decrease ($p < 0.0001$, sign-rank) in HOMA-IR was evident in both groups)						
Univariate models						
Age at 30 weeks (years)	0.028 (−0.18, 0.24)	0.78	0.0019 ($n = 41$)	0.021 (−0.096, 0.14)	0.72	0.0043 (n = 32)
HOMA-IR—30 weeks	**−0.90 (−1.01, −0.78)**	**<0.001**	**0.86 ($n = 41$)**	**−0.71 (−0.85, −0.57)**	**<0.001**	**0.78 ($n = 32$)**
TT (nmol/L) at 30 weeks	0.37 (−0.027, 0.77)	0.067	0.14 ($n = 41$)	0.10 (−0.10, 0.30)	0.32	0.038 ($n = 32$)
ΔTT (nmol/L)	−0.0081 (−0.32, 0.30)	0.96		0.069 (−0.10, 0.24)	0.42	
Baseline cFT (nmol/L)	13.08 (−2.78, 28.93)	0.10	0.064 ($n = 41$)	0.78 (−6.13, 7.69)	0.82	0.066 ($n = 32$)
ΔcFT (nmol/L)	1.15 (−11.40, 13.69)	0.85		0.73 (−4.58, 6.03)	0.78	
E2 (pmol/L) at 30 weeks	0.0030 (−0.034, 0.040)	0.87	0.029 ($n = 40$)	0.00018 (−0.031, 0.032)	0.99	0.086 ($n = 32$)
ΔE2 (pmol/L)	−0.015 (−0.050, 0.021)	0.40		−0.020 (−0.046, 0.0053)	0.12	
SHBG (nmol/L) at 30 weeks	0.0010 (−0.13, 0.13)	0.99	0.0097 ($n = 41$)	−0.032 (−0.11, 0.045)	0.40	0.045 ($n = 32$)
ΔSHBG (nmol/L)	−0.057 (−0.26, 0.15)	0.57		−0.11 (−0.26, 0.035)	0.13	
WC (cm) at 30 weeks	0.030 (−0.14, 0.20)	0.72	0.0090 ($n = 41$)	−0.0039 (−0.088, 0.080)	0.92	0.0004 ($n = 32$)
ΔWC (cm)	0.066 (−0.18, 0.31)	0.58		−0.011 (−0.24, 0.22)	0.93	

Note: Bolded indicates signifance values (*p* valves).

Abbreviations: cFT, calculated free testosterone; E2, oestradiol; HOMA-IR, homeostasis model assessment-insulin resistance; IQR, interquartile range; RCT, randomized controlled trial; SHBG, sex hormone-binding globulin; TT, total testosterone; TU, testosterone undecanoate; WC, waist circumference; Δ, change in.

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4 | DISCUSSION

In our analysis of the Moscow RCT and open-label phases, we evaluated the impact of TU on HOMA-IR in men with MetS/T2DM who were not receiving antiglycaemic agents. As expected, compared to baseline, serum testosterone increased at every time point in both the study and confirmatory cohorts. Compared to baseline, HOMA-IR was significantly reduced at almost every time point in men receiving TU in the study cohort. We cannot explain the increase in HOMA-IR between Weeks 18 and 30 in the RCT (insulin [$p = 0.11$] and fasting glucose [$p = 0.065$] values were not significantly different between these time points), with only three participants dropping out between these visits, but importantly, HOMA-IR remained significantly lower ($p < 0.0001$) at every time point in the open-label phase of the study cohort. Further, in the confirmatory cohort at 30 weeks, reduction in HOMA-IR was significant ($p = 0.0001$) and this trend continued at 66 and 102 weeks ($p < 0.0001$). Our findings highlight a consistent pattern of HOMA-IR decrease in the subgroup of men with MetS and diabetes who were not prescribed antiglycaemic agents.

Compared to baseline, SHBG was also significantly lower at every time point in men receiving TU in the study cohort. This was mirrored in the confirmatory cohort, apart from at Week 66, although significance ($p = 0.013$) was again demonstrated at 102 weeks. This observation has been previously reported, although the mechanism is not clear.[33,34] E2 significantly increased ($p = 0.0040$) at 18 weeks in men in the RCT of the study cohort but was not replicated at subsequent time points or in the confirmatory cohort. Tan et al.[35] evaluated E2 levels post TTh in 34 016 men and found that 20.2% of these men demonstrated elevated E2 levels ≥156 pmol/L (42.6 pg/mL), with the increase being age-dependent.[35]

In accordance with other studies evaluating the effect of TTh on anthropometric outcomes, we found that WC decreased significantly with TU.[36–38] Although WC can only be a considered a surrogate for adiposity (especially visceral), we speculate that the decrease in WC may reflect reduction in adiposity. Interestingly, at Week 18 in the placebo arm, WC decreased significantly, which was perhaps due to participants being motivated to actively engage in the lifestyle programme offered, although this trend did not persist at 30 weeks.

Baseline HOMA-IR was the main predictor of ΔHOMA-IR, with the r^2 values (Tables 2 and 3) suggesting that this accounted for a high degree of the outcome variability observed. In both tables, separate regression analyses were carried out with ΔHOMA-IR as the outcome variable and baseline age, baseline HOMA-IR, baseline and ΔTT, baseline and ΔE2, baseline and ΔSHBG and baseline and ΔWC as dependent variables. Interestingly, ΔHOMA-IR was strongly associated with baseline HOMA-IR in the RCT and open- label phases (study and confirmatory cohort), suggesting higher baseline values of HOMA-IR were associated with a greater reduction in HOMA-IR. Of note, the only other independent variable to be associated with ΔHOMA-IR was baseline WC in men receiving TU in the RCT, with a larger baseline WC predicting greater reduction in HOMA-IR. This association was not evident in the open-label phase of the study cohort. The large dropout rate of participants by 102 weeks ($n = 41$) may have contributed to loss of significance. When baseline HOMA-IR, baseline WC and ΔWC were included as independent variables with ΔHOMA-IR as the outcome, only baseline HOMA-IR remained significant in both cohorts on TU (Table 2).

Our findings suggest baseline HOMA-IR and not baseline WC or ΔWC is the principal predictor of ΔHOMA-IR following TU treatment (Tables 2 and 3). Patients with the highest baseline HOMA-IR demonstrated the greatest improvement following TU in accordance with Wilder's principle.[39] Hence, consideration of baseline characteristics is essential when comparing studies with varying degrees of TTh-associated ΔHOMA-IR.[19–21] A greater decrease in HOMA-IR in the study by Groti et al.[20] was observed compared to the TIMES2 study,[19] perhaps due to the baseline HOMA-IR values being higher in the former. No significant change in HOMA-IR was seen in the study by Gianatti et al.,[21] in which the baseline HOMA-IR was lower.[19,20] Importantly, the TTh preparations differed among these RCTs: transdermal testosterone gel was used in the TIMES2 study and TU in the studies by Groti et al.[20] and Gianatti et al.[21]

An increase in testosterone values following TTh has been shown to improve, energy, mood and walkability.[40] These could also be considered as possible mediators of the improvement in HOMA-IR observed in both the study and confirmatory cohorts following TU treatment. The only quality-of-life measure we had available was the Ageing Male Symptoms Scale (AMSS),[41] although we did not have subscale (somatic, psychological and sexual) data. In the study cohort and confirmatory cohort there was significant improvement ($p < 0.0001$, sign-rank) in the AMSS values following TU treatment (study cohort: median [IQR] AMSS score decreased from a baseline of 42.0 [33.0, 52.0] to 23.0 [17.5, 27.5] after 138 weeks of TU; confirmatory cohort: median (IQR) AMSS score decreased from a baseline of 38.5 [30.0, 49.5] to 27.5 [21.5, 31.0] after 102 weeks of TU). Interestingly a small, but significant ($p = 0.031$) improvement in AMSS score was also observed in the men on placebo, in whom median (IQR) AMSS score decreased from a baseline of 43.5 (34.0, 50.0) to 38.5 (30.0, 49.5) after 138 weeks of TU. The decrease in AMSS score was significantly greater ($p < 0.0001$, rank-sum) in men on TU (study and confirmatory cohorts) compared to those on placebo.

Our findings are mechanistically plausible at a molecular level as testosterone appears to enhance insulin sensitivity in obese men with testosterone deficiency by reducing fat mass, increasing lean mass, lowering free fatty acids, and suppressing inflammation (decreased C-reactive protein, interleukin-1b, tumour necrosis factor-α and leptin).[42,43] Further, TTh has been shown to upregulate insulin receptor β subunit, insulin receptor substrate-1, protein kinase B and glucose transporter type 4 in adipose tissue and adenosine 50-monophosphate-activated protein kinase expression/activity in skeletal muscle.[43,44]

Our study has strengths and weaknesses. It comprised an RCT and an open-label phase, with the latter designed at the outset with measurements performed in one laboratory.[22,29] Whilst we compared TU and placebo in the RCT phase, all men in the open-label phase were on TU, with patient numbers decreasing at each successive time point.

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Regardless, the study design allowed us to use the men on placebo in the RCT phase who were switched to TU in the open-label phase as a confirmatory cohort. Variability in HOMA-IR estimation is accepted, with high dependence on insulin assay performance.[45] However, there was consistency in ΔHOMA-IR in both cohorts, with the trend in ΔHOMA-IR seen at virtually every time point. Unfortunately, as HbA1c was not measured we could not compare our results with those of the T4DM Study,[23] which showed reduction in T2DM progression without significant HbA1c decrease. As TTh appears to be associated with a reduction in SHBG levels,[34] it would have been useful also to have measured free testosterone as opposed to cFT using the method by Vermeulen et al.[46] Furthermore, access to data on changes in body composition in the two groups would have been interesting as ΔE2 could possibly be due to associations with both testosterone levels and adiposity.[47] We would also have wished for data on changes in diet and exercise as these could have been included as potential confounding variables. In view of the relatively small cohort, the follow-up period was insufficient to study associations with cardiovascular disease as an outcome.

In conclusion, this RCT/open-label study in men not on antiglycaemic therapy shows significant decreases in HOMA-IR after only 18 weeks of TU treatment. This pattern was observed in men initially randomized to TU and those switched from placebo to TU after the RCT. The decrease in HOMA-IR in the study group was maintained over 138 weeks of TU. New data from our study show that the principal predictor of ΔHOMA-IR was baseline HOMA-IR. Furthermore, decreases in insulin levels appeared greater than in fasting glucose. Hence, in men with T2DM/MetS not on antiglycaemic therapy, improvements in glycaemic control may be greater than suggested by change in serum glucose. In our study, men with higher baseline HOMA-IR demonstrated greater improvement after TU treatment, with much of the variability in ΔHOMA-IR accounted for by this association. This novel finding will help us to understand the varying results from previous studies with ΔHOMA-IR as the outcome.[19–21] In addition, our findings suggest that screening for testosterone, especially in men suspected to have insulin resistance (e.g., men with MetS, T2DM and central obesity), and subsequent TTh would yield significant healthcare benefit.

AUTHOR CONTRIBUTIONS

Sudarshan Ramachandran, Carola König, Geoffrey Hackett, Amar Mann and Richard Strange: data analysis, manuscript preparation. Yuliya Tishova, George Mskhalaya and Svetlana Kalinchenko: patient recruitment, data collection, maintaining database, manuscript preparation. Amro Maarouf, Mark Livingston: manuscript preparation. Michael Zitzmann: manuscript preparation.

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CONFLICT OF INTEREST STATEMENT

Sudarshan Ramachandran, Geoffrey Hackett, Yuliya Tishova, Svetlana Kalinchenko, George Mskhalaya, Mark Livingston, Amar Mann, Amro Maarouf, Carola König and Michael Zitzmann have no disclosures. Richard Strange acknowledges financial support from the North Staffordshire Medical Institute.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Shipman KE, Strange RC, Ramachandran S. Use of fibrates in the metabolic syndrome: a review. *World J Diabetes*. 2016;7:74-88.
2. Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interim statement of the international diabetes federation task force on epidemiology and prevention; National Heart, Lung, and Blood Institute; American Heart Association; world heart federation; international atherosclerosis society; and International Association for the Study of obesity. *Circulation*. 2009;120:1640-1645.
3. Lee SH, Park SY, Choi CS. Insulin resistance: from mechanisms to therapeutic strategies. *Diabetes Metab J*. 2022 Jan;46(1):15-37.
4. Henkel J, Neuschäfer-Rube F, Pathe-Neuschäfer-Rube A, Püschel GP. Aggravation by prostaglandin E2 of interleukin-6-dependent insulin resistance in hepatocytes. *Hepatology*. 2009;50(3):781-790.
5. Kojta I, Chacińska M, Błachnio-Zabielska A. Obesity, bioactive lipids, and adipose tissue inflammation in insulin resistance. *Nutrients*. 2020; 12(5):1305.
6. Wu H, Ballantyne CM. Metabolic inflammation and insulin resistance in obesity. *Circ Res*. 2020;126(11):1549-1564.
7. Hackett G, Kirby M, Rees RW, et al. The British Society for Sexual Medicine Guidelines on male adult testosterone deficiency, with statements for practice. *World J Mens Health*. 2023;41:508-537. doi: 10.5534/wjmh.221027
8. Antonio L, Wu FCW, Moors H, et al. Vanderschueren D; EMAS study group. Erectile dysfunction predicts mortality in middle-aged and older men independent of their sex steroid status. *Age Ageing*. 2022; 51(4):afac094.
9. Kapoor D, Aldred H, Clark S, Channer KS, Jones TH. Clinical and biochemical assessment of hypogonadism in men with type 2 diabetes: correlations with bioavailable testosterone and visceral adiposity. *Diabetes Care*. 2007;30:911-917.
10. Pye SR, Huhtaniemi IT, Finn JD, et al. Late-onset hypogonadism and mortality in aging men. *J Clin Endocrinol Metab*. 2014;99:1357-1366.
11. Muraleedharan V, Marsh H, Kapoor D, Channer KS, Jones TH. Testosterone deficiency is associated with increased risk of mortality and testosterone replacement improves survival in men with type 2 diabetes. *Eur J Endocrinol*. 2013;169:725-733.
12. Hackett G, Heald AH, Sinclair A, Jones PW, Strange RC, Ramachandran S. Serum testosterone, testosterone replacement therapy and all-cause mortality in men with type 2 diabetes: retrospective consideration of the impact of PDE5 inhibitors and statins. *Int J Clin Pract*. 2016;70:244-253.
13. Shores MM, Smit NL, Forsberg CW, et al. Testosterone treatment and mortality in men with low testosterone. *J Clin Endocrinol Metab*. 2012;97(6):2050-2058.

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14. Hackett G, Jones PW, Strange RC, Ramachandran S, et al. Statin, testosterone and phosphodiesterase 5-inhibitor treatments and age related mortality in diabetes. *World J Diabetes*. 2017;8:104-111.
15. Ottarsdottir K, Nilsson AG, Hellgren M, Lindblad U, Daka B. The association between serum testosterone and insulin resistance: a longitudinal study. *Endocr Connect*. 2018;7(12):1491-1500.
16. Cheung AS, Hoermann R, Dupuis P, Joon DL, Zajac JD, Grossmann M. Relationships between insulin resistance and frailty with body composition and testosterone in men undergoing androgen deprivation therapy for prostate cancer. *Eur J Endocrinol*. 2016 Sep; 175(3):229-237.
17. Aggarwal RR, Ryan CJ, Chan JM. Insulin-like growth factor pathway: a link between androgen deprivation therapy (ADT), insulin resistance, and disease progression in patients with prostate cancer? *Urol Oncol*. 2013;31(5):522-530.
18. Nguyen PL, Alibhai SM, Basaria S, et al. Adverse effects of androgen deprivation therapy and strategies to mitigate them. *Eur Urol*. 2015; 67(5):825-836.
19. Jones TH, Arver S, Behre HM, et al. Channer KS; TIMES2 investigators. Testosterone replacement in hypogonadal men with type 2 diabetes and/or metabolic syndrome (the TIMES2 study). *Diabetes Care*. 2011;34(4):828-837.
20. Groti K, Žuran I, Antonič B, Foršnarič L, Pfeifer M. The impact of testosterone replacement therapy on glycemic control, vascular function, and components of the metabolic syndrome in obese hypogonadal men with type 2 diabetes. *Aging Male*. 2018;21(3):158-169.
21. Gianatti EJ, Dupuis P, Hoermann R, et al. Effect of testosterone treatment on glucose metabolism in men with type 2 diabetes: a randomized controlled trial. *Diabetes Care*. 2014;37(8):2098-2107.
22. Kalinchenko SY, Tishova YA, Mskhalaya GJ, Gooren LJ, Giltay EJ, Saad F. Effects of testosterone supplementation on markers of the metabolic syndrome and inflammation in hypogonadal men with the metabolic syndrome: the double-blinded placebo-controlled Moscow study. *Clin Endocrinol (Oxf)*. 2010;73(5):602-612.
23. Wittert G, Bracken K, Robledo KP, et al. Testosterone treatment to prevent or revert type 2 diabetes in men enrolled in a lifestyle programme (T4DM): a randomised, double-blind, placebo-controlled, 2-year, phase 3b trial. *Lancet Diabetes Endocrinol*. 2021;9(1):32-45.
24. Guo X, Asthana P, Gurung S, et al. Regulation of age-associated insulin resistance by MT1-MMP-mediated cleavage of insulin receptor. *Nat Commun*. 2022;13(1):3749.
25. Grossmann M. Ng Tang Fui M, Cheung AS. *Late-Onset Hypogonadism: Metabolic Impact Andrology*. 2020;8(6):1519-1529.
26. Wallace IR, McKinley MC, Bell PM, Hunter SJ. Sex hormone binding globulin and insulin resistance. *Clin Endocrinol (Oxf)*. 2013;78(3): 321-329.
27. Gibb FW, Homer NZ, Faqehi AM, et al. Aromatase inhibition reduces insulin sensitivity in healthy men. *J Clin Endocrinol Metab*. 2016; 101(5):2040-2046.
28. Tucker LA. Insulin resistance and biological aging: the role of body mass, waist circumference, and inflammation. *Biomed Res Int*. 2022; 9(2022):2146596.
29. ClinicalTrials.gov. The Effect of IM Testosterone Undecanoate on Biochemical and Anthropometric Characteristics of Metabolic Syndrome in Hypogonadal Men (NePlaM3). Available at https://clinicaltrials.gov/ct2/show/NCT00696748 (last accessed on 07/06/2023).
30. Giltay EJ, Tishova YA, Mskhalaya GJ, Gooren LJ, Saad F, Kalinchenko SY. Effects of testosterone supplementation on depressive symptoms and sexual dysfunction in hypogonadal men with the metabolic syndrome. *J Sex Med*. 2010;7(7):2572-2582.
31. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. *Diabetes Care*. 2004;27(6):1487-1495.
32. Schmidt AF, Finan C. Linear regression and the normality assumption. *J Clin Epidemiol*. 2018;98:146-151.
33. Plymate SR, Leonard JM, Paulsen CA, Fariss BL, Karpas AE. Sex hormone-binding globulin changes with androgen replacement. *J Clin Endocrinol Metab*. 1983;57(3):645-648.
34. Ramachandran S, Hackett GI, Strange RC. Testosterone replacement therapy: pre-treatment sex hormone-binding globulin levels and age may identify clinical subgroups. *Andrology*. 2020;8(5):1222-1232.
35. Tan RS, Cook KR, Reilly WG. High estrogen in men after injectable testosterone therapy: the low T experience. *Am J Mens Health*. 2015; 9(3):229-234.
36. Hackett G, Cole N, Mulay A, Strange RC, Ramachandran S. Long-term testosterone therapy in type 2 diabetes is associated with decreasing waist circumference and improving erectile function. *World J Mens Health*. 2020;38(1):68-77.
37. Saad F, Haider A, Doros G, Traish A. Long-term treatment of hypogonadal men with testosterone produces substantial and sustained weight loss. *Obesity (Silver Spring)*. 2013;21(10):1975-1981.
38. Yassin DJ, Doros G, Hammerer PG, Yassin AA. Long-term testosterone treatment in elderly men with hypogonadism and erectile dysfunction reduces obesity parameters and improves metabolic syndrome and health-related quality of life. *J Sex Med*. 2014;11(6): 1567-1576.
39. Messerli FH, Bangalore S, Schmieder RE. Wilder's principle: pretreatment value determines post-treatment response. *Eur Heart J*. 2015;36:576-579.
40. Snyder PJ, Bhasin S, Cunningham GR, et al. Testosterone trials investigators. Effects of testosterone treatment in older men. *N Engl J Med*. 2016;374(7):611-624.
41. Morley JE, Perry HM 3rd, Kevorkian RT, Patrick P. Comparison of screening questionnaires for the diagnosis of hypogonadism. *Maturitas*. 2006;53(4):424-429.
42. Dandona P, Dhindsa S, Ghanim H, Saad F. Mechanisms underlying the metabolic actions of testosterone in humans: a narrative review. *Diabetes Obes Metab*. 2021;23(1):18-28.
43. Dhindsa S, Ghanim H, Batra M, et al. Insulin resistance and inflammation in hypogonadotropic hypogonadism and their reduction after testosterone replacement in men with type 2 diabetes. *Diabetes Care*. 2016;39(1):82-91.
44. Ghanim H, Dhindsa S, Batra M, et al. Testosterone increases the expression and phosphorylation of AMP kinase α in men with hypogonadism and type 2 diabetes. *J Clin Endocrinol Metab*. 2020;105(4): 1169-1175.
45. Mather KJ, Hunt AE, Steinberg HO, et al. Repeatability characteristics of simple indices of insulin resistance: implications for research applications. *J Clin Endocrinol Metab*. 2001;86(11):5457-5464.
46. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab*. 1999;84(10):3666-3672.
47. Rubinow KB. Estrogens and body weight regulation in men. *Adv Exp Med Biol*. 2017;1043:285-313.

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