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Testosterone therapy to prevent type 2 diabetes mellitus in at-risk men (T4DM): Design and implementation of a double-blind randomized controlled trial

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Article title: **Testosterone therapy to prevent type 2 diabetes mellitus in at-risk men (T4DM): Design and implementation of a double-blind randomised controlled trial**

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Abstract

Background: Low circulating testosterone is associated with an increased risk of developing type 2 diabetes (T2DM) in overweight men with impaired glucose tolerance (IGT).

Aim: To determine in a multi-centre, double-blinded placebo-controlled randomised trial, whether testosterone treatment combined with lifestyle intervention (Weight Watchers[®]) relative to lifestyle intervention alone, reduces T2DM incidence and improves glucose tolerance at 2 years.

Study population: Overweight or obese men aged 50–74 years with a serum testosterone of $<14\text{nmol/L}$ and IGT or newly diagnosed T2DM established by an oral glucose tolerance test (OGTT).

Setting, drug and protocol: Six Australian capital city-based tertiary care centres. Participants were randomised 1:1 and injected with testosterone undecanoate (Reandron, Bayer) (1000mg/4ml) or vehicle (4ml castor oil), at baseline, 6 weeks and 3-monthly thereafter.

Primary endpoints: (1) Proportion of participants with 2-hour OGTT $\geq 11.1\text{mmol/L}$ at 2 years. (2) A difference at 2 years $\geq 0.6\text{mmol/L}$ in the mean 2-hour OGTT glucose between treatments.

Secondary endpoints: Fasting insulin, HbA1c, body composition, maximal handgrip strength; sexual function and lower urinary tract symptoms; serum sex steroids and sex hormone binding globulin; mood and psychosocial function; adherence to lifestyle intervention; and healthcare utilisation and costs.

Safety: Overseen by an Independent Data Safety Monitoring Committee. Haematocrit, lipids, and prostate-specific antigen (PSA) are assessed 6-monthly and information relating to haematological, urological and cardiovascular adverse events from each clinic visit.

Sub-studies: (i) Changes in bone density and micro-architecture; (ii) motivation and behaviour; (iii) telomere length; (iv) extended treatment up to 4 years, and (v) hypothalamo-pituitary testicular axis recovery at treatment end.

Trial initiation was January 2013 and last participant enrolment was February 2017.

Trial Registration: ACTRN12612000287831.

Introduction

Obesity is a well-established reversible cause of lowered serum testosterone concentration (T) in men^{1, 2, 3}. A high proportion of men with type 2 diabetes (T2DM) have low T that is inversely associated with obesity, insulin resistance and glycaemia^{4, 5}. In men with T2DM and metabolic syndrome (MetS) mean T is 2.6 nmol/L lower than in controls^{6, 7}. Low T is also associated with an increased risk of incident T2DM in men^{8, 5, 9}. A systematic review with meta-analysis showed that men with T > 15.5nmol/L have a 42% reduced risk of T2DM versus men with T < 15.5nmol/L⁶. There are plausible mechanisms by which obesity-associated reduction in T may induce dysglycaemia. Reducing T to castrate levels in men with prostate cancer increases risk of insulin resistance^{10, 11} and T2DM¹². T effects on insulin sensitivity may be mediated by changes in body composition¹³. Direct effects of T are enhanced catecholamine-induced lipolysis¹⁴ and reduced lipoprotein lipase activity and triglyceride uptake in human abdominal adipose tissue¹⁵. Moreover, T levels correlate positively with mitochondrial indices of insulin sensitivity in human skeletal muscle¹⁶, and modulate pathways regulating skeletal muscle glucose metabolism in mice¹⁷.

In an uncontrolled observational study, 6 years of T treatment in men with T2DM resulted in significant and sustained improvements in weight, glycaemia and overall cardiovascular risk¹⁸. A systematic review of placebo-controlled randomised trials found that T therapy did not lower HbA1c in men with established T2DM, although insulin resistance may have improved, at least over the short term in men with T2DM and/or MetS¹⁹. The limited efficacy of T treatment in men with established T2DM, in contrast to men with MetS, suggests that T treatment may be of most benefit to prevent progression to T2DM and, in established T2DM, benefit may be limited to the earliest stages.

There is international consensus supporting lifestyle intervention in T2DM prevention and management²⁰, and such interventions can increase circulating T. The benefits of T therapy, particularly weight loss²¹, may be greatest when combined with lifestyle interventions²². Whether this is a direct T effect on glucose metabolism, an indirect effect via improved body composition or motivation for lifestyle change²³ remains undetermined. However, there is no large-scale trial assessing T treatment as an adjunct to lifestyle intervention for preventing T2DM in men. Hence, we are undertaking a randomised, double-blind, placebo-controlled trial to determine whether T treatment combined with lifestyle intervention reduces the risk of T2DM at 2 years versus lifestyle intervention alone in ~1000 high-risk men.

Trial design, organisation, monitoring and reporting

Overall organisation

The trial (ACTRN12612000287831) is funded by the Australian National Health and Medical Research Council (NHMRC) (Project Grant1030123), Bayer, Eli Lilly, and the University of Adelaide, with in-kind support from WeightWatchers® (WW) and participating research centres. A Steering Committee of the grant holders, chaired by the Principal Investigator (PI) (GW), maintains academic oversight. Day-to-day trial co-ordination, risk-based monitoring and data management are handled by the NHMRC Clinical Trials Centre (CTC, University of Sydney) in collaboration with the PI.

An Independent Data Safety Monitoring Committee (IDSMC) oversees participant safety. All participating sites received approval from the relevant human research ethics committees (HREC) before commencing recruitment.

The trial was registered in March 2012 and trial initiation was January 2013. The last patient enrolled February 2017. All visits will complete by May 2019.

Study design

This is a 2-year, Phase IIIb, multi-centre, double-blind, randomised, placebo-controlled trial with equal allocation between two treatment arms (Figure 1). There are six Australian capital city-based tertiary care centres: Concord Repatriation General Hospital, Sydney; Queen Elizabeth Hospital, Adelaide; Austin Hospital, Melbourne; Princess Alexandra Hospital, Brisbane; and in Perth the Keogh Institute for Medical Research and Fremantle Hospital, which relocated to the Fiona Stanley Hospital about half-way through enrolment.

[Figure 1 here]

Participants

Inclusion criteria

Participants need to meet all inclusion criteria (Table 1). To maximise the probability of having undiagnosed or developing T2DM at the end of the 2-year study we recruited men aged 50–74 years, with abdominal obesity (waist circumference ≥ 95 cm) and the absence of exclusion criteria, for initial screening laboratory investigations. If the 2-hour plasma glucose was ≥ 7.8 and ≥ 15 mmol/L in response to a 75 g oral glucose tolerance test (OGTT), and serum T was ≥ 14 nmol/L then men were enrolled. If the serum T was < 8 nmol/L endocrine review was undertaken to exclude significant hypothalamo-pituitary-testicular (HPT) axis pathology.

We initially enrolled men with $T < 11$ nmol/L⁵. However, the inverse relationship of insulin resistance with T in men with T2DM does not have a clear breakpoint and remains present with normal range T⁴. We settled on a T cut-off of 14 nmol/L somewhat below that (< 16 nmol/L), at which incident T2DM began to increase in an analysis of longitudinal data from the Florey Adelaide Male Ageing Study (FAMAS)⁵, and 15.5 mmol/L in the prospectively followed cohorts

in the meta-analysis of Ding et al. (2006)⁶. This is reflected in the Study Protocol V1.5, approved by the lead HREC on 20 April 2013.

Exclusion criteria

Exclusion criteria (Table 1) included previously diagnosed T2DM or use of any oral or injectable antidiabetic pharmacotherapy. We initially excluded men newly diagnosed with T2DM by the OGTT. However, given the arbitrary nature of the 2-hour OGTT cut-off for diagnosing T2DM²⁴, and because the objective was to remediate glucose tolerance, we amended the protocol (V2.0, approved by the HREC 19 March 2014) to increase the 2-hour glucose for exclusion to > 15mmol/L. This was a level below which we reasoned that more aggressive management than provided in the trial could be safely delayed. Current or planned treatment of obesity within 6 months precluded participation.

We excluded men treated with T any time in the preceding 12 months, or with established HPT axis pathology where treatment with T was, or was likely to be, required. We also excluded men who used any medication affecting T production (e.g., opioids, gonadotropin-releasing hormone (GnRH) analogues) or action (e.g., spironolactone, finasteride, dutasteride), or production of sex hormone binding globulin (SHBG) (growth hormone, antiepileptics and thyroxine (unless on stable dose thyroxine for > 3 months)) or with an underlying condition likely to require such treatments within 2 years.

We took a conservative approach to the presence of cardiovascular disease, requiring no events or significant symptoms in the preceding 6 months, no cerebrovascular disease (transient ischaemic attack (TIA) or stroke) in the preceding 3 years, and blood pressure (systolic/diastolic) \leq 160/100 mmHg at screening. Because of the association of T treatment with thrombosis²⁵ and increased red cell mass²⁶ we excluded men with a significant personal or first-degree family

history of thrombophilia and those with haematocrit $> 50\%$. Given T administration by deep intramuscular injection, we excluded men taking anticoagulants other than low dose aspirin (< 150 mg) and/or clopidogrel²⁷.

Other exclusions are: Ongoing major depression or other significant psychiatric disorder, known infection with human immunodeficiency or hepatitis virus, malignancy current or past (other than non-melanomatous skin cancer), abnormal liver (alanine transaminase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), bilirubin ≤ 3 times upper limit of normal) or renal dysfunction (estimated glomerular filtration rate (eGFR) < 30 mL/minute/1.73m²).

If there was a score > 19 on the International Prostate Symptom Score (IPSS) (Questions 1–7), indicating severe voiding (lower urinary tract) symptoms (LUTS) entry was conditional on urological review excluding significant pathology. Despite the cautions of some clinical guidelines, a recent systematic review concludes there is scant evidence that T treatment is contraindicated in men with severe LUTS²⁸. A history of prostate cancer precluded enrolment.

All trial participants who consumed more than two standard drinks of alcohol a day²⁹ were counselled to reduce their intake. Those men considered by the site investigator at the time of screening to have problem drinking were excluded. Men using recreational drugs, or with major non-malignant disease requiring medical intervention and/or likely to lead to serious illness or death within 2 years, were excluded.

Participant recruitment

Strategies to recruit participants included mass media coverage, radio advertising, articles in health insurance company newsletters, direct approaches to general practitioners, online marketing and staged mail-outs by the Australian Department of Human Services (DHS) to men

in the target age group. Prospective participants were directed to a study-specific website (www.diabetesprevention.org.au) or centralised phone line to obtain information.

Screening

After providing consent for screening, initial eligibility was established by questionnaire responses. Eligible men had a consent form and laboratory request forms mailed or made available online ahead of presenting to the clinic in the morning after an overnight fast. The clinics were members of a network of collection centres from a single national pathology service provider (Sonic Healthcare Ltd, Sydney). Consent forms were checked and filed. Following a baseline blood draw a further sample was taken 2 hours after 75 g oral glucose. The baseline full blood count (FBE), HbA1c by HPLC (Biorad D-100) and baseline and 2-hour glucose were assayed. If men were eligible based on haematocrit and 2-hour glucose, then serum T was assayed. If this was ≤ 14 nmol/L (Electrochemiluminescence Immunoassay, T II, Roche Diagnostics, GmbH, Mannheim), the remaining assays needed to confirm eligibility were run. All screening test results were electronically transferred from the pathology service to the central data management system. Participants were notified as to their eligibility or not. Site staff were notified electronically, and the study nurse called eligible participants to schedule an appointment for the remaining screening and enrolment processes (Table 2).

Enrolment and randomisation

At the initial appointment written consent for enrolment into the study was obtained, a standardised medical assessment performed, and the participant's eligibility verified by a site investigator. Eligible men were randomised in a concealed allocation via a centralised, web-based randomisation system using the method of minimisation in a 1:1 ratio to the intervention and

placebo arms (Flexetrial v5.9.4, Sydney, Australia). Randomisation was stratified by centre, age group (50–59 years, 60–74 years), waist circumference (95–100 cm, 101–115 cm, >115 cm), 2-hour glucose on OGTT (7.8–9.5 mmol/L, 9.6–11.0 mmol/L, 11.1–15.0 mmol/L), currently smoking (yes, no) and first-degree family history of T2DM (yes, no).

Baseline questionnaires were completed. Body weight, un-shod and in light clothing, and waist circumference in a horizontal plane midway between the iliac crest and ribs and read in the mid-axillary line during expiration, were measured. Peak handgrip muscle strength of each hand (mean of three measures on each occasion) was assessed using either a Jamar (Patterson Medical, Warrenville, Illinois, US) or Smedley (Stoelting Co., Wood Dale, Illinois, US) hand dynamometer. Venous blood was drawn for the central laboratory, participants were enrolled in WW and the first study injection administered. Pre-departure, participants were provided with an appointment for a dual energy X-ray absorptiometry (DXA) scan to assess regional and whole-body bone mineral density, muscle and fat mass. DXA scanners are Hologic and Hologic Discovery A (Hologic Inc., Marlborough, Massachusetts, US) and GE Lunar Prodigy, GE Lunar Prodigy Advance and GE Lunar iDXA (GE Healthcare Lunar, Madison, Wisconsin, US). Wherever possible DXA scans for each participant use the same machine.

On study

Participants attend study visits at 6 weeks and 3 months, and 3-monthly thereafter for further injections, clinical review, blood collection and questionnaire completion. They also separately attend for a further DXA scan at 2 years. Study procedures and schedule of assessments are summarised in Table 2. Study sites are masked to all safety laboratory investigations unless there is a pre-specified flag (Table 3).

Treatment

Injectable T undecanoate (Reandron®, Bayer) (1000 mg/4ml) in a 4 ml castor oil vehicle or 4 ml vehicle alone in identical ampoules (Bayer), was administered to participants by slow deep intramuscular injection in the upper outer gluteal region by clinic nurses at randomisation, 6 weeks (+/- 1 week), and 3-monthly (+/- 2 weeks) thereafter. Adherence to the treatment schedule was monitored in the central data management system. This schedule maintains physiological circulating T concentrations³⁰ and obviates the need for monitoring adherence and drug exposure. Serum from all visits is stored frozen (-80°C) and sex steroids measured (as described below) in batches as participants complete the trial. Masking is maintained, and no dosage adjustment made during treatment. T undecanoate is registered in Australia for treatment of male hypogonadism (ARTG 106946).

Lifestyle intervention

Collaboration with WW facilitates the standardisation of, and ease of access to, a lifestyle intervention that is acceptable to men³¹ and effective for T2DM prevention, including in an Australian context³². At enrolment, a temporary membership card, program information, and a telephone number for the WW call centre were provided. Participants were encouraged to attend weekly WW meetings. An interactive website provides diet and activity guidelines, and self-monitoring tools that allow men to log food, physical activity and weigh-in details. Men are encouraged to achieve a 5% reduction in body weight each year and to monitor and record their own body weight weekly because frequent self-weighing is helpful for weight loss³³. Adherence with the overall lifestyle program is monitored via website logins and activity, meeting attendance and information collected at 3-monthly clinic visits.

Outcome measures

Primary endpoints and power calculation

Rationale for two endpoints: The initial decision was to enrol obese men with prediabetes (2-hour glucose ≥ 11 mmol/L on the OGTT) and examine reduction in progression to T2DM at 2 years based on a repeat OGTT. However, 12 months into the 4-year recruitment period we amended the protocol and obtained ethics approval to include men with a 2-hour glucose of up to 15 mmol/L. The cut-offs used to define T2DM on the OGTT are somewhat arbitrary and poorly reproducible³⁴. Furthermore, T2DM of relatively recent onset is reversible with weight loss³⁵, and improvements in³⁶, and even normalisation of³⁷, glycaemia has been reported in observational studies of T treatment. Accordingly, we originally defined the primary outcome as the proportion of patients with OGTT ≥ 11.1 mmol/L at 2 years, accepting some loss of precision by using a categorical variable for a continuous measurement. For example, a 2-hour glucose of 9 mmol/L at baseline and 10 mmol/L at 2 years represents a poor outcome compared to a 2-hour glucose of 10 mmol/L at baseline and 8 mmol/L at 2 years, but both would have been classed as the same outcome according to our initially planned endpoint (percentage non-diabetic).

Hence, to reduce the reliance on the categorical endpoint of percentage non-diabetic, we introduced a continuous variable as a second primary endpoint and will assess the mean change in OGTT between the treatments at 2 years. The primary outcome of the trial will be considered positive if either of the two co-primary endpoints is met (Table 4).

Power calculation

Based on our 5-year follow-up of the FAMAS cohort, the estimated 2-year incidence rate of T2DM for men aged ≥ 50 years with a T ≥ 14 nmol/L and prediabetes is 23% (unpublished). This rate was adjusted by 30% to account for lifestyle intervention benefits^{36,38}, providing an estimated incidence of 16.1% over 2 years. Using methods given by Machin (2009)³⁹, a sample size of 1000

participants will have a 80% power to detect a difference in the proportions of participants with OGTT \geq 11.1 mmol/L at 2 years of 7.1% (16.1% in the control group to 9.0% in the T-group). This sample size will have a 88% power to detect a difference in the reduction of OGTT from baseline to 2 years between groups of 0.6 mmol/L based on a standard deviation of 2.4 mmol/L. Since the anticipated correlation between the two endpoints is 0.75, the significance levels for the individual endpoints were adjusted⁴⁰ using numerical methods⁴¹ in the ACCoRD (Analysis of Censored and Correlated Data) software package v8.5.3 (Eastwood, Australia). The overall study will have $>$ 80% power to detect the stated differences with a significance level of 3.5% for the endpoint of OGTT \geq 11.1 mmol/L and 2.5% for the endpoint of change in OGTT of at least 0.6 mmol/L. This level of significance will have an overall (family-wise) significance level of 5%. This sample size allows for non-compliance of 5% and attrition of 15%.

Subgroup analysis of primary endpoints

A subgroup analysis will determine whether T2DM incidence and the degree of change of OGTT from baseline to 2 years among those receiving T relative to placebo varied according to whether the participant was pre-diabetic or newly diagnosed T2DM at baseline (baseline OGTT $<$ 11.1 or \geq 11.1 mmol/L).

Secondary endpoints: Secondary endpoints, listed in Table 4, and the reason for inclusion are now described.

- (i) Additional markers of glycaemic status: The percentage of men with normal glucose (2-hour glucose $<$ 7.8 mmol/L) at 2 years; the initiation of antidiabetic pharmacotherapy established by participant report and Pharmaceutical Benefits Scheme records, and measurement of fasting plasma glucose (FPG) and HbA1c;

- (ii) Mechanism/s of effect of testosterone may relate to either or both of:
- a. Improved body composition reflected by a decrease in total and or abdominal fat mass and increase in lean mass (DXA) and muscle strength (hand grip dynamometry) at 2 years, and in insulin sensitivity by the measurement of FPG and insulin and assessment of insulin resistance (IR) via the homeostasis model assessment of IR (HOMA-IR)⁴².
 - b. Enhanced adherence to the WW program as reflected by attendance at groups, use of the online program, or both, and its relationship to weight loss.
 - c. Increase in physical activity as assessed by the Active Australia questionnaire.
- (iii) Treatment-specific benefits: may be attributable either to T, improvement in health behaviours and weight loss⁴³ or an interaction between T and the latter factors. These outcomes include: erectile function, sexual desire and LUTS as assessed by the International Index of Erectile Function (IIEF-5), Sexual Desire Inventory, and IPSS respectively, as used in the Men in Australia Inflammation Lifestyle, Environment and Stress (MAILES) study⁴⁴.
- (iv) Treatment impact on psychosocial factors:
- a. Health-related quality of life (HRQoL) assessed by the Short-Form Health Survey (SF-12) testing the hypotheses that HRQoL will improve over time and be greater at study completion for men receiving T verses placebo;
 - b. Psychosocial function and motivation for lifestyle change assessed by the MacArthur Scale of Subjective Social Status⁴⁵, Pearlin's Personal Mastery Scale⁴⁶, and Sense of Coherence⁴⁷ addressing the following hypotheses: subjective social status, mastery, and sense of coherence will improve over time in men and be greater at study end for men receiving T verses placebo;

- (v) To determine whether psychosocial measures mediate the impact of T on glycaemic status: Hypotheses being tested are that: improvements in subjective social status, mastery, and sense of coherence will partially or fully mediate (account for, in the causal pathway) the impact of t on glycaemia;
- (vi) To determine whether the relationship between T and glycaemic status varies by socio-demographic measures: this framing acknowledges that T will not influence socio-demographic status but that such measures could modify T impact on glycaemia; it also explicitly explores moderation rather than simply statistically controlling for socio-demographic measures (including highest education, household income (adjusted for employment status), occupation, and marital or cohabitation status) as covariates. The hypothesis is that greater education, greater household income, higher status occupation, or being married or cohabitating will strengthen the relationship between T and glycaemic status and, conversely, lesser values for these measures will be associated with a lesser strength of the relationship between T and glycaemic status;
- (vii) Association of outcomes with baseline and change in sex steroid concentrations. Circulating T, dihydroT (DHT), oestradiol (E2) and oestrone (E1) will be measured by stable isotope dilution liquid chromatography–tandem mass spectrometry (LC-MS/MS; API-5000)⁴⁸ at baseline, 18, 66, and 102 weeks. Serum SHBG, follicle stimulating hormone (FSH) and luteinising hormone (LH) will be measured by automated electrochemiluminescence immunoassay (Roche Diagnostics, GmbH, Mannheim);
- (viii) Treatment impact on health care expenditure will be based on costs of prescribed pharmaceuticals from the PBS, costs of hospitalisations from the database of Australian

Refined Diagnostic Related Groups (AR-DRGs) and costs of GP visits from the database of the Medical Benefits Schedule (MBS). The theoretical cost of the T and lifestyle program will also be estimated at every 3rd follow-up. An economic evaluation will be undertaken between groups for incremental costs of the intervention per unit of health service resource used. Based on trial data, a cost-effectiveness analysis will be undertaken to determine the incremental cost-effectiveness ratio (ICER) of T therapy. The ICER is simply the net cost (cost of intervention minus cost savings from illness prevention) divided by the net change in health outcome. The 'trial-based' ICER will be expressed as net cost per incident T2DM prevented. Using epidemiological and cost data regarding T2DM, modelling will then estimate ICERs in terms of net cost per death prevented, life year gained and quality-adjusted life year (QALY) gained.

- (ix) Biobanking: aliquots of whole blood, plasma and serum from each participant are stored for future assays, including genomics, covered by the initial consent.

Safety

Table 3 summarises the pre-specified safety reasons for study drug discontinuation. A haematocrit $\leq 54\%$ requires a confirmatory test without fasting within 2 weeks before withdrawal. If a significant increase in haematocrit occurs with symptoms (e.g., new onset or worsened ischaemic symptoms (TIA or angina)) then venesection may be offered in addition to drug withdrawal. If prostate-specific antigen (PSA) levels increase above the age-specific normal range, the test will be repeated, ensuring factors that may lead to a false positive are eliminated, for instance, infection or bicycling, following which there is referral to a urologist whose decision it would be to either monitor or biopsy. Withdrawal will be based on the urologist's advice.

The IDSMC, comprising a cardiologist, endocrinologist, pharmacologist, and biostatistician meets every 6 months. The IDSMC functions independently of the T4DM trial conduct and is responsible for monitoring participant safety, trial conduct and emerging results. To date, the trial has been permitted to proceed as per protocol.

Sub-studies

Three sub-studies aim to determine the effects of T treatment on: (i) bone micro-architecture (high resolution peripheral quantitative computed tomography (HR-pQCT), Austin Health only) and density (DXA) (T4Bone); (ii) motivation and behaviour (T4M&B) and (iii) telomere length (T4Telomeres). Two additional sub-studies determine effects of extended treatment with T for up to 4 years (T4DM run-on), and rate of HPT axis recovery at the end of treatment (T4DM run-off), respectively.

Statistical methods

Treatment effects on primary and secondary endpoints will be analysed according to a modified intention-to-treat principle. Baseline characteristics will be summarised using N and % for categorical variables, and medians and interquartile ranges for skewed continuous variables. The primary analysis will be an unadjusted analysis of the proportion of participants with T2DM at 2 years and treatment comparisons will utilise the chi-squared test. For the mean change in glucose level from baseline, a two-sample t-test will be used. These analyses will be unadjusted. As these two endpoints are correlated, a significance level of 0.035 will be used for the 2-hour glucose ≥ 11.1 mmol/L and 0.025 for the mean change in glucose. Where appropriate, secondary analyses adjusted for covariates will be conducted using appropriate regression methods (i.e., Cox regression, logistic regression, generalised estimating equations (GEE)). Baseline T by LC-

MS/MS (< 8, 8–11 and 11.1+ nmol/L) and stratification variables of centre, age group (50–59 y and 60–74 y), waist (95–100, >100–115, >115), 2-hour glucose (7.8–9.5, >9.5–11, >11–15), current smoking and first-degree family history of T2DM will be adjusted for. Additional baseline factors as potential covariates are weight, serum testosterone and use of selective serotonin reuptake inhibitors (SSRIs). A missing data analysis will use logistic regression to explore associations of baseline OGTT results and whether an OGTT at 2 years was obtained. Secondary outcomes will be analysed using chi-squared or t-tests where appropriate, and for repeated measures with binary outcomes, a GEE (exchangeable correlation structure) and a log link function will be used. For subgroup analyses of pre-diabetic and newly diagnosed T2DM, an interaction term between this and treatment will be fitted in a GEE model and the relative risk and associated 95% confidence interval will be reported for each level of the subgroup. The nominal p-value for significance for secondary endpoints is 5% and all comparisons will be two-sided. The statistical analysis plan will be agreed upon and database locked before unmasking of treatment allocation.

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Conflict of interest statement

GW has received research funding from Bayer, Lilly, Lawley Pharmaceuticals and Weight Watchers, and speaker honoraria from Bayer, Lilly and Besins Health Care.

CA has received honoraria from Besins Health Care and is an advisory board member for Ferring.

MG has received research funding from Bayer, Novartis, Weight Watchers, Lilly and speaker's honoraria from Besins Healthcare and Otsuka.

DJH has received institutional grants for investigator-initiated studies of testosterone pharmacology (Lawley, Besins Healthcare) but no personal income and has provided expert testimony to anti-doping and professional standards tribunals and testosterone litigation.

BBY has received speaker honoraria and conference support from Bayer, Lilly and Besins Healthcare, research support from Bayer, Lilly and Lawley Pharmaceuticals, and has been a member of advisory committees for Lilly and Besins.

EA, KB, AC, MD, WH, VG, WI, AJ, RM, KR and BS declare no relevant conflicts of interest.

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Figure 1: T4DM study design

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Table 1: Eligibility criteria

<p>Inclusion criteria</p> <ul style="list-style-type: none"> • Men aged e50 and d74 years • Waist circumference e95 cm • Serum testosterone d14 nmol/L • 2-hour plasma glucose e7.8 & d15 mmol/L on 75g OGTT • Willing to participate in a lifestyle program co-ordinated by Weight Watchers® • Able and willing to meet all protocol-required procedures and visits • Able to read and understand the Participant Information and Consent Form and provide informed consent to participate
<p>Exclusion criteria</p> <ul style="list-style-type: none"> • Significant hypothalamo-pituitary-gonadal (HPG) pathology • Testosterone treatment in the past 12 months, or history of anabolic steroid abuse at any time • Medications affecting testosterone or SHBG in the previous month, or conditions indicating potential future use of such medications • Previously diagnosed T2DM, use of medication to lower blood glucose • 2-hour glucose > 15mmol/L on OGTT, or symptoms of hyperglycaemia at any level • Treatment with anti-obesity drugs or any investigational medication within 6 months prior to informed consent, previous or planned bariatric surgery • Major cardiovascular event in previous 6 months or active cardiac disease, including cardiac failure with NYHA classification e2, angina, or arrhythmias • BP systolic e160, diastolic e100 • TIA or stroke within the previous 3 years • Any malignancy besides non-melanomatous carcinoma of the skin • Excessive alcohol intake (>14 standard drinks/week) or recreational drug use in the previous 6 months • Ongoing episode of major depression or other significant psychiatric disorder

Table 2: Schedule of assessments

Study visit	Screen, enrol and baseline	On study			End of study
		Week 6	12-weekly for 2–4 years (except 2-year visit)	2 years	
Informed consent	X				
Eligibility screen	X				
Medical history, physical exam, DRE	X				
Demographics	X		X	X	X
Height	X				
BP, pulse, weight, waist circumference	X		X	X	X
Hand grip assessment	X		Every 2 nd visit	X	X
DEXA scan	X			X	
Questionnaires: Physical activity (Active Australia), Macarthur Scale of Subjective Social Status, Quality of Life (SF-12), Personal Mastery Scale (Pearlin), 13-item Sense of Coherence Questionnaire, IIEF 15, IPSS, PSQI	X		Every 2 nd visit	X	X
CES-D depression questionnaire	X		At 1 year	X	
Adverse events, concomitant medications	X	X	X	X	X
Weight Watchers compliance		X	X	X	
OGTT	X			X	
Screening blood tests	X				
FPG	X		Every 2 nd visit	X	X
HbA1c	X		X	X	X
Safety monitoring blood tests	X		X	X	X
Blood collection for central lab	X		Weeks 18 and 66 only	X	X

Table 3: Pre-specified reasons for early discontinuation of study treatment

<ul style="list-style-type: none"> • ALT measurement >3-fold the upper limit of normal (ULN) • Haematocrit level \leq 54 % • PSA > age-specific ULN • Development of contraindications to testosterone • Allergic or severe adverse reaction to any component of the investigational product • The participant needs to take concomitant drugs that interfere with the investigational product • The participant is no longer able to participate for other medical reasons • The participant withdraws consent • At the discretion of the investigator

Table 4: Study endpoints

Co-primary endpoints
<ul style="list-style-type: none"> • 2-hour glucose in the diabetic range (\leq 11.1mmol/L on 75g OGTT) at 2 years • Change in 2 hour glucose at 2 years from baseline
Secondary endpoints
<ul style="list-style-type: none"> • Normalised blood glucose (2 hour glucose < 7.8mmol/L) • Initiation of antidiabetic pharmacotherapy • Glucose metabolism: FPG, insulin and HbA1c • Anthropometrics: body weight, waist circumference • Body composition: using DEXA measurements for whole-body and regional fat and lean mass • Muscle strength: peak handgrip • Sex steroid hormone profile: testosterone, dihydroT (DHT), estrone (E1), estradiol (E2) and SHBG • Sexual function and lower urinary tract symptoms (LUTS) • Biomarkers for metabolic function: lipids (total cholesterol, LDL, trigs, HDL) • Psychosocial function • Compliance with lifestyle intervention program • Health care expenditure

Eligible participants

- Men aged 50-74 years
- Pre-diabetic or newly diagnosed diabetic
- Serum T ≤ 14 nmol/L
- Waist circumference ≥ 95 cm

Stratification:

- Centre
- Age group -50-59y, 60-74y
- Waist circumference (cm)
 - 95-100;
 - 101-115;
 - >115.
- 2 hour glucose on OGTT (mmol/L)
 - 7.8-9.5;
 - 9.6-11.0;
 - 11.1-15.0.
- Currently smoking - yes, no
- First-degree family history of T2D - yes, no.

