Recovery of Male Reproductive Function After Ceasing Prolonged Testosterone Undecanoate Injections

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Keywords:	Testosterone, LH, FSH, male sexual function, quality of life

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Recovery of Male Reproductive Endocrine Function After Ceasing Prolonged Testosterone Undecanoate Injections

Short title: Recovery of Serum Testosterone, LH & FSH

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Abstract

Context: The time course of male reproductive hormone recovery after stopping injectable testosterone undecanoate (TU) treatment is not known.

Objective: To investigate rate, extent, and determinants of reproductive hormone recovery over 12 months after stopping TU injections.

Methods: Men (n=303) with glucose intolerance but without pathologic hypogonadism who completed a 2-year placebo(P)-controlled randomized clinical trial of TU treatment were recruited for a further 12 months while remaining blinded to treatment. Sex steroids (T, DHT, E_2, E_1) by LCMS, LH, FSH and SHBG by immunoassays and sexual function questionnaires (Psychosexual Diary Questionnaire (PDQ), International Index of Erectile Function (IIEF), SF-12) were measured at entry (three months after last injection) and 6, 12, 18, 24, 40 and 52 weeks later.

Results: In the nested cohort of TU-treated men, serum T was initially higher but declined to 12 weeks remaining stable thereafter with serum T and SHBG 11% and 13%, respectively, lower than P-treated men. Similarly, both questionnaires showed initial carryover higher scores in T-treated men, but after weeks 18 showed no difference between T and P treated men. Initially fully suppressed serum LH and FSH recovered slowly towards the participant's own pre-treatment baseline over 12 months since last injection.

Conclusions: After stopping 2 years of 1000 mg injectable TU treatment, full reproductive hormone recovery is slow and ~~,~~ progressive over 15 months since last testosterone injection but ~~eventually complete~~may take ~~taking~~ longer than 12 months ~~since the last dose~~to be complete. Persistent proportionate reduction in serum SHBG and T reflects lasting exogenous T effects on hepatic SHBG secretion rather than androgen deficiency.

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Introduction

Testosterone (T) production in men is under tight negative feedback control[1]. Exogenous androgens suppress the hypothalamo-pituitary testicular (HPT) axis, markedly suppressing circulating LH and FSH as well as T (for androgens other than T). For example, recovering male androgen abusers display profound suppression of endogenous T, serum LH and FSH for 9 to 18 months after cessation of androgens[2-4]. Men with a normal HPT axis treated with exogenous T even at physiological replacement doses will suppress endogenous T production and may display androgen withdrawal symptoms for some time after drug cessation leading to androgen dependence[5]. This understudied issue is relevant to the 100-fold increase in T prescribing over the last three decades[6] for reasons other than pathological hypogonadism[7-10]. These men often cease T treatment after relatively short periods[11] risking iatrogenic androgen withdrawal effects. This may lead into a self-reinforcing cycle of androgen dependence[5]. Hence, the natural history of male reproductive hormone recovery after ceasing exogenous T and its determinants are clinically important, but not reported.

Few well designed, placebo (P)-controlled studies of T treatment report detailed follow-up observations on recovery of male reproductive endocrine function after cessation. After 12 months of injectable testosterone undecanoate (TU) serum T elevation persisted at 56 weeks returning to baseline by 82 weeks[12]. Another study of daily transdermal gel reported that after 6 months treatment, serum T returned to pre-treatment baseline at 6 months after ceasing treatment[13]. There was no follow-up reported of the daily transdermal T gel in the T Trials[14], the next largest after T4DM placebo-controlled trial of T treatment. Therefore, this study aimed to investigate the rate, extent, and determinants of recovery of reproductive endocrine function hormones in men following prolonged (2-year) treatment with injectable TU based on a nested randomized, placebo-controlled clinical trial.

Materials and Methods

Participants

The Runoff sStudy aimed to investigate the rate and extent of recovery in reproductive hormones (serum T, LH, FSH) and change in psychosexual function after ceasing 2-years of regular TU injections. Participants were recruited from the Testosterone for Diabetes Mellitus (T4DM) Study[15, 16]. Briefly, the T4DM study recruited men 50 to 74 years with impaired glucose tolerance (IGT) or newly diagnosed type 2 diabetes mellitus (diabetes) but without pathologic hypogonadism to be randomized to 2-years of treatment with either TU (1000 mg in 4 ml oil) or matching oil vehicle P (supplied by Bayer; clinical trial registration ACTRN12612000287831)[15, 16]. Consistent with entry criteria for the T4DM study[16], pathology of the hypothalamo-pituitary testicular (HPT) axis, defined as recognised organic pathology of the HPT axis, was excluded. A subgroup volunteered to continue in the T4DM study for up to an extra 2 years. All injections were administered by study personnel at three-month intervals following a loading dose at 6 weeks after the first dose as per the product marketing approval. The Runoff study had ethics approval from each centre with participants providing written informed consent pre-entry.

The Runoff study was designed in 2016 after most participants had enrolled in the T4DM study (ethics approval 3 July 2012, first enrolment 5 Feb 2013, last enrolment 27 Feb 2017) aiming to investigate recovery while participants remained masked to their T4DM treatment allocation. All T4DM participants were invited to join the Runoff study recognizing that it prolonged their study involvement. Of 1007 T4DM participants, 303 accepted the invitation of further study visits. The

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major reason for declining was the inconvenience of a further 12 months of study visits. Participants in the T4DM and Runoff studies received no reimbursement for participation. Of these 303 men, 215 men were included in the hormone analyses if they completed at least 5 of the 7 scheduled visits (91 men with all 7 visits, 60 with 6 visits, 64 with 5 visits). Sera from those completing 4 or fewer visits did not have Runoff study hormones measured.

Study design and procedures

Entry date into the Runoff study was defined as the date of the first missed injection (i.e. 3 months after the last T4DM study injection) and outcomes were evaluated at both timepoints (entry to Runoff study, time since last injection). All Runoff study data were collected prior to participants unmasking of T4DM treatment allocation. Participants attended at entry and 6, 12, 18, 24, 40 and 52 weeks later with body weight recorded, an 8 ml blood withdrawn with serum frozen until assay and two sexual function questionnaires completed. The Psychosexual Diary Questionnaire (PDQ) and International Index of Erectile Function (IIEF) were completed online (73%) or on paper at each visit. The modified PDQ[17] analysis comprised eight variables using responses for the time window framed as "over the last week" but omitting Q3 Mood questions. The self-rated questions comprised Q1 (Overall Sexual Desire), Q2A (Sexual Enjoyment without a Partner), Q2b (Sexual Enjoyment with a Partner), Q5 (% Full Erection), Q6 (Erection Sustained), Q7 (Lack of Sex Drive is a Problem for Me) and Q8 (Overall Satisfaction with Sex Life) were scored using a sliding scale from 0-100%. The Q4 (Sexual Activities) comprised 12 specified sexually oriented activities which were scored as Yes (1) or No (0) with the sum as the response with a range 0-12. The IIEF was scored as described from the 15 item responses into five domains Erectile Function, Orgasmic Function, Sexual Desire, Intercourse Satisfaction and Overall Satisfaction[18, 19].

At Runoff study entry, participants were also asked (a) "Which treatment do you think you were receiving during the T4DM study?" and (b) "What changes made you think which treatment you were receiving during the T4DM study?") with possible responses (all could be selected) comprising "physical (eg energy, endurance, strength)", "sexual (eg sexual desire or activity)", "psychological (eg mood, motivation)" and "other" effects.

Hormone analysis

Serum steroids were measured in a single batch by previously validated liquid chromatography-mass spectrometry (LCMS) method[20] modified to change extraction solvent and incorporate ultrapressure chromatography[21]. The limits of quantitation and reproducibility (within-run coefficient of variation CV) over three levels of quality control (QC) samples- were for testosterone (T, 174 pM or 50 pg/ml, 5%), dihydrotestosterone (DHT, 345 pM or 100 pg/ml, 10%), estradiol (E_2, 18 pM or 5 pg/ml, 8%) and estrone (E_1, 19 pM or 5 pg/ml 7%)~~were for testosterone (T, 174 pM or 50 pg/ml, 5%), dihydrotestosterone (DHT, 345 pM or 100 pg/ml, 10%), estradiol (E_2, 18 pM or 5 pg/ml, 8%) and estrone (E_1, 19 pM or 5 pg/ml 7%)~~. Serum LH, FSH and SHBG were measured by Roche immunoassays with CVs of <2% for LH and FSH (over three levels of QC) and <3% for SHBG (over two QC) with detection limits of 0.1 IU/L for LH and FSH and 2 nmol/L for SHBG.

Genetic analysis

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The 2 genetic polymorphisms with known influence on testosterone metabolism or action were analyzed by polymerase chain reaction (PCR). Uridine 5'-diphospho-glucuronosyltransferase 2B17 (UGT2B17) genotyped ~~as described~~[22, 23]. The androgen receptor CAG triplet repeat polymorphism was genotyped by PCR and capillary electrophoresis (3730xl DNA analyser) as described[24] with the forward primer labelled with 6-carboxyfluorescein (FAM) AR 1162: 5' – FAM - CACCTCCCGGCGCCAGTTTGCTGCTGCTGC – 3' and an unlabeled reverse primer AR B112: 5' – CTGCTGCTGCTGCCTGGGGCTAGTCTCTTG – 3'. CAG repeat lengths were calibrated against samples with known CAG triplet lengths determined by Sanger sequencing.

Data analysis

Data are presented as mean and standard error of the mean (SEM). Undetectable serum LH and FSH were imputed by the limits of detection (0.1 IU/L) divided by √2 as described[25]. Recovery of serum LH and FSH was based on return to the participant's own pre-treatment baseline serum LH and FSH. The survival analysis findings were verified by an alternative analysis using the return to the population mean baselines for serum FSH and LH determined by pooling all samples from pre-treatment baseline (n=955) and P-treated men (n=661). Recovery by survival analysis was analysed using the *survivor*, *survminer* and *ggplot2* packages (https://cran.r-project.org/).

Serial data of continuous variables (hormones, SHBG) were analysed by linear mixed model regression with repeated measures employing an autoregressive within-subject covariance matrix, which was optimal according to minimizing Akaike Information Criterion (NCSS 2021). The main effect was treatment (testosterone vs placebo) with within-subject effect of time (since Runoff entry or last injection as specified) together the time*treatment interaction. Potential covariables comprised baseline (all from pre-T4DM) age, height, measures of overweight (weight, body mass index (BMI), fat mass (kg) and abdominal fat mass (%) from dual-energy X-ray absorptiometry (DEXA) body composition analysis), body surface area (BSA, Gehan & George formula[26]), duration of treatment (number of injections), hand grip strength, baseline serum testosterone, hemoglobin (Hb), systolic blood pressure (SBP) or diastolic blood pressure (DBP), glomerular filtration rate (GFR) and UGT2B17 and CAG triplet repeat genotypes. Models were run with interpretable two-way interactions deleting non-significant interactions (P<0.01) from the final model. Post-hoc tests used repeated measures analysis of variance with pre-set linear contrasts to define early and later timepoints in the time-course.

Results

Men in the Runoff study did not differ from men in the main T4DM study in any of 23 baseline anthropometric or demographic variables (all p>0.13, table 1) or in variables that were measured at the end of 2 years T4DM participation prior to entry to Runoff study (table 2). Participants in both groups had a median of 9 injections with 7% having fewer than 9 (range 5 to 8) due to premature discontinuation and 24% more than 9 (range 10-17) injections due to participation in the subgroup which extended study treatment beyond 24 months. At entry to T4DM, men with diabetes (n=35, 19%) had higher HbA_1C (6.1 ± 0.1 % vs 5.7 ± 0.03 %) but did not differ from men with IGT (n=180) in other variables (age, height, weight, pulse, systolic and diastolic blood pressure, BMI, BSA, serum T, DHT, E_2, E_1, SHBG, hemoglobin and glomerular filtration rate; data not shown). There was no difference between men treated with T or P in androgen receptor CAG triplet repeats (both median

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21 repeats) nor in the UGT2B17 polymorphism (overall 10% homozygous deletor, 44% heterozygous, 46% homozygous wild-type genotypes).

Serum hormones

Serum T was significantly higher in T-treated men at entry but at all timepoints from 12 weeks onwards serum T was consistently 11% lower in T treated than in P treated men (figure 1). The significantly lower mean serum T (pooling weeks 18~~24~~ to 52) in T vs P treated men (78 ± 3 % vs 89 ± 4%, p=0.026) was no longer significantly different after adjustment for mean serum SHBG (treatment p=0.79, treatment*serum SHBG interaction p=0.34).

The overall time course of serum T demonstrated a significant downward trend for both T and P groups (p<0.0001) but steeper for T treated men (time*treatment interaction p<0.0001). From 18 weeks onward, however, serum T was stable without significant change over time. The only significant univariate covariable predictor on serum T time course were ~~as~~ positive effects of (i) baseline T (p=<0.0001), an ~~the~~ effect ~~of which was~~ nullified by including BMI as a covariate and (ii) diabetes for which a higher serum T (p=0.008) was nullified by including weight change as a covariate. No other potential covariates (baseline weight, BSA, DEXA fat mass, abdominal fat mass), number of injections, SBP, DBP, GFR or the UGT2B17 or CAG triplet repeat polymorphism had significant impact on the serum T time course.

Averaged over all timepoints in Runoff study, mean s~~S~~erum SHBG (figure 1) displayed a stable pattern of lower concentrations in T treated men (p=0.011) with no significant effect of time or time*treatment interaction. ~~Averaged over all timepoints,~~ SHBG was significantly lower in T vs P-treated men expressed relative to participant's own pre-T4DM baseline (93 ± 3% vs 106 ± 3%, p=0.005). There were significant positive covariates effects on serum SHBG concentration of weight loss (P<0.0001), age (p=0.002), baseline T (p=0.003) and Hb (p=0.027). Other significant covariates with positive effects on the time course of serum SHBG were hemoglobin (p=0.016), GFR (p=0.007) and SBP (p<0.001) but not DBP or diabetes (vs IGT). Serum SHBG did not differ between men randomised to T or P (respectively, 37.8 ± 13.8 vs 37.4 ± 13.6, p=0.67) or those who did or did not participate in the Runoff study (respectively, 36.6 ± 12.7 vs 38.0 ± 14.1, p=0.13; table 1).

Mean serum LH and FSH concentrations recovered gradually to reach the group mean pre-treatment baseline levels at 24 weeks after Runoff entry (ie 36 weeks after last injection) (F~~f~~igure 1). Median time to recovery to the participant's own pre-treatment baseline was 33.9 weeks [95% confidence limits 31.0 – 36.0 weeks] for serum FSH and 33.9 weeks [31.1 – 36.0 weeks) for serum LH and both took 63 weeks to reach 90% of their own pre-treatment baseline (F~~f~~igure 2). Expressing recovery as time to reach the mean of grouped baseline (n=1616, serum FSH 7.2 ± 0.3 IU/L, LH 5.6 ± 0.2), the timings were similar. Both serum LH and FSH displayed highly significant effects of time, treatment, and time*treatment interactions (all p<0.0001) on the time course of their recoveries with additional effects of serum SHBG alone (p=0.026 and 0.030, respectively) and in its interaction with age (both p=0.005). There were no other significant covariables (including diabetes vs IGT) with any influence on the time course of recovering serum LH or FSH concentrations.

For serum DHT, there was a downward profile over time in both treatment groups (time, p=0.015), being steeper in the T treated men (time*treatment interaction, p=0.021) (F~~f~~igure 3). The only other significant covariate effect was a positive effect of serum SHBG (p<0.0001). For both serum E_2 and E_1, there was a downward profile with time in both treatment groups (time, p=0.001; time*treatment interaction=0.40) but no significant effects of T compared with P treatment or any

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significant covariates (Ffigure 3). None of serum DHT, E_2 or E_1 were significantly influenced by diabetes (vs IGT).

Psychosexual Quality of Life

For the IIEF questionnaire, there were effects of time and, of treatment or treatment x time interactions, for all measures (Erectile Function, Orgasmic Function, Sexual Desire, Intercourse Satisfaction, Overall Satisfaction). At entry and for weeks 6 and 12, the IIEF questionnaire showed higher scores for sexual function for T-treated compared with P-treated men for all measures due to carry-over of T treatment (Ffigure 4). Apart from Overall Satisfaction at week 18, for all other measures of sexual function from weeks 18 onwards displayed there was no significant difference between T and P treatment effects apart from Overall Satisfaction at week 18.

For the PDQ questionnaire, there were significant effects of time for seven of eight measures (Overall Sexual Desire, Sexual Enjoyment with Partner, Daily Sexual Activities, % Full Erection, Lack of Sexual Desire a Problem, Erection Sustained, Overall Satisfaction with Sex Life) with only Sexual Enjoyment without Partner showing divergent effects between treatment groups (figures 5 & 6). There were also effects of treatment or treatment x time interaction for six out of eight measures other than Erection Sustained and Sexual Enjoyment with Partner. At entry, the PDQ questionnaire showed higher scores for sexual function in T-treated compared with P-treated men for four of eight measures due to carryover of T treatment (figures 5 & 6). Other than Overall Satisfaction, which remained higher in T treated men until week 18 then reduced so that from week 12 onwards it was not significantly different from P treatment.

Perceptions of Treatment

Overall, 131/303 (43%) of participants guessed correctly whether they were receiving T or P, 64/303 (21%) guessed incorrectly and 108/303 (36%) were unable to pick which treatment they were on (Ttable 32). Among those receiving T treatment, 37% guessed correctly whereas 50% of those on P guessed correctly. The prevalence of reported perceived symptomatic effects that participants considered as helping guess treatment (whether correct or not) were physical (24%), sexual (23%), psychological (13%) and other (73%). Of those, sexual effects (ratio 2.7 [95% confidence interval 1.5-5.0]) and "other" effects (odds ratio 0.6 [0.35-0.93]) were significantly more and less, respectively likely than by chance to distinguish between T and P treatments whereas physical and psychological effects were not.

Discussion

This randomised, placebo-controlled study, a nested sub-cohort of the T4DM study[16], provides for the first time placebo-controlled data on the natural history of recovery of male reproductive endocrine function following cessation after of prolonged injectable testosterone treatment. The T4DM study population comprised men aged 50 to 74 years with metabolic dysfunction (impaired glucose tolerance or recently diagnosed type 2 diabetes) but without pathologic androgen deficiency randomised to injectable TU or placebo treatment for two years. During the T4DM study, the Runoff study recruited T4DM participants to an originally unplanned extension of their unpaid involvement for a further year of recovery while remaining masked to their treatment allocation. The participants in the Runoff study , who did not differ from the T4DM study population in any of 23 demographic and anthropometric variables at baseline or after 2 years of participating in T4DM. The present findings indicate a slow and progressive but eventually complete recovery of testicular endocrine

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function over the 15 months since last testosterone injection towards the participants own pre-treatment baseline but may not be complete by 12 months with the tempo of recovery~~,~~ determined mainly by time since last ~~cessation of~~ testosterone dose~~treatment~~. Whether there remain any detrimental effects on androgen-sensitive non-reproductive functions after full recovery of testicular endocrine function requires further follow-up.

The time course of serum T recovery had distinctive features. Firstly, as expected the serum T concentration on entry to the Runoff study was higher among the TU-treated compared with P treated men reflecting carry-over effects from the T4DM study treatment. That timing of carryover from testosterone injections is comparable with the approved therapeutic inter-injection interval for TU injection treatment reflecting ~~so likely represent~~ ongoing release of TU from its injection depot. A second feature was the unexpected downward trend in P treated men, albeit less steep than in T treated men. This decline in serum T during the Runoff study in the P treated men may reflect recurring deleterious effects of weight (re)gain on T secretion after the T4DM study ended. A third feature was the consistently lower serum T concentrations from 12 weeks onward in T treated compared with P treated men. The ~~11%~~ reduction of mean serum T concentration from 12 weeks onward was proportionate to the stable ~~13%~~ reduction in serum SHBG throughout the study. These findings reflect the importance of SHBG as the principal circulating protein carrier of the hydrophobic T molecule[27]. Although serum SHBG is sensitive to large changes in body weight [28], the small regain of body weight during the Runoff study (reflecting cessation of T4DM lifestyle interventions) did not differ between treatment groups and could not explain the reduced serum SHBG during the Runoff study. The persistent long-term effects of T treatment on serum SHBG resembles that seen in men recovering from androgen abuse[3, 4] who have similar persistent reduction in serum SHBG[29]. These lasting effect of androgens on hepatic SHBG secretion resemble those ~~is~~ seen with the physiological surge in T secretion of male puberty[30], an effect replicated by TU treatment for delayed puberty[31-33]. Importantly, the proportionate reduction in circulating SHBG and T does not imply significant T deficiency.

The two independent sexual function questionnaires displayed carry-over in T effects on sexual function early in this study consistent with the higher serum T in T-treated men. Beyond 18 weeks however, both QoL questionnaires showed no significant differences between T and P treated men thereby excluding symptomatic T deficiency of which sexual symptoms are characteristic[29, 34]. Furthermore, the Runoff participants limited ability to guess correctly they received was partly dependent on perceived effects on sexual function, but not on physical or psychological effects, findings resembling those of the European Male Ageing Study's findings that circulating T had weak but significant inverse relationship with sexual symptoms but not with a wide range of physical or psychological symptoms[34]. Men's symptoms of organic androgen deficiency occur at highly reproducible blood T levels within an individual although the specific, leading symptoms vary widely between individuals[29]. By contrast, grouping androgen deficient men by symptoms reduces the power of blood T level to discriminate such symptoms[35]. Hence in concert, in the second half of the study the proportionate reductions in serum SHBG and T together with the QoL evidence make it likely the men's endogenous T production had recovered without significant androgen deficiency.

The slow and parallel recovery of serum LH and FSH is a salient finding. While recovery from suppression by exogenous T is expected, the delayed recovery signifies a prolonged depot effect of injectable TU and lingering effects of hypothalamic suppression by exogenous T treatment. Similar findings of slow, progressive and parallel recovery of serum LH and FSH is observed in recovery of HPT axis function in former androgen abusers[36]. These findings suggest that in this context of a recovering HPT axis, serum LH and FSH operate as androgen sensors comparable to the way in which

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serum TSH operates as a sensor of thyroid function status in otherwise healthy individuals[37]. Furthermore, due to study population, it cannot be excluded that impaired hypothalamic function in men with metabolic disturbances may contribute to the slow recovery. Although the survival analyses of serum LH and FSH do not achieve a plateau at 12 months since last testosterone injection, among T treated men the mean serum LH and FSH show no sign of further increases from 24 weeks onward making it unlikely that there is a further progressive increase in serum LH and FSH after 12 months. There are no controlled studies to compare with our findings. A previous uncontrolled series of 4 cases with congenital hypogonadotropic hypogonadism treated with injectable TU for at least 3 years reported an average of 30 weeks for serum T to decline to hypogonadal levels after ceasing injections[38].

In the present hormonal analyses, the dominant effect on time course of HPT axis recovery was the duration since cessation of T exposure. This finding is constrained by the use of a standard TU dose so we cannot exclude that supraphysiological androgen doses may exert more prolonged suppression; however, this does not appear to occur with former androgen abusers who characteristically use massive androgen doses in short, repetitive cycles[3, 4]. Of note, the time course of recovery from androgen abuse dosing was also primarily influenced by the time since cessation rather than androgen dose or duration[4].

This study did not examine recovery of sperm production but the time course of HPT axis recovery is similar to the recovery of sperm output in hormonal male contraceptive investigations using both T alone and T plus progestin regimens [39, 40]. In those studies of healthy young men, rigorously screened for normal sperm output prior to entry, recovery to fertile sperm output was achieved in 4-5 months [40] whereas recovery of sperm output to the participants own baseline was only 83% at 12 months and taking 24 months to reach 100% after ceasing testosterone ~~post-cessation of~~ treatment ~~and reaching 100% at 24 months~~[39]. While the recovery of endogenous T was not investigated in those prototype male hormonal contraceptive studies, the low intratesticular T threshold required to support spermatogenesis in mice[41, 42] and men[43, 44] suggests the time to full recovery of endogenous T production may be longer than for restoration of spermatogenesis although contrary findings are reported for men recovering from androgen abuse[36].

Study strengths include the randomized, placebo-controlled design of the underlying T4DM study which provides an unprecedented objective time course of recovery over 12 months following cessation of 2-years of injectable TU treatment. The treatment injections were administered by study personnel, thus ensuring ~~so the design ensuring~~ high adherence with a known physiological T dose and frequency. The duration of treatment was not a significant covariate for any of the hormonal recovery endpoints. Participants were not reimbursed in this or the T4DM study so that financial motives were unlikely to influence ~~colour~~ their participation or responses. During recovery, sex steroids were profiled by LCMS and two well-validated psychosexual function questionnaires were employed that displayed requisite sensitivity to carry-over T effects from the main T4DM study and showed similar sexual function to P-treated men beyond weeks 12-18.

Study limitations include that the steroid LCMS measurements used cannot distinguish exogenous from endogenous circulating T, which might provide more direct insight into the tempo of recovery of endogenous T secretion in the presence of exogenous T. In theory, carbon isotope ratio mass spectrometry, used with urine in antidoping laboratories[45], might provide such insight if improved sensitivity allows application to small serum samples. This limitation is overcome by the indirect but sensitive measures of recovery in serum LH and FSH providing a direct read-out of hypothalamic suppression of the HPT axis. The same limitation applies to the recovery from endogenous T suppression following cessation of other T products, notably transdermal T delivery, which feature

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faster onset and offset of action so that analogous recovery studies would be of interest. Another limitation was that this follow-on study was only conceived after men had entered the 2-year T4DM study so that only 30% of unpaid volunteers agreed to another year of follow-up creating ~~with~~ potential for participation bias. Yet according to all anthropometric and demographic measures available, this nested cohort was representative of the full T4DM cohort, ~~itself the~~ largest and equally longest ~~ever~~ randomized placebo-controlled study of T treatment. The present study's findings in Australian men ~~over~~ 50 to 74 years ~~years~~ of age with abdominal obesity, dysglycemia but without pathologic hypogonadism, may not be directly extrapolated to men of different age, medical status, or countries. This profile closely resembles the participants in the Testosterone Trials which also recruited a majority of obese, hypertensive ex-smokers with one third having diabetes[14]. T prescribing for such men has increased dramatically over recent decades[6, 7] making the findings clinically relevant and important. When T treatment is ceased, they risk androgen withdrawal symptoms, so the time course of recovery is of importance in their ongoing medical care. Finally, this study only followed men for one year and even long-term beneficial or detrimental effects could not be determined and would require longer to be determined or excluded. ~~follow-up.~~

We conclude that after stopping 2 years of a standard therapeutic doses of injectable TU treatment in middle-aged men with metabolic disturbance but not pathologic hypogonadism, recovery of testicular endocrine function is slow and ~~,~~ progressive over 15 months since the last testosterone injection but may take longer than 12 months to be complete. ~~eventually complete.~~ Over the first ~~Full recovery takes~~ 12 months since the last dose, there is a ~~including~~ persistent mild, proportionate reduction in serum SHBG and T without evidence of testosterone deficiency. The study also supports the hypothesis that the dominant factor in the tempo of recovery from androgen-induced suppression of the HPT axis is the time since cessation of exogenous androgen exposure.

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Figure Legends

Figure 1 – Time course of serum testosterone (upper left), SHBG (upper right), LH (lower left) and FSH (lower right) plotted as mean and standard error of the mean on the y axis over time in weeks on the x axis. Time in this figure is the time in weeks since entry to the Runoff study – this entry occurs 12 weeks after the last T4DM study injection. Inset are the results of the analysis of time, treatment, and their interaction.

Figure 2 – Kaplan-Meier survival analysis of the time to recovery of serum LH (left) and FSH (right) to the participants own pre-treatment baseline. Time in this survival analysis is from the time of the last injection – which is 12 weeks earlier than entry to the Runoff study. Events are marked with vertical bars and the shading around the survival line represents 95% confidence interval at that point on the curve.

Figure 3 – Time course of net weight loss from entry to T4DM study (upper left), serum dihydrotestosterone (upper right), estradiol (lower left) and estrone (lower right) plotted as mean and standard error of the mean on the y axis over time in weeks on the x axis. Time in this figure is the time in weeks since entry to the Runoff study – this entry occurs 12 weeks after the last T4DM study injection. Inset are the results of the analysis of time, treatment, and their interaction. Net weight loss is the difference at the time of observation in this study from baseline weight prior to entry to the T4DM study (ie remaining net weight loss carryover from the T4DM study).

Figure 4 - Plot of the five principal domains of the International Index of Erectile Function (IIEF-15). Erectile function (upper left), orgasmic function (upper middle), sexual desire (upper right), intercourse satisfaction (lower left) and overall satisfaction (lower middle) are plotted as mean and standard error of the mean with the outcome on the y axis, time on the x axis. Time in this figure is the time in weeks since entry to the Runoff study – this entry occurs 12 weeks after the last T4DM study injection. Inset are the results of the analysis of time, treatment, and their interaction.

Figure 5 - Plot of the Psychosexual Diary Questionnaire (PDQ). Overall sexual desire (upper left), sexual enjoyment with partner (upper right), sexual enjoyment without partner (lower left) and daily sexual activities (lower right) are plotted as mean and standard error of the mean with the outcome on the y axis, time on the x axis. Time in this figure is the time in weeks since entry to the Runoff study – this entry occurs 12 weeks after the last T4DM study injection. Inset is the results of the analysis of time, treatment, and their interaction.

Figure 6 - Plot of the Psychosexual Diary Questionnaire (PDQ). Fullness of erection (upper left), lack of sex drive a problem (upper right), erection sustained (lower left) and overall satisfaction with sex life (lower right) are plotted as mean and standard error of the mean with the outcome on the y axis, time on the x axis. Time in this figure is the time in weeks since entry to the Runoff study – this entry occurs 12 weeks after the last T4DM study injection. Inset is the results of the analysis of time, treatment, and their interaction.

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Table 1: Baseline characteristics

	T4DM not in study	In Runoff study	P-value
Randomised patients	704	303	.
Treated with testosterone	349/704 (49.6%)	155/303 (51.2%)	0.645
Medical baseline variables			.
Glucose tolerance			
• Normal (2 hour glucose <7.8)	7/704 (1.0%)	3/303 (1.0%)	0.860
• Prediabetic (7.8 <= 2 hour glucose < 11.1)	554/704 (78.7%)	243/303 (80.2%)	.
• Diabetic (2 hour glucose ≥ 11.1)	143/704 (20.3%)	57/303 (18.8%)	.
Serum testosterone			
Baseline testosterone (LCMS, nmol/L)	(n=668) 13.7 (4.4)	(n=291) 13.7 (4.4)	0.909
Grouped baseline testosterone (LCMS, nmol/L)			.
• <8nmol/L	47/668 (7.0%)	25/291 (8.6%)	0.646
• 8 to <11nmol/L	148/668 (22.2%)	60/291 (20.6%)	.
• ≥11nmol/L	473/668 (70.8%)	206/291 (70.8%)	.
Screening testosterone (immunoassay, nmol/L)	(n=704) 9.9 (2.5)	(n=303) 10.0 (2.6)	0.723
Serum SHBG (nmol/L)	38.0 (14.1)	36.6 (12.7)	0.131
Anthropometric variables			
Waist circumference (cm)	(n=704) 118.4 (12.6)	(n=303) 117.4 (11.0)	0.258
Weight (kgs)	(n=704) 108.0 (17.6)	(n=303) 107.6 (16.4)	0.748
Height (cm)	(n=704) 176.3 (6.5)	(n=303) 176.0 (6.1)	0.628
BMI (kg/m^2)	(n=704) 34.7 (5.2)	(n=303) 34.7 (4.9)	0.903
• Normal (18.5-25)	8/704 (1.1%)	1/303 (0.3%)	0.219
• Overweight (25-30)	130/704 (18.5%)	48/303 (15.8%)	.
• Obese (30-35)	250/704 (35.5%)	128/303 (42.2%)	.
• Severely obese (35-40)	207/704 (29.4%)	86/303 (28.4%)	.
• Very severely obese (>40)	109/704 (15.5%)	40/303 (13.2%)	.
BSA (m^2)	(n=704) 2.3 (0.2)	(n=303) 2.3 (0.2)	0.741
Family Medical History			.
Type 2 diabetes	276/704 (39.2%)	123/303 (40.6%)	0.679
Prostate cancer	74/703 (10.5%)	42/303 (13.9%)	0.129
Demographic baseline variables			.
Age (years)	(n=704) 59.5 (6.3)	(n=303) 60.1 (6.4)	0.151
Aboriginal/Torres Strait Islander descent	6/703 (0.9%)	2/303 (0.7%)	0.751
Ethnicity			.
• Australian/New Zealand	453/703 (64.4%)	205/303 (67.7%)	0.400
• European	198/703 (28.2%)	82/303 (27.1%)	.

	T4DM not in study	In Runoff study	P-value
• Other	52/703 (7.4%)	16/303 (5.3%)	.
Antidepressant (SSRI) usage	46/704 (6.5%)	18/303 (5.9%)	0.723
Current smoker	41/703 (5.8%)	12/303 (4.0%)	0.223
Relationship status			.
• Married/De facto	594/703 (84.5%)	263/303 (86.8%)	0.687
• Separated/Divorced	65/703 (9.2%)	21/303 (6.9%)	.
• Widowed	10/703 (1.4%)	4/303 (1.3%)	.
• Single	34/703 (4.8%)	15/303 (5.0%)	.
Occupational status			.
• Employed	359/704 (51.0%)	156/303 (51.5%)	0.987
• Self-employed	131/704 (18.6%)	58/303 (19.1%)	.
• Working without pay in the family business	4/704 (0.6%)	1/303 (0.3%)	.
• Retired or on pension	180/704 (25.6%)	75/303 (24.8%)	.
• Unemployed	30/704 (4.3%)	13/303 (4.3%)	.
Current shift worker	68/703 (9.7%)	23/303 (7.6%)	0.291
Age left school (years)	(n=698) 16.6 (1.4)	(n=302) 16.7 (1.2)	0.126

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Table 2 Matching of Participants after 2 years in T4DM study prior to entry into Runoff Study

	Placebo treated		Testosterone treated	
	Enter Runoff Study	Not in Runoff Study	Enter Runoff Study	Not in Runoff Study
Randomised patients	148	355	155	349
2hr glucose on OGTT (mmol/L)	(n=145) 8.7 (3.4)	(n=268) 8.8 (3.1)	(n=152) 7.6 (2.7)	(n=291) 8.2 (2.7)
Testosterone (nmol/L)	(n=142) 14.7 (5.7)	(n=237) 14.4 (5.2)	(n=148) 17.8 (6.3)	(n=271) 16.0 (6.4)
Waist circumference (cm)	(n=148) 111.7 (12.4)	(n=262) 113.0 (11.5)	(n=154) 109.2 (12.2)	(n=287) 111.7 (13.0)
Weight (kgs)	(n=148) 103.7 (16.8)	(n=262) 103.5 (16.6)	(n=154) 101.4 (16.6)	(n=286) 103.4 (17.8)
BMI (kg/m^2)	(n=148) 33.2 (5.1)	(n=262) 33.2 (5.0)	(n=154) 32.9 (5.2)	(n=286) 33.3 (5.3)

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Table 3 – Participants Perceptions About Their Treatment

	PARTICIPANT PERCEPTIONS			
TREATMENT	Testosterone	Placebo	Unsure	Total
Testosterone	57 (37%)	45 (29%)	53 (34%)	155
Placebo	19 (13%)	74 (50%)	55 (37%)	148
TOTAL	76	119	108	303

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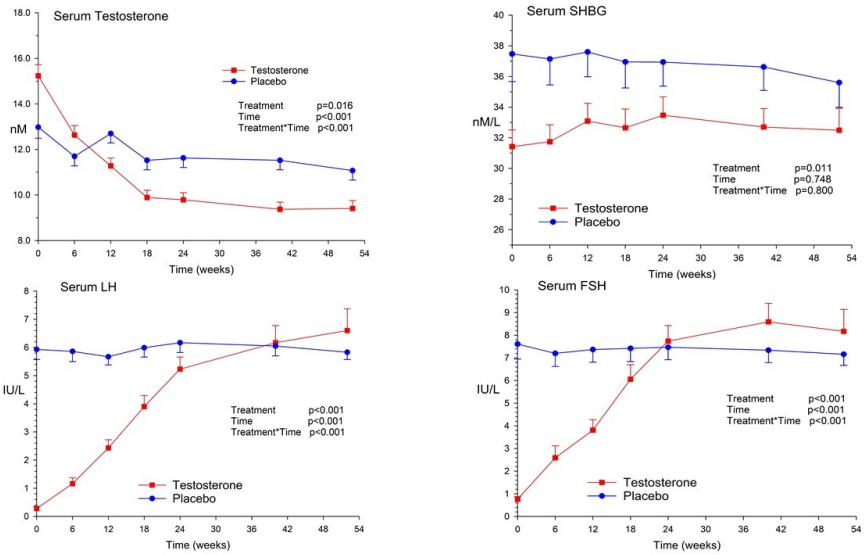

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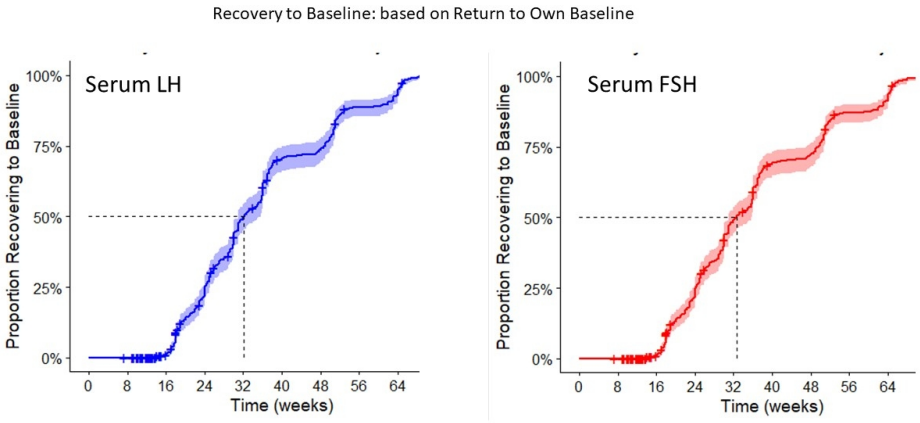

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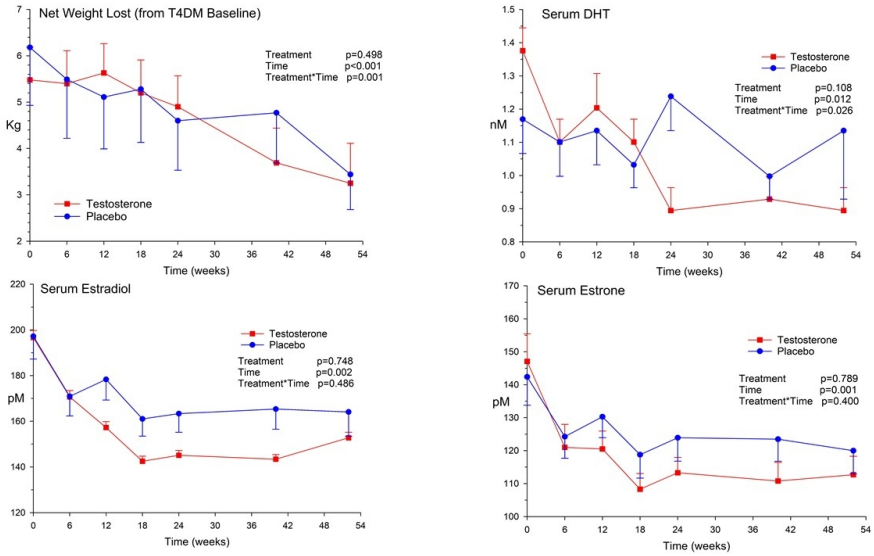

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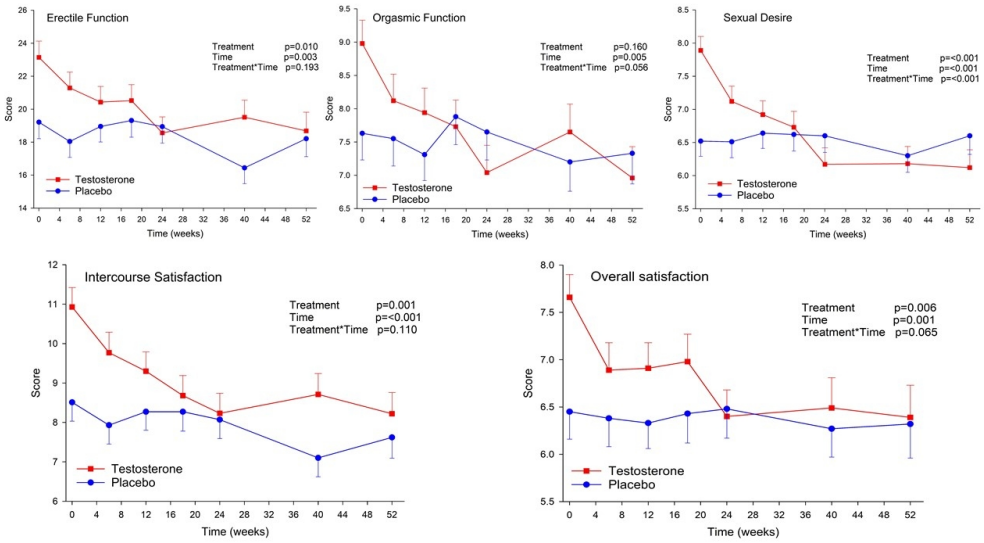

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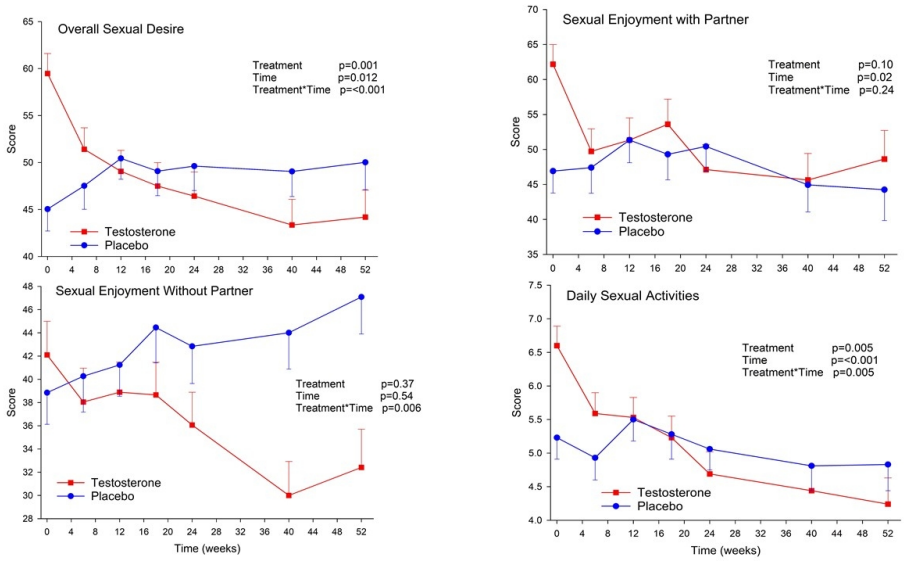

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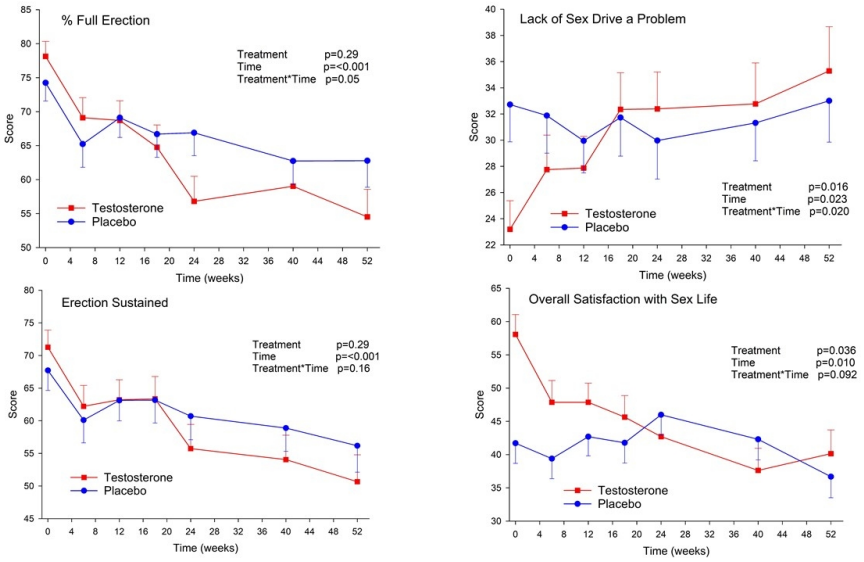

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