Research article

Lean tissue mass and energy expenditure are retained in hypogonadal men with spinal cord injury after discontinuation of testosterone replacement therapy

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Objective: To determine whether favorable changes to lean tissue mass (LTM), resting energy expenditure (REE), and testosterone (T) that occurred with 12 months of physiological testosterone replacement therapy (TRT) were retained 6 months after discontinuing treatment.
Design: Prospective, open-label, controlled drug intervention trial.
Setting: Metropolitan area hospitals.
Subjects: Eugonadal ($n = 11$) and hypogonadal ($n = 13$) men with chronic spinal cord injury (SCI).
Interventions: Hypogonadal subjects received a 5 or 10 mg transdermal T patch daily for 12 months, with adjustment of the dose to normalize the serum T concentration; TRT was discontinued after 12 months (TRT-12M) and subjects were followed for an additional 6 months and re-evaluated (Post-TRT). Total body dual energy X-ray absorptiometry and blood draws were performed at baseline (BL) prior to TRT, TRT-12M, and Post-TRT. Eugonadal subjects did not receive treatment and were evaluated at comparable time points.
Results: There were no significant differences between groups prior to TRT at BL for any of the study endpoints. In the hypogonadal group, a significant increase in LTM was observed from BL to TRT-12M (50.2 ± 7.4 vs. 52.9 ± 6.8 kg, $P < 0.01$), which persisted Post-TRT compared to BL (52.2 ± 7.8 kg, $P < 0.05$). The increase in REE from BL to TRT-12M (1283 ± 246 vs. 1410 ± 250 kcal/day) was also retained at Post-TRT (1393 ± 220 kcal/day). These sustained improvements in LTM and REE after termination of anabolic hormonal therapy may be associated with persistent beneficial effects on health and physical function of hypogonadal men with chronic SCI.

Keywords: Spinal cord injury, Lean tissue mass, Hypogonadism, Testosterone

Introduction

Paralysis after spinal cord injury (SCI) leads to a dramatic loss of sub-lesional lean tissue mass (LTM) during the first year after acute injury; with increasing duration of injury (DOI), declines in supra-lesional compartments are also reported to occur.[1] Even the maintenance of favorable body composition is difficult to achieve because of the inability to engage in normal levels of physical activity, with greater reductions in energy expenditure reported in persons with higher levels of neurological impairment.[2] Disuse atrophy and physical inactivity each contribute to decreased energy expenditure, and without appropriate caloric restriction, result in increased adiposity that may eventually lead to

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DOI 10.1179/2045772314Y.0000000206

adverse metabolic consequences.[3,4] These body composition and metabolic outcomes could negatively affect physical function, health and wellness, social integration, quality of life and may likely be associated with increased cost of care. Dysfunction of the hypothalamic–pituitary–gonadal axis (HPGA) in persons with SCI may reduce levels of serum testosterone (T), which may further result in the adverse composition changes.[5–11] Low serum T levels are observed in 39–46% of men with SCI and percent of low levels are increased for each decade of life compared to the able-bodied male population.[7,12] Serum T concentrations have been reported to be inversely related to DOI.[5,13]

Testosterone replacement therapy (TRT) in able-bodied men results in favorable changes in body composition, with increased LTM and decreased fat tissue mass (FTM).[14,15] In a previous report from our group, 12 months of TRT in hypogonadal men with SCI was demonstrated to be safe and effective at increasing LTM and energy expenditure.[16] As such, in men with chronic SCI, appropriately monitored TRT offers a logical and efficacious approach to treat clinically low T and improve body composition. The question not addressed in our initial report, however, was whether or not the favorable changes persisted once TRT is discontinued. Previous work performed by the administration of anabolic agents in the elderly resulted in conflicting findings with regard to the persistence of the favorable changes on body composition of T and growth hormone administration.[17,18] The primary objective of this report is to describe the changes to LTM and energy expenditure 6 months after discontinuation of TRT; additional participants were added to the treatment group in this study from our prior report.[16]

Materials and methods

Participants

Twenty-four healthy non-ambulatory men (American Spinal Injury Association Impairment Scale (AIS) with sensorimotor impairment (AIS A, B, or C)) between the ages of 18 and 65, >1 year post-SCI, were recruited for participation from SCI Service of the James J. Peters Veterans Affairs Medical Center, Bronx, NY, and the Kessler Institute for Rehabilitation, West Orange, NJ. One subject in the treatment group and two subjects in the control group were lost to follow-up prior to completion of the study. As such, 12 subjects in the treatment group and 9 subjects in the control group completed the entire protocol, including the visit after discontinuation of TRT. Participants were excluded from the study if they had a history of prostate cancer or elevated prostate-specific antigen (PSA ≥ 4.0 μg/l), elevated hematocrit (Hct) $\geq$55%, abnormal liver function tests (LFT; $\geq$2.5 times the upper limit of normal), abnormal digital rectal examination (DRE) revealing prostatic enlargement or possible malignancy, heart and/or vascular disease, acute or chronic illness of any etiology, cancer, significant psychological and/or sleep disorders. The study was approved by the Institutional Review Boards of the James J. Peters VA Medical Center and the Kessler Institute for Rehabilitation. Written informed consent was obtained from each subject prior to study participation.

Procedures

A prospective, controlled, 12-month TRT intervention trial was performed in a cohort of hypogonadal men with SCI. All subjects who received TRT were discontinued from therapy and followed for an additional 6 months, at which time repeated measurements for body composition, energy expenditure, and pertinent laboratory values were obtained. Participants were instructed to maintain their usual diet and physical activity/exercise schedule over the course of the study. The total time for participation in the study for hypogonadal and eugonadal subjects was 24 months. Eugonadal subjects were evaluated for study endpoints in conjunction with their scheduled routine annual medical examinations at baseline, 12 and 24 months. After enrollment, hypogonadal subjects had a 6-month lead-in period to confirm their hypogonadal status prior to the initiation of TRT, 12 months of TRT (TRT-12M), and then a 6-month follow-up observation without TRT (Post-TRT). There were no statistical differences between outcome variables collected at the start of the lead-in period from those obtained immediately prior to initiating TRT; because these lead-in time points were essentially redundant, only a single baseline time point representing testing acquired immediately prior to initiating TRT has been reported. The time point at 24 months from baseline in the eugonadal group was used to compare to the time point at 18 months from baseline in the hypogonadal group (a 6-month lead-in period was only performed in the TRT group).

Of the 24 individuals enrolled, 13 subjects were designated hypogonadal (treatment group) and 11 subjects, eugonadal (control group), as determined by serum total T concentrations. For the purposes of this study, hypogonadism was defined as a serum total T concentration $\leq$11.3 nmol/l, and eugonadal, $\geq$11.4 nmol/l, a threshold value not in accordance with the current

Endocrine Society definition for hypogonadism;[19] this value for low T was also employed in the Baltimore Longitudinal Study of Aging.[20] Our investigation was initiated prior to the publication of the 2006 recommendations by the Endocrine Society,[21] which was the first consensus document to provide guidance on clinical treatment of hypogonadism. All blood collections during the study were performed between 8:00 and 10:00 a.m. For screening purposes, all participants had blood drawn for total T on three separate occasions and the average concentration was used to determine group assignment (e.g., hypogonadal or eugonadal). Because of interruption to somatosensory innervation, somatic symptoms of hypogonadism are often not perceived by persons with SCI, or cannot be differentiated from the consequences of SCI (e.g., depression, decreased libido, and a decreased sense of well-being are not uncommon symptoms in persons with SCI). Thus, there was the absence of the ability to correlate levels of serum T to the presence or absence of symptoms of hypogonadism *per se* as one of the determining factors prior to initiating TRT.

After the 6-month lead-in period, TRT was initiated in the treatment group for 12 months using a daily 5 mg T patch (Androderm, Watson Pharma Inc., Corona, CA). At 6 months, all 13 participants receiving TRT had T concentrations within the expected physiological range. Because of difficulty coordinating bathing and placement of the T patch, the time of placement varied. Subjects were instructed to place the patch on a clean, dry, and shaved, if necessary, area of the skin on the thighs or abdomen. Placement in the evening was recommended but accomplished in only 4 of 13 participants (~21:00 hours), and placement of the patch in the others was performed in the morning (~09:00 hours). After 2 months of TRT, the serum concentration of T was determined 9–12 hours after patch placement. If serum T levels were not within normal physiologic range, the dose was increased to 10 mg (two patches) daily for the remaining 10 months of treatment. After 2 months, 6 of the 13 subjects in the treatment group required titration to a dose of 10 mg daily. To prevent skin irritation, T patch application sites were rotated among various sites anatomically located on the quadriceps and abdomen, with a 10-day interval between repeated uses of the same placement location. Subjects were instructed on the use of a potent topical corticosteroid (e.g., triamcinolone) cream at the application site if skin irritation developed. Patch compliance was monitored and determined by inventory of the number of patches returned each month. The average rate of compliance was 97% (±3.3%) for the entire treatment period. Total body dual energy X-ray absorptiometry (DXA) scans, comprehensive physical examination, and laboratory work were performed at baseline prior to initiating TRT, after TRT-12M, and Post-TRT. Laboratory values and a comprehensive physical examination were also obtained at the 2- and 6-month time point after initiation of TRT and have previously been reported.[16] Subjects in the control group completed the same laboratory work and body composition testing at baseline, 12 and 24 months.

Laboratory analysis

Analysis for serum T and serum PSA were performed by Quest Diagnostics, Inc. (Teterboro, NJ, USA) using the ADVIA Centaur XP Immunoassay Chemiluminescent System according to the manufacturer's specifications (Bayer Healthcare Diagnostics, Bohemia, NY, USA). Hemoglobin (Hb) and hemotocrit (Hct) were obtained from the complete blood count with an ACT 10 Hematology Blood Analyzer (Beckman Coultur, Brea, CA). Lipid profile (total cholesterol (TC), triglycerides (TG), and high density lipoprotein cholesterol (HDL-C) with low density lipoprotein cholesterol (LDL-C) levels estimated using the Friedewald equation),[14] and LFTs (aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltransferase (GGT), alkaline phosphatase (AP), and albumin) were determined in the General Chemistry Laboratory of the VA Medical Center using an ADVIA 1650 automatic chemistry analyzer, following procedures recommended by the manufacturer (Bayer Diagnostics, Newbury, UK).

Dual energy X-ray absorptiometry

Soft tissue body composition was measured in the morning after an overnight fast with subjects wearing the minimum amount of clothing that was appropriate for the procedure. A total body scan was performed in accordance with manufacturer guidelines using fan beam DXA (GE LUNAR Prodigy Advance, software version 11.4 and 12.2, Madison, WI, USA). Total body scans were performed and the energy level (scan mode) used for each total body scan was based on subject thickness (e.g., thin, standard, or thick). To analyze the results of each total body scan, proprietary software algorithms were used to segment the body into trunk, pelvis, and upper and lower extremities using the standard regions of interest. Over the length of the study, as a part of the daily quality assurance, a lumbar spine phantom was scanned >200 times with the coefficient of variation (CV%) determined to be 0.9% (reference value 30% fat). In accordance with ISCD guidelines,[22] total body scans were repeated on

30 individuals with SCI by the 'on-and-off-the-table' method (i.e. subjects were repositioned between scans)[23]; our precision error (RMS − %CV) was calculated to be 1.2% for LTM and 2.2% for FTM.

Energy expenditure

Resting energy expenditure (REE) was measured by indirect calorimetry using dilution technology. A metabolic cart (V-Max Encore 229, CareFusion, Yorba Linda, CA, USA) was used to measure exhaled air from subjects for fractions of mixed expired oxygen (O_2) and carbon dioxide (CO_2; FEO_2 and $FECO_2$). Using the dilution technique, a high-flow pump pulls expired air that is captured by a ventilated plexiglass canopy that covers the head. The flow rate of the pump was determined by the fraction of CO_2 in the air. The pump rate was adjusted by the technician to maintain an $FECO_2$% between 0.5 and 1.0% (room to expired air ratio of 4:1) as the VE fluctuated. A mass-flow sensor was used to measure volume and air flow, and was calibrated before each testing session with a certified 3-l calibration syringe. A measured stroke volume ±3% at various flow rates was required to achieve successful calibration. Gas analyzers were calibrated prior to each testing session by using two calibration gas concentrations (Cal 1: 16% O_2 and 4% CO_2; Cal 2: 26% O_2 and 0% CO_2) and room air (20.94% O_2 and 0.05% CO_2) and calibration was completed when O_2 and CO_2 concentrations were no greater than ±0.05% of the expected values.

Subjects arrived at the laboratory for testing between the hours of 8:00 and 10:00 a.m., following a 12-hour fast, with a minimum of 24 hours free from any type of exercise. Subjects were transferred to comfortable bed and instructed to lie still in a quiet, softly lit room for 20 minutes prior to testing. Data were collected under steady-state conditions defined as ±5% change in respiratory quotient and ±10% change in VO_2 and V_E for 5 consecutive minutes. Predicted basal energy expenditure (BEE) was determined by the Harris-Benedict equation,[24] and the percentage of predicted was calculated as follows: % predicted energy expenditure = REE/BEE × 100. VO_2 and VCO_2 were continuously measured, averaged every 20 seconds, and converted to REE using the abbreviated Weir equation[25]: $REE = VO_2\ (3.941) + VCO_2\ (1.106)$. Calculation of VO_2,[26] VCO_2,[27] and respiratory exchange ratio (RER)[27] were performed, as referenced.

Statistical analysis

Values are expressed as group mean ± SD. Unpaired *t*-tests were performed to identify group (treatment vs. control) differences for demographic (age, height, weight, BMI, DOI, screening serum T), laboratory (T, albumin, PSA, GGT, AST, ALT, AP, TC, HDL-C, LDL-C, TG, Hb, Hct), body composition [weight, LTM (for total, trunk, leg, and arm), FTM (for total, trunk, leg, and arm), total body % fat], and energy expenditure (VO_2, RER, REE, REE/kg, and BEE) continuous variables. To identify interventional changes, a mixed model analysis of variance (ANOVA) with 1 between factor (group: treatment, control) and 1 within factor (time: baseline, TRT-12M, and Post-TRT) was performed to determine the change or the retention of soft tissue body composition and energy expenditure endpoints; the anticipated rise in serum T with TRT and fall in serum T concentration after termination of TRT was also analyzed by this approach. To further characterize significant main and interaction effects, post-hoc paired *t*-tests were performed within groups. An *a priori* level of significance was set at $P \leq 0.05$. Statistical analyses were completed using Statview 5.0 (SAS Institute, Inc., Cary, NC, USA).

Results

Demographic data for subjects in the treatment and control groups are provided (Table 1). The current report that includes follow-up observations after TRT consists of 12 subjects in the treatment group and 9 subjects in the control group; the study herein has one additional subject in the treatment group and two fewer subjects in the control group than our prior report, which describes our observations limited to TRT.[16] Subjects in the treatment group were taller and older ($P < 0.01$) than those in the control group. The groups were similar for weight, BMI, and DOI. No significant (NS) group differences were found among the body composition variables at baseline prior to initiating TRT in the hypogonadal group (Table 2).

Body composition

There were no significant differences between the groups for soft tissue body composition at baseline (i.e., FTM and LTM), regions of interest (i.e., total body, trunk, leg, and arm), and total body % fat (Table 2). For LTM from baseline during TRT, a significant interaction effect ($P = 0.02$), and subsequent post-hoc test revealed that the treatment group had significant gains in total body LTM (+2.7 kg; $P < 0.01$), which persisted 6 months after discontinuation of TRT (+2.0 kg; $P < 0.05$). Significant interaction effects were not achieved in the trunk ($P = 0.09$) or legs ($P = 0.11$). However, in the treatment group the trunk increased during TRT (+1.5 kg; $P < 0.01$) and remained elevated 6 months after discontinuation of TRT (+1.2 kg; $P < 0.05$)

Table 1 Characteristics of the study groups

	Control group ($T \geq 11.4$ nmol/l)	Treatment group ($T < 11.3$ nmol/l)
n	11	13
Screening *T* (nmol/l)	17.4 ± 4.8	9.2 ± 3.1*
Age (years)	35 ± 9	44 ± 6†
Height (m)	1.74 ± 0.04	1.81 ± 0.07†
Weight (kg)	79.6 ± 9.2	82.4 ± 12.5
BMI (kg/m^2)	26.3 ± 3	25.3 ± 3.3
DOI (years)	12.2 ± 8.6	14.5 ± 9.7
Paraplegia/tetraplegia (*n*)	9/2	7/6
Motor complete (*n*)	9	8
Motor incomplete (*n*)	2	5

Abbreviations: SD, standard deviation; T, testosterone; BMI, body mass index; DOI, duration of injury. Data are presented as group mean ± SD.
*P < 0.0001 for treatment vs. control; †P < 0.01 for treatment vs. control.

compared to the baseline values (Fig. 1). The leg compartment had an increased group mean response during TRT (+0.8 kg; P = 0.08), which remained elevated at Post-TRT (+0.4 kg; NS) compared to the baseline values, but neither of these changes achieved statistical significance (Fig. 1). Subjects in the control

Table 2 Soft tissue body composition and energy expenditure findings at baseline, after TRT, and discontinuation of TRT

					P value		
		Baseline	TRT-12M	Post-TRT	Group	Visit	Interaction
Weight (kg)	Treatment	82.6 ± 12.9	83.7 ± 14.8	84.1 ± 15.5	–	–	–
	Control	82.8 ± 6.3	82.9 ± 7.8	81.3 ± 8.5	–	–	–
LTM (kg)					–	–	–
Total body	Treatment	50.2 ± 7.4	52.9 ± 6.8*	52.2 ± 7.8§	–	–	0.02
	Control	52.7 ± 8.6	50.9 ± 6.7	51.0 ± 7.3	–	–	–
Trunk	Treatment	24.6 ± 4.0	26.1 ± 3.7*	25.8 ± 4.3§	–	0.02	0.09
	Control	25.3 ± 3.2	25.2 ± 3.1	25.7 ± 3.7	–	–	–
Leg	Treatment	14.7 ± 2.7	15.5 ± 2.9†	15.1 ± 3.5	–	–	0.12
	Control	14.2 ± 3.8	13.9 ± 3.4	13.9 ± 3.2	–	–	–
Arm	Treatment	7.5 ± 2.2	7.8 ± 2.0†	7.6 ± 2.2	–	0.05	–
	Control	8.0 ± 1.7	8.2 ± 1.7	7.8 ± 1.4	–	–	–
FTM (kg)					–	–	–
Total body	Treatment	29.3 ± 9.7	27.7 ± 10.9	28.8 ± 11.2	–	–	0.10
	Control	27.2 ± 6.9	29.1 ± 6.7‡	27.4 ± 7.9	–	–	–
Trunk	Treatment	16.8 ± 5.8	16.0 ± 6.6	16.8 ± 7.1	–	–	0.09
	Control	14.6 ± 3.4	16.6 ± 4.3‡	15.7 ± 4.2	–	–	–
Leg	Treatment	8.9 ± 3.3	8.5 ± 3.6	8.6 ± 3.6	–	–	–
	Control	8.7 ± 2.7	8.6 ± 2.5	8.2 ± 3.3	–	–	–
Arm	Treatment	2.5 ± 1.0	2.3 ± 1.1	2.5 ± 1.1	–	–	0.09
	Control	2.9 ± 1.3	2.9 ± 1.3	2.6 ± 1.4‡	–	–	–
Total body % fat	Treatment	35.1 ± 8.6	32.2 ± 9.1	33.5 ± 9.1	–	–	0.05
	Control	33.8 ± 7.4	34.3 ± 7.0	33.6 ± 8.2	–	–	–
VO_2 (ml/kg/min)	Treatment	2.28 ± 0.31	2.46 ± 0.35	2.49 ± 0.38	–	0.10	–
	Control	2.26 ± 0.19	2.31 ± 0.32	2.38 ± 0.57	–	–	–
RER	Treatment	0.86 ± 0.10	0.92 ± 0.14	0.84 ± 0.10	–	0.01	–
	Control	0.91 ± 0.12	0.91 ± 0.11	0.85 ± .08	–	–	–
REE (kcal/day)	Treatment	1283 ± 246	1410 ± 250	1393 ± 220	–	0.04	0.17
	Control	1341 ± 105	1350 ± 137	1378 ± 214	–	–	–
REE/kg (kcal/kg/day)	Treatment	27.2 ± 3.4	26.6 ± 3.3	26.9 ± 3.3	–	–	–
	Control	25.8 ± 3.3	26.8 ± 3.6	27.3 ± 3.8	–	–	–
BEE (kcal/day)	Treatment	1753 ± 182	1760 ± 188	1761 ± 201	–	–	–
	Control	1794 ± 107	1827 ± 176	1786 ± 136	–	–	–
BEE (% pred)	Treatment	73 ± 10	79 ± 11	79 ± 10	–	0.08	–
	Control	75 ± 6	74 ± 7	77 ± 10	–	–	–

Abbreviations: TRT-12M, testosterone replacement therapy for 12 months; Post-TRT, discontinued testosterone replacement therapy for 6 months; kg, kilograms; LTM, lean tissue mass; FTM, fat tissue mass. RER, respiratory exchange ratio; REE, resting energy expenditure; REE/kg, REE per kilogram of LTM; pBEE, predicted basal energy expenditure; % pred, percent predicted data are expressed as group mean ± SD. Data are expressed as group mean ± SD. P-value represents significant group main effects. Change from baseline in treatment group: *P < 0.01; §P < 0.05; †P = 0.08; change from baseline in the control group: ‡P ≤ 0.05.

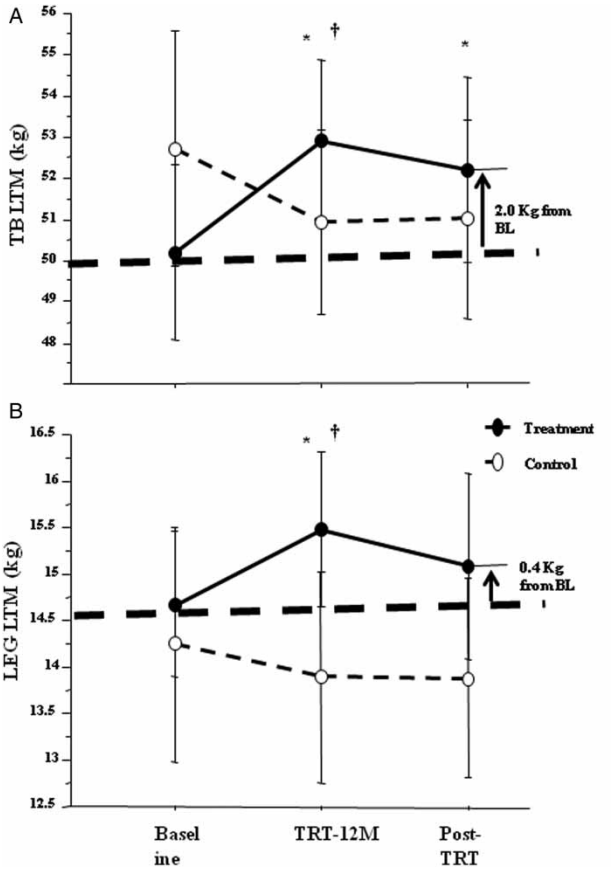

Figure 1 Increase of LTM in the (A) total body (TB) and (B) leg after 12 months of T replacement therapy (TRT-12M), and retention of LTM in TB and leg 6 months after discontinuation of TRT (Post-TRT). The darker circles represent the treatment group and the lighter circles, the control group. *P < 0.05 for change from baseline, †P < 0.05 for treatment vs. control.

group remained consistent throughout the trial or had marginal decreases from baseline to 12 or 24 months.

For FTM, there were no significant group or visit main effects, suggesting no change between groups over the intervention. A significant interaction effect ($P = 0.045$) was observed for total body % fat. Total body % fat in the treatment group was reduced during treatment and remained low after Post-TRT compared to that in the control group, whose values remained relatively consistent over 24 months. Trends toward a significant interaction effect for FTM was observed in the total body ($P = 0.10$), trunk ($P = 0.09$), and arm ($P = 0.09$) (Table 2).

Energy expenditure

The groups were not significantly different at baseline for energy expenditure (Table 2). Significant visit main effects were observed for RER ($P < 0.01$) and REE ($P < 0.05$). Post-hoc analysis revealed a significant change ($P < 0.05$) from baseline to month 12 for each variable in the treatment group, but not in the control group. In the treatment group, REE at Post-TRT was not statistically different from TRT-12M, but was significantly elevated ($P < 0.05$) from the baseline level (Table 2). To corroborate this treatment and retention effect on energy expenditure, similar patterns (e.g. increases from baseline to month 12, no significant change between TRT-12M and Post-TRT, and Post-TRT levels remained elevated compared to baseline) were observed in the treatment group for VO_2 and % predicted energy expenditure.

Laboratory and adverse effects

By study design at baseline, serum T concentrations in the hypogonadal group were statistically and clinically lower than that in the control group ($P < 0.01$). With TRT, serum T concentration increased into the normal range in the treatment group ($P < 0.01$), but remained unchanged in the control group. At Post-TRT, serum T concentrations decreased significantly from the month 12 to baseline value ($P < 0.01$; Table 3). In the treatment group, serum HDL-C level increased at month 12 ($P < 0.05$) and remained at a similarly higher concentration at Post-TRT (Table 3). In both groups, there were no additional significant differences in lipoprotein parameters at baseline or over the course of the study, and all other values remained within the normal range. The hepatic panel, Hb, Hct, and PSA values were within the normal range and did not change significantly in either group over the course of the study. There were no statistical differences in mean laboratory values between the treatment and control groups, except for AST ($P < 0.05$), which was greater in the treatment group, but values remained within normal limits. There were no abnormal DRE findings noted in either group at baseline or during the duration of the study (Table 3).

In the treatment group, there were minor adverse events that were possibly or probably related to the TRT intervention. Two of the 13 subjects developed minor skin irritations that resolved with the use of corticosteroid cream, and one subject developed body acne that resolved during continued treatment. An additional four subjects developed mild lower extremity edema that was continually monitored and clinically managed by the use of compression stockings. In one subject, elevated serum LFTs (e.g. ALT and GGT) were noted at month 12 that then returned to within normal limits at Post-TRT; no other abnormal LFTs were observed in this or other subjects. One treatment subject had elevated Hb and Hct levels at month 12, which returned

Table 3 Results of laboratory values and digital rectal exams at baseline, after TRT, and discontinuation of TRT

Laboratory	Normal range	Group	Baseline	TRT-12M	Post-TRT	P value		
						Group	Visit	Interaction
Total T (nmol/l)	11.4–27.8	Treatment	9.8 ± 2.2	15.2 ± 8.5*	9.9 ± 3.1**	0.006	0.02	–
		Control	16.0 ± 4.0	17.6 ± 6.0	16.5 ± 4.4	–	–	–
Albumin (g/l)	0.3–0.55	Treatment	0.41 ± 0.03	0.41 ± 0.02	0.42 ± 0.03	–	–	–
		Control	0.39 ± 0.03	0.42 ± 0.03	0.41 ± 0.03	–	–	–
PSA (μg/l)	0–4.0	Treatment	1.2 ± 0.85	1.4 ± 0.93	1.0 ± 0.58	–	–	–
		Control	1.4 ± 1.1	1.32 ± 0.71	1.1 ± 1.1	–	–	–
Hepatic panel						–	–	–
GGT (IU/l)	0–51	Treatment	38 ± 36	38 ± 40	28 ± 13	–	–	–
		Control	18 ± 7	18 ± 8	20 ± 10	–	–	–
AST (IU/l)	0–41	Treatment	29 ± 7†	27 ± 6	27 ± 7	–	–	0.06
		Control	22 ± 6	26 ± 10	24 ± 8	–	–	–
ALT (IU/l)	30–50	Treatment	28 ± 11	28 ± 12	27 ± 10	–	–	–
		Control	19 ± 12	22 ± 10	21 ± 12	–	–	–
AP (IU/l)	30–115	Treatment	83 ± 26	82 ± 32	84 ± 21	–	–	–
		Control	64 ± 19	61 ± 15	71 ± 15	–	–	–
Lipid panel						–	–	–
TC (mmol/l)	2.95–6.22	Treatment	4.43 ± 0.83	4.32 ± 0.83	4.63 ± 0.78	–	–	–
		Control	4.40 ± 0.83	4.45 ± 0.93	4.33 ± 0.93	–	–	–
HDL-C (mmol/l)	>0.9	Treatment	0.99 ± 0.26	1.12 ± 0.23*	1.11 ± 0.19	–	0.03	–
		Control	0.90 ± 0.22	0.99 ± 0.20	0.99 ± 0.14	–	–	–
LDL-C (mmol/l)	0–3.4	Treatment	2.77 ± 0.62	2.46 ± 0.70	2.79 ± 0.57	–	–	–
		Control	2.90 ± 0.85	2.69 ± 0.75	2.59 ± 0.75	–	–	–
TG (mmol/l)	0.25–1.80	Treatment	1.48 ± 0.60	1.27 ± 0.69	1.60 ± 0.96	–	–	–
		Control	1.39 ± 0.87	1.64 ± 0.78	1.74 ± 1.25	–	–	–
Hb (g/l)	1.32–1.71	Treatment	1.32 ± 0.22	1.37 ± 0.28	1.35 ± 0.19	–	–	–
		Control	1.31 ± 0.20	1.36 ± 0.11	1.25 ± 0.36	–	–	–
Hct (%)	38.5–50	Treatment	39.0 ± 6.3	41.0 ± 7.7	40.1 ± 4.9	–	–	–
		Control	39.9 ± 3.4	39.2 ± 2.7	39.1 ± 5.1	–	–	–
Abnormal DRE		Treatment	0	0	0	–	–	–

Abbreviations: DRE, digital rectal exam; TRT-12M, testosterone replacement therapy for 12 months; Post-TRT, discontinued testosterone replacement therapy for 6 months; PSA, prostate specific antigen; GGT, gamma glutamyltransferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AP, alkaline phosphatase; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; Hct, hematocrit. Data are expressed as group mean ± SD. P-value represents significant group main effects. Change from baseline in treatment group: *$P < 0.05$. Change from 12 months in treatment group: **$P < 0.05$. †$P < 0.05$ for treatment vs. control.

to within normal limits Post-TRT. There were no reports of mood fluctuations, depression, or other feelings of anxiety during the course of TRT. Similarly, there were no adverse events reported after T was discontinued.

Discussion

This report, which has expanded upon our prior findings,[16] demonstrated in hypogonadal men with chronic SCI that discontinuation of TRT resulted in an expected fall in serum T to pre-treatment levels, but what was not anticipated was the retention of LTM 6 months after discontinuation of TRT. Because clinical trials are designed to test a therapeutic intervention, and not usually designed to describe endpoints after discontinuation of a treatment modality, there have been a limited number of clinical trials that have addressed the retention of favorable changes in body composition induced by TRT.[17,18] Sattler *et al.* recruited 108 community-dwelling elderly men (71 ± 4 years) who were placed on transdermal T and recombinant growth hormone therapy for 16 weeks with subsequent evaluation at week 28; in those subjects who had attained serum T levels in the 'youthful' range on TRT and who also had improvements in body composition measures above for LTM or below for FTM of these respective group medians at study week 17, significant retention of favorable changes in body composition persisted at week 28[17]; no additional time points were evaluated in this study for retention of favorable changes after 28 weeks. Thus, the Sattler *et al.* study raises the possibility that if the decline in physical function in the frail elderly can be sufficiently reversed, then the gains in muscle mass and strength may be retained by improved functional capacity, breaking the downward cycle of ever increasing weakness and debility. In contrast, in 242 frail elderly men administered TRT for 6 months (74 ± 6 years), O'Connell *et al.* [18] failed to find retention of favorable changes in body composition 6 months after stopping anabolic therapy, without any interim measurements obtained 6–12 months after discontinuation of the hormonal intervention. It should be

appreciated that the pathophysiology of the frail elderly is distinct from that of a stable, relatively young individuals with SCI, which includes those participants in our study who were 33–49 years old. Despite the return of serum T concentration in our SCI treatment cohort to baseline levels 6 months after discontinuation of TRT, the beneficial changes to body composition and energy expenditure persisted for 6 months after discontinuation of treatment. If the TRT-induced gains in muscle mass are attained in younger persons with SCI, then it may be quite possible to consolidate, and possibly build upon, these gains by the associated hormonally induced improvements in level of energy, mood, and functional capacity. In addition, a multi-disciplinary approach that incorporates appropriate diet and exercise paradigms during and after the TRT may be speculated to offer the possibility of having the greatest and most enduring impact on body composition and functional outcomes.

TRT has been shown to have positive effects on body composition, with associated increases of LTM and decreases of FTM.[28–30] Details of the effects of TRT on soft tissue body composition changes that led to overall weight gain have been previously reported.[16] It should be noted that the magnitude of this change in body soft tissue was the result of increased total body LTM (+2.7 kg), which, by comparison with the anabolic treatment cohort in the Sattler *et al.*[17] article, would place our SCI participants in the upper end of the 'high responder' group – that is, those most likely to retain their beneficial changes in body composition at least 3 months after discontinuation of the anabolic intervention. The increase observed in LTM of the trunk and legs was an unexpected finding because of the presence of muscle paralysis and immobility in the lower extremities and portions of the trunk. The presence of increased and retained REE in the treatment group 6 months after discontinuing TRT compared to the baseline value serves to further confirm the positive and persistent changes observed to LTM, whereas if REE had not been concomitantly increased, then an argument may have been reasonable advanced that extracellular water had confounded our measurement.[31] The implications of increasing LTM and energy expenditure in the paralyzed portion of the body was not determined in our current study, but perhaps such soft tissue body composition changes may reduce the propensity for abnormal metabolic changes,[9,32] a possibility that was not able to be assessed with any sense of certainty in our current study. Our data suggest a trend for reduction in total body percent fat, but absolute changes in FTM after TRT were not consistent or significant, possibly due to the limited size and the inherent diversity of our study sample.

Positive correlations between serum HDL-C and T concentrations have been observed in an able-bodied cohort.[33] A recent systematic review and meta-analysis of 51 studies suggested that TRT results in a small decrease in the level of serum HDL-C (−0.49 mg/dl; 95% CI, 1.35–5.01), as well as an increase in hemoglobin concentration (0.3.18%; 95% CI, −0.85 to −0.13).[34] Our treatment group had an approximate 13% improvement in mean serum HDL-C concentration during TRT that persisted after discontinuation of TRT, despite T concentrations reverting to baseline levels; it should be appreciated that over the same time interval the control group had a 10% improvement in mean serum HDL-C concentration. Because the difference in mean serum HDL-C between the T and control groups from baseline to the time point after discontinuation of TRT for 6 months was essentially negligible, it would not be prudent or appropriate, in the authors' opinion, to further discuss the potential beneficial effects of TRT on serum HDL-C in our report. Persons with SCI have been reported to have an increased risk for coronary artery disease[35–37] and serum HDL concentrations are frequently depressed, sometimes markedly.[38,39] In the general population, the association between low T and increased risk of cardiovascular disease is currently being established.[40] In consideration of the elevated prevalence of low serum T concentration in men with SCI[7] and the aforementioned risk for cardiac-related disease,[35–37] it may be prudent to consider screening for depressed serum T levels in the routine evaluation of men with chronic SCI. In our relatively limited cohort, the average hemoglobin concentration was not changed during or after discontinuation of TRT.

Extended longitudinal investigations in persons with SCI are difficult to perform because of frequent acute illness, access to transportation, and, as in the able-bodied population, compliance with medication and follow-up visits. These considerations contributed to our unbalanced study design, with a few subjects terminating participation prior to the final study time point. Although salutary changes to body composition, energy expenditure, and possibly serum HDL-C concentration resulted from TRT in our SCI cohort, the translation and/or relevance of these findings as they may relate to functional gain, mood, strength, and sexual function could not be addressed with any degree of certainty in this report. Compared to similar treatment trials in the general population, our prospective clinical trial was relatively small and this limited our ability to

address other endpoints of interest. As such, future randomized, controlled clinical trials should be performed to confirm our findings, as well as extend them to relevant clinical outcomes. Similarly, to maximize further the changes observed in soft tissue body composition by administration of TRT (e.g. favorable and opposite changes in lean and fat tissue mass), the possibility may be considered of concomitantly employing appropriate nutritional counseling and/or exercise training.

Conclusion

Twelve months of clinician-supervised TRT in hypogonadal men with chronic SCI was safe and effective with beneficial changes observed to body composition, energy expenditure, and, possibly, serum HDL-C levels. Six months after discontinuation of TRT, when serum T concentration returned to pre-treatment levels, beneficial changes persisted to LTM, energy expenditure, and, possibly, serum HDL-C concentration. Larger prospective randomized-controlled trials in hypogonadal men with SCI are required to determine whether TRT is safe and effective at maintaining concentrations of T in the normal physiological range with associated potential favorable outcomes of improved carbohydrate and lipid metabolism, higher functional performance, heightened mood, greater independence, and enhanced social integration.

Disclaimer statements

Contributors All authors had significant contributions to the study design, performance, data analysis/interpretation, manuscript development and approval of the final document.

Funding Veteran Affairs Rehabilitation Research and Development Service (#B9212-C, #B4162-C, and #B2468-C) and the James J. Peters VA Medical Center. Clinical Trial Registration Number: NCT00266864.

Conflicts of interest None.

Ethics approval Clinical Trial Registration Number: NCT00266864. The study was approved by the respective regulatory committees at the James J. Peters VA Medical Center, and the Kessler Institute for Rehabilitation.

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