Research Article

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Cirrhosis

Testosterone therapy increases muscle mass in men with cirrhosis and low testosterone: A randomised controlled trial

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Background & Aims: Low testosterone and sarcopenia are common in men with cirrhosis and both are associated with increased mortality. Whether testosterone therapy in cirrhosis improves muscle mass and other outcomes is unknown.

Methods: We conducted a 12-month, double-blinded, placebo-controlled trial of intramuscular testosterone undecanoate in 101 men with established cirrhosis and low serum testosterone (total testosterone <12 nmol/L or free testosterone <230 pmol/L) in a single tertiary centre. Body composition was assessed using dual-energy X-ray absorptiometry at baseline, 6 and 12 months.

Results: At study completion, appendicular lean mass was significant higher in testosterone-treated subjects, with a mean adjusted difference (MAD) of +1.69 kg, (CI +0.40; +2.97 kg, $p = 0.021$). Secondary outcomes included a substantially higher total lean mass in the active group (MAD +4.74 kg, CI +1.75; +7.74 kg, $p = 0.008$), matched by reduced fat mass (MAD −4.34 kg, CI −6.65; −2.04, $p < 0.001$). Total bone mass increased (MAD +0.08 kg, CI +0.01; +0.15 kg, $p = 0.009$) as did bone mineral density at the femoral neck (MAD +0.287 points, CI +0.140; +0.434, $p < 0.001$). Haemoglobin was higher with testosterone therapy (MAD +10.2 g/L, CI +1.50; +18.9 g/L, $p = 0.041$) and percentage glycosylated haemoglobin (HbA1c) lower (MAD −0.35%, CI −0.05; −0.54, $p = 0.028$). Mortality was non-significantly lower in testosterone-treated patients (16% *vs.* 25.5%, $p = 0.352$). There was no increase in adverse events in testosterone-treated subjects.

Conclusion: Testosterone therapy in men with cirrhosis and low serum testosterone safely increases muscle mass, bone mass and haemoglobin, and reduces fat mass and HbA1c. This is the first evidence-based therapy for sarcopenia in cirrhosis and thus requires larger-scale investigation into its potential impact on mortality.

Lay summary: Both low testosterone and muscle wasting are associated with increased risk of death in men with severe liver disease. Administering testosterone to men with liver disease who have low testosterone levels significantly increases their muscle mass. In addition, testosterone has non-muscle beneficial effects which may be able to increase survival in this population.

Clinical trial number: Australian New Zealand Clinical Trials Registry trial number ACTRN 12614000526673.

Introduction

Serum testosterone is reduced in up to 90% of men with cirrhosis [1,2]. Sarcopenia is similarly common in cirrhosis, with an estimated prevalence of 40–70%. This prevalence appears to be greater in men, at 50–70%, as compared to 20% in women [3–5]. Both low testosterone and sarcopenia have been associated with increased mortality in men with cirrhosis, independent of the Model for End-stage Liver Disease (MELD) score [5]. Reversal of sarcopenia in an observational cohort has been associated with improved survival [6], and thus it is logical to anticipate a mortality-benefit if we can treat sarcopenia in cirrhosis. However, there are currently no interventions supported by randomised controlled data to increase muscle mass in this population. Testosterone is known to increase muscle mass and reduce body fat in both hypogonadal and eugonadal men [7,8], but it cannot be assumed that the same is true in cirrhosis, due to multiple other factors contributing to the pathogenesis of sarcopenia in this population [9].

Previous studies investigating the potential therapeutic effects of testosterone therapy in men with cirrhosis have been limited by such issues as inclusion of women and eugonadal men, variable follow-up durations, and inappropriate drug delivery such as oral administration, with inconclusive findings [10–13]. A Cochrane review of testosterone therapy in men with liver disease included a high proportion of non-cirrhotic subjects with alcoholic hepatitis who ceased alcohol ingestion during the study period, and thus the failure to identify benefit is uninterpretable [14]. Previous studies have not examined effects on muscle mass, which has only relatively recently been identified as an important prognostic factor in cirrhosis.

Keywords: Cirrhosis; Sarcopenia; Liver disease; Muscle.
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Abbreviations: MAD, mean adjusted difference; APLM, appendicular lean mass; HbA1c, percentage glycosylated haemoglobin; MELD, model for end-stage liver disease; DEXA, dual-energy X-ray absorptiometry; SHBG, sex hormone binding globulin; INR, international normalised ratio; LH, luteinising hormone; TUG, timed-up-and-go; SF36, short form 36; ADAM, androgen deficiency of the ageing male; IPAQ, international physical activity questionnaire; IQR, interquartile range.

We hypothesised that testosterone treatment can improve muscle mass in men with cirrhosis and low testosterone levels, and therefore conducted a randomised placebo-controlled trial of appropriately delivered intramuscular testosterone confined to men with established cirrhosis and low testosterone levels, to examine the effects of testosterone in this population.

Patients and methods

This was a randomised, double-blinded, placebo-controlled clinical trial of testosterone undecanoate in men with cirrhosis and low baseline testosterone conducted between May 2013 and November 2015 at a single tertiary referral centre. All patients provided signed informed consent. The trial was approved by the Austin Hospital Human Research Ethics Unit and registered with the Australian New Zealand Clinical Trials Registry (ACTRN 12614000526673) after enrolment of the first patient due to a clerical error. No analysis was conducted prior to registration and there were no alterations in study outcomes following initial patient enrolment. All authors had access to study data and approved the final manuscript. An independent external consultant endocrinologist had access to unblinded trial data during the course of trial to ensure there were no major safety concerns, and all adverse events were immediately reported to the Research Ethics Unit.

Patients

Men with cirrhosis of any aetiology were recruited from general hepatology and pre-liver transplant outpatient consultations. The diagnosis of cirrhosis was made on a combination of clinical, biochemical, radiological and histological features. Supportive radiological findings included a nodular liver contour with signs of portal hypertension, biochemical findings included thrombocytopenia, low albumin and raised international normalised ratio (INR), and suggestive clinical findings were spider naevi, caput medusa or ascites. In those patients with histology, cirrhosis was defined as Metavir grade 4 fibrosis. In patients with equivocal findings, cirrhosis was diagnosed by a valid transient elastography reading of >15 kPa with other supporting features or >20 kPa without. This cut-off is higher than that quoted by the manufacturer to avoid the inadvertent inclusion of non-cirrhotic patients.

Low testosterone was defined as total testosterone <12 nmol/L as measured by hospital immunoassay, or Vermeulen-calculated free testosterone <230 pmol/L [15,16]. These levels were chosen because they correspond to the lower limits of normal reported for healthy young men [16]. Patients were required to have 2 eligible morning samples prior to trial enrolment. Only men below the age of 70 years were included due to the recognised age-related reduction in testosterone levels [17]. Patients were required to be up-do-date with standard surveillance investigations including abdominal ultrasound prior to study entry.

Exclusion criteria included hepatocellular carcinoma or other known malignancy, prostate disease, known hypersensitivity to testosterone therapy, polycythaemia (haematocrit >55%), uncontrolled hypertension (>160/90 mmHg despite treatment), uncontrolled obstructive sleep apnoea, severe renal dysfunction (estimated glomerular filtration rate <30 ml/min), uncontrolled epilepsy, migraine, or significant cardiac insufficiency (New York Heart Association class III or IV). Given that drug administration was intramuscular, patients with a platelet count below 30×10^9/L were excluded, as were patients taking warfarin. Enoxaparin therapy was not an exclusion if the indication for its use allowed it to be withheld on the day of trial drug administration.

Patients who were eligible at baseline but subsequently developed any of the exclusion criteria during the trial period were withdrawn from the trial but included in intention-to-treat analyses.

Trial design

This was a 54-week trial conducted at the Austin Hospital, Melbourne, Australia, with visits at 0, 6, 18, 30, 42 and 54 weeks. Recruitment took place between May 2013 and October 2014 and the final follow-up visit was November 2015. All patients continued routine clinical care throughout the study including receiving advice on exercise, protein intake, and late night snacks to minimise protein catabolism.

Randomisation and intervention

Eligible participants were randomly assigned in a concealed 1:1 allocation to either testosterone or placebo stratified by Child Pugh Class (A, B or C) at the block level, using permuted blocks with a block size of 4. The randomisation sequence was generated by a statistician and implemented by the Austin Health clinical trials pharmacy. Pharmacists, trial investigators and participants were blinded to intervention allocation. Intramuscular testosterone undecanoate 1000 mg or a visually identical placebo injection (4 ml volume, both in oily base) was injected into the upper, outer quadrant of the buttock at 0, 6, 18, 30 and 42 weeks according to manufacturer recommendations.

Sample size determination

The sample size is based on an estimate of normal appendicular lean mass (APLM) of 28.7 ± 4.5 kg [18]. As the cirrhotic cohort was expected to be sarcopenic their mean APLM was assumed to be 1 standard deviation below the normal (24.2 ± 3.8 kg). To be likely not to miss a difference at 12 months of 2.4 kg (10%) between the two groups, we arrived at a size of 40 subjects required in each group, using a two sided *t* test, significance level set at 0.05 and power of 0.8. To allow for 20% dropout by 6 months, we aimed to recruit 100 patients.

Main outcome measures

The primary outcome was APLM by dual-energy x-ray absorptiometry (DEXA); the sum of lean mass of the arms and legs, which correlates well with total body muscle mass by magnetic resonance imaging [18]. APLM was chosen as the primary outcome as it measures functionally important muscles, and is not confounded by the presence of ascites.

Secondary outcome measures included total body lean mass, fat mass, bone mass (in kg) and bone density at the femoral neck and lumbar spine.

Measurement of body composition

Body composition was measured using DEXA (Prodigy Version 13.6 GE Lunar, Madison, WI) at baseline, 6 and 12 months. DEXA body composition is highly accurate and reproducible, and provides a breakdown of each body compartment into lean mass, fat mass and bone mass [19]. It has been specifically validated in cirrhotic patients [20]. The coefficient of variation for lean mass in healthy subjects is 0.7% [21].

DEXA was chosen in preference to computerised tomography (CT) at the 3rd lumbar vertebrae due to technical difficulties in repeating the CT scan at the exact same transverse level for accurate comparison. Slight variations in transverse slice level drastically alter the measurement of muscle area, and thus confound results.

Haematology and biochemical parameters

Blood was taken for analysis at each trial visit. Serum testosterone was measured using the access testosterone assay (Beckman Coulter, Inc, Fullerton, CA, USA, minimum detection limit of 0.35 nmol/L, reference range 10–27.6 nmol/L) [22]. Free testosterone was calculated according to Vermeulen's formula by mass-action equation using total testosterone and SHBG [15]. Serum estradiol was measured using the Cobas® immunoassay (Roche diagnostics, Indianapolis, USA, reference range 28–156 pmol/L). SHBG levels were determined with the Immulite 2000 analyser (Diagnostics Products Corporation, Los Angeles, CA, USA, reference range 13–71 nmol/L).

Other biochemistry was measured using standard methodologies at the Biochemistry Department, Austin Health. This included full blood examination, serum creatinine and electrolytes, INR, liver function, alfa-fetoprotein, ammonia and prostate specific antigen. Given the known association between hypogonadism and insulin resistance [23] we measured glycosylated haemoglobin (HbA1c) as a measure of glucose homeostasis.

Clinical parameters

Handgrip strength of the dominant hand was measured using a calibrated digital Jamar® hand dynamometer at baseline, 0 and 6 months and averaged over 3 successful attempts. This measure has been previously shown to be a useful

prognostic marker in cirrhosis [24]. The timed-up-and-go (TUG) was performed to assess mobility, with a normal value of less than 10 seconds. The TUG has been shown to be prolonged in cirrhotics experiencing higher rates of falls [25].

A history and clinical examination was performed at each visit to assess the presence and severity of gynecomastia, ascites and hepatic encephalopathy, and a thorough review of medical records was conducted to assess for such complications in the 12 weeks between study visits. Number of hospital admissions and admission for infection (according to the North American Consortium for the Study of End-Stage Liver Disease (NACSELD criteria)) were recorded [26].

Quality of life questionnaires

Quality of life was measured using the short form 36 (SF36 form), which evaluates both physical and mental quality of life [27]. The short international physical activity questionnaire (IPAQ) relies on patient self-reporting to quantify the average weekly energy expended [28]. The androgen deficiency in the ageing male (ADAM) questionnaire is a sensitive but not specific method of identifying symptoms of androgen deficiency [29]. These questionnaires were performed at baseline, 6 months and 12 months.

Statistics

Descriptive statistics are shown as median plus interquartile range (IQR). Comparison of baseline characteristics was based on Wilcoxon rank-sum test or chi-squared test with Yates' correction for continuity in case of categorical variables. In case of low frequencies, Fisher's exact test was used. No adjustments were made for multiple comparisons on explanatory variables.

Differences in outcomes between the treatment and placebo group were assessed using a linear mixed model fitted by restricted maximum likelihood [30]. Fixed effects included baseline values of the variable assessed, treatment group (testosterone *vs.* placebo as a categorical variable), categorical time points, as represented by three visits at 0, 30 weeks (referred to as 6 months), and 54 weeks (referred to as 12 months), and the interaction term of visit x treatment group. Repeated measure by subject was added as a random effect. The interaction term was of particular interest, representing the change across groups over time. We show the mean adjusted difference (MAD) and report its profiled 95% confidence interval between the groups from baseline to 6 months, and baseline to 12 months, and the overall *p*-value for follow-up. This follows the intention-to-treat protocol, including all randomised subjects who were enrolled in the trial.

A sensitivity analysis was performed including only patients that completed the study and adhered to protocol. As a measure of change, we used the unadjusted median (IQR) observed difference and tested for significance between groups by Wilcoxon rank-sum test. All tests were two-tailed with $p < 0.05$ denoting statistical significance. Analyses were conducted using R for Mac version 3.2.2 with the added packages JGR 1.7-18, Deducer 0.7-7 and lme4 1.1-10 [31].

Results

Patients receiving testosterone and placebo were well matched with no significant differences at trial commencement including MELD score and muscle mass (Table 1). Median age was 55.0 years [51.0; 60.0] and most men were caucasian (91.1%). The median MELD score was 14 [10; 17]. 19.8% of patients were classified as Child-Pugh A, 35.6% Child-Pugh B and 44.6% Child-Pugh C. At baseline, 52.5% of patients overall had suffered recent (within 3 months) moderate or severe hepatic encephalopathy (56% of the testosterone group, 49% of the placebo group), and 34.7% of subjects required recent abdominal paracentesis for ascites (34% testosterone, 35% placebo).

The commonest aetiology of liver disease was alcohol abuse, with 30.7% of cases attributed to alcohol alone and a further 8.9% to the combination of alcohol and hepatitis C (HCV) infection. HCV alone was responsible for 28.7% of cases, non-alcoholic fatty liver disease 12.9%, primary sclerosing cholangitis 6.9%, hepatitis B 5% and the remainder due to other causes. There were no significant differences in aetiology between the two groups, including history of alcohol abuse (Table 1).

101 patients entered the trial; 50 on active therapy and 51 on placebo. 66 reached 6 months (34 on active treatment, 32 on placebo) and 47 reached 12 months (22 on active treatment, 25 on placebo). There were 21 deaths at 12 months (8 on testosterone therapy (16%) and 13 on placebo (25.5%). This reduced mortality on testosterone treatment was not statistically significant ($p = 0.352$). There were 15 transplants, 8 on testosterone (16%) and 7 on placebo (13.7%), $p = 0.967$ (Supplementary Fig. 1).

Other dropouts were for the following reasons: compliance issues [10], development of hepatocellular carcinoma [5], pain at injection site [2], diagnosis of lymphoma [1] diagnosis of sleep apnoea [1] and patient perception of lack of effect [1] (Supplementary Fig. 1). There was no significant difference in numbers of dropouts on testosterone therapy *vs.* placebo therapy ($p = 0.91$).

Impact on testosterone levels

Testosterone treatment increased total testosterone levels (MAD +22.9 nmol/L (CI +18.8; +27.0 nmol/L) at 6 months and +19.3 nmol/L (CI +14.6; +24.0 nmol/L), $p < 0.001$ at 12 months, as well as free testosterone (MAD +345 pmol/L (CI +278; +413 pmol/L) at 6 months and +319 pmol/L (CI +241; +396 pmol/L) at 12 months, $p < 0.001$).

Estradiol levels also increased on testosterone therapy (MAD +105 pmol/L (CI +26; +184 pmol/L) at 6 months and +145 pmol/L (CI +54; +236 pmol/L) at 12 months, $p = 0.003$). The estradiol-to-testosterone ratio was lower in actively treated patients which reflects a predominance of androgen effect (MAD −60.6 pmol/L/nmol/L (−103.5; −17.6) at 6 months and −39.7 pmol/L/nmol/L (−89.0; +10.3) at 12 months, $p = 0.019$). LH was suppressed in testosterone-treated patients (median 7.3 IU/L [3.9, 9.9] at baseline reduced to median 0.1 IU/L [0.1, 0.1] at 12 months, whereas LH rose in patients on placebo (median 5.6 IU/L [3.5, 8.7] rose to median 6.4 IU/L [4.7, 11.9] at 12 months.

Primary outcome

APLM was significantly higher in the active group than the placebo group at both 6 and 12 months. The MAD was +1.13 kg at 6 months (CI +0.014; +2.24 kg) and +1.69 kg (CI +0.40; +2.97 kg), $p = 0.021$ overall. MAD results for APLM and other variables are displayed in Table 2 (intention-to-treat analysis). The separation in APLM is demonstrated in Fig. 1. When adding the presence of ascites as a categorical binary covariate to the mixed model the APLM result was not confounded.

Secondary outcomes: Body composition

There was an increase in total body lean mass at both 6 and 12 months in the testosterone-treated group compared to placebo. The effect size was substantial, with the median difference in total lean mass reaching +4.74 kg at 12 months ($p = 0.008$) (Fig. 2A; Table 2A). Total fat mass was significantly lower in the active group compared to placebo, with the fat mass reduction (−4.34 kg, $p < 0.001$) approximately matching the lean mass gain at 12 months (Fig. 2B; Table 2A). As a result, body mass index (BMI) did not significantly change over the 12 months (MAD −0.21 kg/m^2 at 12 months, $p = 0.80$).

Total bone mass increased in testosterone-treated compared to placebo subjects during the study period (MAD +0.08 kg (CI

Table 1. Baseline demographics by treatment group.

	Overall (n = 101)	Active (n = 50)	Placebo (n = 51)	*p* value	n
Age (years)	55.0 [51.0;60.0]	55.5 [52.0;60.0]	54.0 [50.0;59.0]	0.612	101
Caucasian, n (%)	92 (91.1%)	45 (90.0%)	47 (92.2%)	0.741	101
Alcohol, n (%)	40 (39.6%)	20 (40.0%)	20 (39.2%)	1.000	101
SHBG (nmol/L)	88.0 [53.0;119]	90.0 [61.0;127]	76.0 [47.0;107]	0.066	101
Testosterone (nmol/L)	9.10 [2.80;14.7]	9.25 [3.92;17.0]	9.10 [2.70;12.7]	0.431	101
Free T, (pmol/L)	104 [51.0;164]	113 [42.8;164]	90.0 [52.0;164]	0.870	101
LH (IU/L)	6.20 [3.60;9.60]	7.30 [3.92;9.88]	5.60 [3.45;8.65]	0.115	101
Estradiol (pmol/L)	158 [114;221]	148 [119;198]	170 [111;227]	0.354	94
Spironolactone, n (%)	65 (64.4%)	33 (66.0%)	32 (62.7%)	0.894	101
BMI (kg/m^2)	28.8 [26.0;32.5]	29.3 [25.8;32.7]	28.1 [26.2;32.1]	0.699	101
Gynecomastia, n (%)	66 (67.4%)	32 (64.0%)	36 (70.6%)	0.731	101
Grip strength (kg)	33.0 [27.2;39.7]	31.8 [27.2;37.5]	33.9 [26.9;40.5]	0.506	100
TUG (seconds)	10.0 [8.00;13.0]	9.25 [8.00;12.0]	11.0 [8.00;13.0]	0.233	101
Haemoglobin (g/L)	119 [102;140]	115 [101;139]	121 [102;140]	0.711	101
Sodium (mmol/L)	136 [132;140]	138 [133;140]	136 [131;140]	0.239	101
Albumin (g/L)	30 [25; 33]	30 [26; 32]	29 [24; 33]	0.860	101
Vitamin D (nmol/L)	69.0 [46.0;102]	64.5 [37.2;101]	80.0 [51.0;108]	0.201	99
MELD score	14.0 [10.0;17.0]	13.0 [9.00;17.0]	15.0 [11.0;17.5]	0.296	101
Child Pugh score	9.00 [7.00;11.0]	9.00 [7.00;10.0]	9.00 [7.50;11.0]	0.409	101
APLM (kg)	24.3 [21.5;27.7]	24.5 [21.7;27.0]	24.0 [21.4;28.0]	0.844	101

SHBG, sex hormone binding globulin; T, testosterone; LH, luteinising; BMI, body mass index; TUG, timed-up-and-go; Hb, haemoglobin; MELD, model for end-stage liver disease; APLM, appendicular lean muscle.

+0.02; +0.14 kg), $p = 0.009$ overall. This translated to a statistically significant increase in femoral neck T score (MAD +0.287 points, CI +0.140; +0.434, $p < 0.001$) and a trend to increased T score at the lumbar spine (+0.22 points, CI +0.04; +0.411, $p = 0.063$) (Table 2B).

Secondary outcomes: Muscle function and quality of life

Grip strength was higher in patients on testosterone therapy ($p = 0.07$) suggesting an association. TUG was non-significantly lower in actively treated patients (Table 2B) and the physical activity component of the quality of life questionnaire (SF36-PCS) was non-significantly higher in actively treated patients ($p = 0.394$, Table 2B). There were no differences in the mental health component of the SF36, the androgen deficiency questionnaire (ADAM) or the physical activity (IPAQ) scores.

Secondary outcomes: Haematology and biochemistry

Haemoglobin (Hb) levels increased significantly on testosterone treatment compared to placebo (MAD +10.2 g/L at 12 months (CI +1.50; +18.9 g/L), $p = 0.041$. There were no occurrences of polycythaemia (Table 2). At 12 months, the HbA1c was significantly lower in actively treated patients than placebo (MAD −0.35%, CI −0.54; −0.05), $p = 0.028$ (Table 2B).

The prostate specific antigen rose slightly on treatment, with the MAD +0.04 (CI −0.21; +0.30) at 12 months, $p = 0.003$ overall. One patient (on placebo) developed new lower urinary tract symptoms requiring insertion of an indwelling urinary catheter. There was no significant difference in any other haematological or biochemical parameter including serum alpha-fetoprotein, circulating ammonia, sodium, albumin, coagulation profile and platelet count.

Secondary outcomes: Clinical outcomes

20% of all men suffered major infection during the study period. 16% of men on testosterone suffered major infection as compared to 23.5% on placebo but this was not statistically significant, $p = 0.696$. There were no significant differences in hospitalisation rate, severity of ascites or hepatic encephalopathy, or change in either the Child-Pugh or the MELD score during the study period. As above, mortality was non-significantly lower in testosterone-treated subjects (16% *vs.* 25.5%, $p = 0.352$).

Sensitivity analysis

A per protocol analysis of study completers was performed (Table 3). Results were similar to intention-to-treat analysis. The MAD in APLM (n = 45) was +1.56 kg at 6 months (CI +0.14; +2.97 kg) and +1.78 kg at 12 months (CI +0.37; +3.20 kg), $p = 0.029$ overall. This sensitivity analysis confirms the significant effect of testosterone therapy on APLM and other body composition and haematological parameters.

The dropout rate of this study was high, and patients who dropped out were sicker than study completers (Table 4). Although MELD score was higher in patients who dropped out, the difference in APLM in active patients did not correlate with MELD score (correl +0.045, $p = 0.323$) and thus severity of liver disease did not impact on the anabolic effect of testosterone. Similarly, difference in APLM did not correlate with severity of testosterone deficiency (correl +0.162, $p = 0.323$).

Safety

There were no cardiovascular events in either group, and no infections, haematomas, or bleeding at injection site in any patient. Moderate injection site pain (impacting on daily activi-

Table 2. Mean adjusted difference (MAD) in active patients as compared to placebo for body composition outcomes at 6 and 12 months, which represents intention-to-treat analysis (A). MAD in secondary outcomes at 6 and 12 months (B).

A

	MAD at 6 months	MAD at 12 months	*p* value overall
APLM (kg)	+1.13 (CI +0.014 to +2.24)	+1.69 (CI +0.40 to +2.97)	0.021
Total lean mass (kg)	+2.23 (CI -0.38 to +4.85)	+4.74 (CI +1.75 to +7.74)	0.008
Total fat mass (kg)	-3.24 (CI -5.24 to -1.24)	-4.34 (CI -6.64 to -2.04)	<0.001
Total bone mass (kg)	+0.08 (CI +0.02 to +0.14)	+0.08 (CI +0.01 to +0.15)	0.009

B

	MAD at 6 months	MAD at 12 months	*p* value overall
Femoral T score (points)	+0.258 (CI +0.130 to +0.386)	+0.287 (CI +0.140 to +0.434)	<0.001
Lumbar T score (points)	+0.11 (CI -0.06 to +0.27)	+0.22 (CI +0.04 to +0.411)	0.063
Handgrip strength (kg)	+2.69 (CI +0.19 to +5.19)	+2.30 (CI -0.43 to +5.03)	0.072
TUG (seconds)	-0.95 (CI -2.02 to +0.11)	-0.27 (CI -1.27 to +0.74)	0.220
Hb (g/L)	+7.33 (CI -0.25 to +14.9)	+10.2 (CI +1.50 to +18.9)	0.041
HbA1c (%)	+0.13, (CI -0.15 to +0.41)	-0.35 (CI -0.68 to -0.02)	0.028
SF36-PCS (points)	+0.23 (CI -4.47 to +4.95)	+3.55 (CI -1.69 to +8.77)	0.394

APLM, appendicular lean mass; TUG, timed-up-and-go; Hb, haemoglobin; HbA1c, percentage glycosylated haemoglobin; SF36-PCS, short form survey physical activity component.

ties) was observed in 2 patients on placebo therapy who requested to be withdrawn from the study. No patients required analgesia. 2 patients receiving enoxaparin therapy had enoxaparin withheld the day of trial drug administration, with no adverse effects experienced. There was no increase in adverse events in the testosterone-treated group (Table 5). Importantly there was no difference in hepatocellular cancer incidence or new portal vein thrombosis, and no patients developed polycythaemia or prostate disease. Of the 21 deaths, 2 were Child-Pugh A patients (infection; sub-arachnoid haemorrhage), 8 were Child-Pugh B and 11 were in Child-Pugh C patients; all of which were liver failure-related deaths.

Discussion

Sarcopenia is now recognised as an important independent predictor of adverse outcome in patients with liver cirrhosis [5–7]. We have shown for the first time that in cirrhotic men with low testosterone levels, testosterone therapy significantly increased muscle mass as assessed by quantification of both appendicular and total lean body mass. We also demonstrated reduced fat mass and increased bone density. Remarkably, these anabolic effects of treatment on muscle and bone were observed in a relatively short time period and were not influenced by the severity of liver disease or the severity of testosterone deficiency at baseline. Although this therapy is only applicable to men, sarcopenia is far less common in cirrhotic women, and the associated increased mortality risk has only been reported for men [3].

As far as we are aware this is the first RCT of a targeted therapy in cirrhosis which has successfully increased muscle mass. The ability of testosterone therapy to increase muscle mass in this setting is notable given the multiple factors that contribute to sarcopenia in cirrhosis. These include malnutrition, portal hypertension, elevated inflammatory mediators, reduced insulin-like growth factor-1 (IGF-1), myostatin upregulation [32] and a shift to muscle protein breakdown for energy use as a result of reduced hepatic glycogen synthesis and storage [33–37]. Testosterone specifically targets androgen receptors in existing muscle cells to promote growth and in satellite cells to trigger differentiation into new myocytes [38], but it may also act on other pathways including down-regulation of myostatin [39] and upregulation of IGF-1 [40], which may contribute to its efficacy in cirrhosis. Serial muscle biopsies however would be required to investigate the molecular mechanisms of testosterone action, which would confer excessive risk in decompensated liver disease.

Given the strong association between sarcopenia and the risk of mortality in patients with cirrhosis, it is likely that therapies that increase muscle mass can improve survival. Data to support this hypothesis come from an uncontrolled study in patients with portal hypertension who had undergone a transjugular intrahepatic portosystemic shunt (TIPS) procedure for the purpose of controlling ascites. This study showed that survival was significantly greater in those whose muscle mass increased as compared to those whose did not [6]. We observed a lower mortality rate in testosterone-treated patients (16% *vs.* 25.5%) but this did not reach significance as this trial was not designed or powered to evaluate this outcome.

In addition to the potential mortality-benefit of attenuating sarcopenia, the non-muscle effects of testosterone therapy observed in our study could positively influence long–term outcomes. We observed a significant increase in haemoglobin in an anaemic cohort without causing any polycythaemia, and in other populations, anaemia has been associated with increased all-cause mortality [41]. We also showed that testosterone replacement reduced HbA1c which is a surrogate marker for insulin resistance, which is in keeping with trials in other hypogonadal populations demonstrating reduced insulin resistance with testosterone therapy [42]. Reducing insulin resistance may reduce liver fibrosis progression and reduce the incidence of hepatocellular carcinoma in our population [43,44].

The positive impact of testosterone therapy on bone mineral density was particularly important given that there are no therapies that have been demonstrated to increase in BMD in cirrhotic

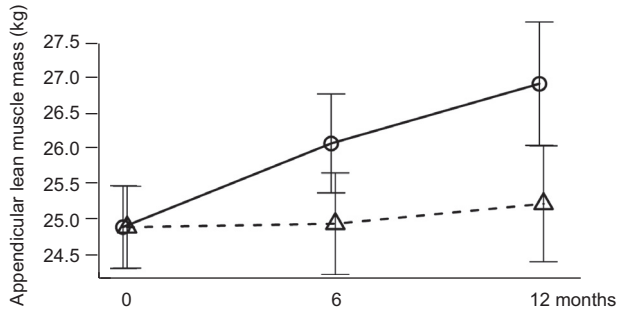

Fig. 1. Mean adjusted difference in appendicular lean mass. Appendicular lean mass progressively increased in the testosterone therapy group, while remaining stable in the placebo group. ○, testosterone therapy; Δ, placebo.

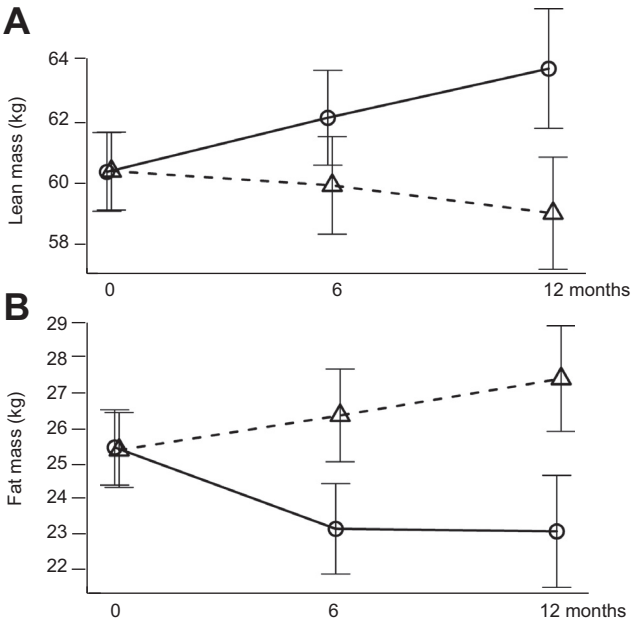

Fig. 2. Whole body composition changes. (A) Mean adjusted difference in total lean and fat mass. Total lean mass progressively increased in the testosterone therapy group, while decreasing in the placebo group. ○, testosterone therapy; Δ, placebo. (B) Mean adjusted difference in fat mass. Fat mass was progressively reduced in the testosterone therapy group, while increasing in the placebo group. ○, testosterone therapy; Δ, placebo.

populations, although our group has previously shown that bisphosphonates are effective post-liver transplant [45]. In cirrhosis bone health is impaired due to multiple factors, including vitamin K and D deficiency, hyperbilirubinemia, elevated osteoprotegerin, low insulin-like growth factor 1 levels and hypogonadism [46]. Testosterone therapy is known to increase bone mineral density in other populations of hypogonadal men [47,48]. This effect may be mediated by estradiol, and thus would not be observed if non-aromatisable androgens were to be administered [49,50]. This trial was not powered to assess fracture risk, which is ultimately the goal of any intervention in bone health. Larger and longer trials are needed to investigate the fracture event rate in cirrhotics.

Our study overcomes limitations of previous trials of testosterone therapy in men with liver disease. It included only patients with established cirrhosis as opposed to alcoholic hepatitis, which comprised the majority of patients included in the previous negative Cochrane review [14]. Intramuscular delivery removes the risk of potentially harmful oral formulations of testosterone [51]. In addition, our study included only men with low baseline testosterone levels. The inclusion of women and eugonadal men in previous studies likely underestimated the beneficial effects of testosterone [10,11]. The single trial to assess the effects of testosterone on muscle strength in hypogonadal cirrhotic men demonstrated an increase in handgrip strength [12], but there was no control group for comparison. The current study is the only placebo-controlled trial in hypogonadal men with cirrhosis, and the only trial to comprehensively examine body composition.

We acknowledge that this trial had a relatively short treatment duration. However, if anything this may have led to an underestimation of the effect size. The observed increase in APLM of 1.69 kg did not return all active patients to a "normal" value of 28.7 kg, but muscle mass appeared to increase progressively throughout the trial and thus with longer treatment duration we may have observed further benefit. Similarly, for bone mass, the ceiling of the testosterone effect may not have been reached. Furthermore, the use of calculated free testosterone for recruitment (as opposed to the gold standard equilibrium dialysis), which may have reduced accuracy in the context of altered albumin and SHBG levels [52], may have resulted in inclusion of subjects with relatively mild testosterone deficiency. However, the lack of correlation between baseline testosterone level and change in APLM suggests that testosterone therapy was similarly effective regardless of the baseline testosterone profile.

The high dropout rate in this trial was not surprising in an advanced cirrhotic cohort, however this reduced the available data for analysis. Despite this, both the intention-to-treat and the per protocol analyses yielded the same, statistically significant results, and thus we believe our findings are robust and reproducible. The dropout rate however means the trial was underpowered to reliably assess effects on muscle function, but the data suggest a positive treatment effect ($p = 0.07$). Handgrip testing requires patient effort and cooperation which could vary measurements in a cohort prone to encephalopathy. The data are in fact similar to testosterone trials in non-cirrhotic men, where lean mass typically improves more consistently than muscle strength [53]. It is also important to note that there is as yet no "gold standard" for quantifying muscle mass. We chose APLM as DEXA scanning, although highly reproducible, cannot differentiate between muscle and water. In this RCT, addition of ascites as a binary covariate in the mixed model demonstrated that it did not impact on the change in APLM, and thus fluid status did not appear to affect results.

Finally, the relatively small trial size means that we cannot exclude the possibility of any potential rare side effects of testosterone therapy in this population, such as increased hepatocellular carcinoma (HCC) risk. Superseded 17α-alkylated formulations of testosterone had indeed been associated with liver tumours [54] but reassuringly there have been no reports of HCC from current formulations and no increase in HCC incidence in previous trials of testosterone therapy in cirrhosis [14].

In conclusion, this trial demonstrates for the first time that testosterone therapy can safely increase muscle mass in men with cirrhosis who have low baseline testosterone levels and thus represents the first evidence-based therapy for sarcopenia in cirrhosis. Testosterone therapy in this setting also has anabolic effects on bone mineral mass, and reduces total fat mass. Testosterone therapy increases haemoglobin and reduces HbA1c; both

Table 3. Median changes of parameters from baseline to end-of-trial in study completers (n = 47), which represents per protocol analysis.

	All	Testosterone	Placebo	*p* value
APLM (kg), n = 45	0.59 [-0.65;2.13]	+1.69 [0.81;2.49]	-0.05 [-0.89;0.61]	0.014
Lean mass (kg), n = 45	0.54 [-1.83;4.06]	+3.43 [0.54;5.34]	-0.77 [-2.07;0.89]	0.017
Fat mass (kg), n = 45	1.05 [-3.04;4.06]	-2.42 [-5.27;1.56]	1.62 [0.97;6.26]	0.008
Bone mass (kg), n = 45	0.02 [-0.10;0.11]	+0.03 [-0.05;0.13]	-0.01 [-0.14;0.06]	0.092
Lumbar T score, n = 45	0.00 [-0.20;0.30]	0.10 [-0.10;0.40]	-0.10 [-0.30;0.22]	0.105
NOF T score, n = 45	-0.10 [-0.30;0.20]	0.10 [-0.10;0.30]	-0.20 [-0.41;0.10]	0.006
Hb (g/L), n = 47	2.00 [-3.00;16.0]	5.00 [1.00;17.0]	0.00 [-5.25;11.2]	0.055
HbA1c (%), n = 42	0.00 [-0.40;0.20]	-0.25 [-0.68;0.10]	0.00 [-0.30;0.30]	0.130

Results displayed as median [95% confidence interval]. APLM, appendicular lean mass; NOF, neck of femur; Hb, haemoglobin; HbA1c, percentage glycosylated haemoglobin.

Table 4. Comparison of median baseline parameters between study completers and non-completers.

	Non-completers	Study completers	*p* value
MELD score	15.0 [12, 19]	12 [9, 15]	0.002
Child Pugh score	10 [8, 11]	8 [6, 10]	0.001
Total testosterone	6.5 [2.0, 13.9]	11.2 [6.3, 17.2]	0.038
Serum sodium	134 [131, 138]	139 [135, 141]	0.006
APLM	23.4 [21.2, 27.3]	24.9 [21.9, 27.9]	0.314
Testosterone treatment	52%	47%	0.76

Table 5. Adverse outcomes between the two groups were well matched.

	Testosterone	Placebo	*p* value
Cardiovascular event	0	0	1
Moderate/severe injection site pain	0	2 (4%)	0.49
Haematoma	0	0	1
Lower urinary tract symptoms	0	1 (2%)	1
Obstructive sleep apnoea	1 (2%)	0	0.47
Hepatocellular carcinoma	3 (6%)	2 (4%)	0.678
New clot	2 (4%)	1 (2%)	0.617
Mortality	8 (16%)	13 (25.5%)	0.352
Transplantation	8 (16%)	7 (13.7%)	0.967
Major infection	8 (16%)	12 (23.5%)	0.696

likely to be advantageous in advanced cirrhosis. Further larger-scale trials are required to quantify the impact of these beneficial outcomes of testosterone therapy on mortality and other clinical endpoints including infection risk and hospitalisation.

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Conflict of interest

Mathis Grossmann has received research funding from Bayer Schering, Novartis, Weight Watchers, Lilly and speaker's honoraria from Besins Healthcare. Marie Sinclair, Paul Gow, Peter Angus and Rudolf Hoermann have no conflicts of interest to declare pertaining to this work.

Authors' contributions

Marie Sinclair, Mathis Grossmann, Peter Angus and Paul Gow contributed to planning of the trial, drafting and finalising of the manuscript. Day-to-day trial proceedings were conducted by Marie Sinclair. Dr Rudolf Hoermann provided statistical support

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhep.2016.06.007.

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