Endocrine CONNECTIONS

RESEARCH

A potent liver-mediated mechanism for loss of muscle mass during androgen deprivation therapy

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Abstract

Context: Androgen deprivation therapy (ADT) in prostate cancer results in muscular atrophy, due to loss of the anabolic actions of testosterone. Recently, we discovered that testosterone acts on the hepatic urea cycle to reduce amino acid nitrogen elimination. We now hypothesize that ADT enhances protein oxidative losses by increasing hepatic urea production, resulting in muscle catabolism. We also investigated whether progressive resistance training (PRT) can offset ADT-induced changes in protein metabolism.
Objective: To investigate the effect of ADT on whole-body protein metabolism and hepatic urea production with and without a home-based PRT program.
Design: A randomized controlled trial.
Patients and intervention: Twenty-four prostate cancer patients were studied before and after 6 weeks of ADT. Patients were randomized into either usual care (UC) ($n = 11$) or PRT ($n = 13$) starting immediately after ADT.
Main outcome measures: The rate of hepatic urea production was measured by the urea turnover technique using $^{15}N_2$-urea. Whole-body leucine turnover was measured, and leucine rate of appearance (LRa), an index of protein breakdown and leucine oxidation (Lox), a measure of irreversible protein loss, was calculated.
Results: ADT resulted in a significant mean increase in hepatic urea production (from 427.6 ± 18.8 to 486.5 ± 21.3; $P < 0.01$) regardless of the exercise intervention. Net protein loss, as measured by Lox/Lra, increased by $12.6 \pm 4.9\%$ ($P < 0.05$). PRT preserved lean body mass without affecting hepatic urea production.
Conclusion: As early as 6 weeks after initiation of ADT, the suppression of testosterone increases protein loss through elevated hepatic urea production. Short-term PRT was unable to offset changes in protein metabolism during a state of profound testosterone deficiency.

Introduction

Sarcopenia, the age-related loss of muscle mass and function, presents an escalating public health burden, contributing to increased falls, disability and loss of functional independence (1). In men, testosterone is critical for maintaining muscle mass and function, bone mass and body composition (2), and its gradual decline with aging plays an important role in the development of sarcopenia (1, 3). Testosterone levels begin to decrease from the third decade of life, with the prevalence of hypogonadism of approximately 20% in men over the age of 60 years, reaching 50% in men over 80 years (4, 5). In older men, testosterone replacement increases lean body mass (LBM), reduces fat mass (FM) and improves physical function (2). Androgen deprivation therapy (ADT) used for the treatment of prostate cancer suppresses testosterone to castrate levels. This results in a rapid loss of muscle mass far exceeding that of normal aging (6, 7). Thus, ADT offers a unique model to study the effects of hypogonadism on muscle and its role in the pathogenesis of sarcopenia, as well as providing insight into the physiological actions of testosterone in maintaining body composition in healthy older men.

We previously showed that testosterone has a protein anabolic effect on the liver (8, 9), with the recent discovery that the urea cycle may be the intra-hepatic pathway mediating this process (10). Administration of testosterone in hypogonadal men significantly reduced the rate of hepatic urea production, paralleled by a reduction in protein loss (10). This effect precedes any changes in muscle mass, indicative of a direct effect on the hepatic urea cycle by testosterone.

Progressive resistance training (PRT) is a key therapy in the treatment of sarcopenia, contributing to significant improvements in muscle mass and strength (11). Similarly, PRT in prostate cancer patients on ADT can mitigate adverse changes in body composition (12). A supervised exercise program instituted at the start of ADT has been found to preserve appendicular lean mass and prevent gains in FM over 3 months (13). Thus, PRT initiated at the start of ADT may prevent its adverse effects on muscle mass and function.

Thus, the primary aim of this study was to investigate whether an increase in hepatic urea production is a determining factor in mediating protein oxidative losses under conditions of profound testosterone withdrawal during ADT. The secondary aim was to determine whether the introduction of PRT at the start of ADT can mitigate changes in protein metabolism, thus offsetting its accelerated effects on muscle catabolism.

Subjects and methods

Subjects

Twenty-four men with prostate cancer scheduled to receive conventional ADT with gonadotrophin-releasing hormone (GnRH) analogs were recruited from the Crown Princess Mary Cancer Centre, Westmead Hospital and the Blacktown Cancer and Haematology Centre, Blacktown Hospital, Australia. Inclusion criteria included men aged between 50 and 80 years with histologically confirmed prostate cancer of early or locally advanced stage or metastatic disease with bone involvement only (≤5 sites of metastases) and Eastern Cooperative Oncology Group (ECOG) 0 performance status. Exclusion criteria were concurrent chemotherapy or anti-androgen therapy, previous ADT within the last 12 months or any musculoskeletal, cardiovascular or neurological disorders which prevent participants from undertaking upper and lower limb exercises. This study was approved by the Western Sydney Local Health District Human Research Ethics Committee. The study was conducted in accordance with the principles of the Declaration of Helsinki. All participants gave written informed consent. The study was registered with the Australian and New Zealand Clinical Trials Registry (ACTRN12616001311448).

Study design

In this prospective study, 24 men were studied at baseline and after 6 weeks of ADT. The main endpoint measurement was rate of hepatic urea synthesis. Other endpoint measurements included (1) whole-body leucine rate of appearance (LRa) and oxidation (Lox), which are indices of whole-body protein turnover and oxidative losses, respectively (2) energy expenditure, (3) body composition and (4) other biochemical markers, including serum levels of testosterone, urea, sex hormone-binding globulin (SHBG) and prostate-specific antigen (PSA). Participants were studied after an overnight fast in the Blacktown Clinical School and Research Centre, Australia. At each visit fasting blood samples were collected at 08.30–09.00h, placed on ice and plasma and serum were separated and stored at −80°C until analysis.

To determine whether exercise would offset the negative effects of ADT on protein metabolism, participants were randomly assigned to two arms: PRT or UC using a computer random assignment program. Participants randomized to the UC group received no intervention. All participants maintained standard

medical care for the treatment of prostate cancer and were instructed to maintain their usual activity and dietary patterns throughout the intervention period.

PRT intervention

Participants assigned to the PRT group undertook 6 weeks of home-based resistance training starting immediately after their first ADT injection. Resistance training was performed three times per week, with 8–10 exercises targeting the major muscle groups using adjustable dumbbells or body weight loading (calisthenics). Patients performed three sets per exercise with 8–12 repetitions maximum per set. The difficulty of each number of calisthenics exercise and/or the loading was advanced with strength adaptation (14). One week of exercise supervision (two sessions) was provided at baseline to instruct patients in proper lifting techniques and loading progressions. Online instructional videos and a printed training manual was provided for each exercise. Compliance to exercise was recorded in a training log book by the participants. Overall activity level of all participants was monitored by physical activity questionnaires and total step count for 1 week (via a pedometer) prior to their study visit.

Methods

Protein turnover

Whole-body protein metabolism was measured using the leucine turnover technique as previously described (10). In brief, after an overnight fast, an intravenous (IV) priming dose of $NaH^{13}CO_3$ was followed by a primed constant 3-h IV infusion of 1-$[^{13}C]$ leucine (Cambridge Isotope Laboratories, Woburn, MA, USA). On each visit, blood and breath samples were collected before and during the leucine infusion. α-ketoisocaproic acid (KIC) was used as a surrogate marker of leucine as it more accurately reflects the intracellular environment (15). KIC was extracted from plasma as described by Nissen *et al*. (16) and plasma KIC enrichment with ^{13}C was measured by gas chromatography-mass spectrometry (GCMS; MSD 5971A, Hewlett-Packard Co., Palo Alto, CA, USA). CO_2 enrichment with ^{13}C in breath samples was measured at University of Surrey UK on a Delta Plus XP isotope ratio mass spectrometer fitted with a Gas Bench II inlet system (Thermo Fisher Scientific). Leucine is either oxidized or re-incorporated into protein, and the fractional partitioning between these two pathways of disposal is determined from the fraction of infused isotope that appears in breath. Rates of leucine appearance (LRa) and leucine oxidation (Lox)

were calculated as previously described (17). Based on our previous experiences, coefficients of variation (CVs) for LRa and Lox are 3.5 and 6.1% respectively (18).

Hepatic urea production

The rate of urea production was measured by the urea turnover technique using stable isotope methodology with $^{15}N_2$-Urea as tracer, as described in detail previously (10). In brief, after an overnight fast, an IV priming dose of $^{15}N_2$-urea was given, followed by a continuous IV infusion of the tracer for 4h. On each visit, blood samples were collected before and during the primed infusion, when steady state was reached. Plasma was separated immediately and stored at −80°C until analysis. $[^{13}C, {}^{15}N_2]$-urea was added to plasma samples as an internal control, and samples were prepared for analysis as previously reported (10). Enrichments of $[^{15}N_2]$ and $[^{15}N_1]$-urea were determined by GCMS (MSD 5971A, Hewlett-Packard Co.). Rate of hepatic urea production is an inverse measure of isotopic enrichment of $[^{15}N_2]$-urea in blood and was calculated as a product of the rate of urea infusion and the trace-to-tracee ratio. Based on our experiences, day-to-day variation in urea production is 5.5%, inter-assay CV 3.5% and intra-assay CV 1.8%.

Energy expenditure

Whole-body energy expenditure and substrate oxidation were measured by indirect calorimetry. This involved using an open-circuit ventilated hood system (ParvoMedics, Sandy, UT, USA), calibrated against standard gases before each study. The participants rested on a bed for at least 30 min. A clear plastic hood was then placed over their head for a 20-min period. The measurements were taken during two 20-min periods and averaged.

Body composition

LBM and FM were assessed by dual x-ray absorptiometry (DXA; GE Healthcare Lunar Prodigy Pro) and Bioelectrical Impedance Spectroscopy (BIS) using the ImpediMed Ltd SFB7 analyzer (ImpediMed Ltd, Qld, Australia). (19) Change in body cell mass (BCM), a functional component of lean body mass, was estimated by subtracting extracellular water (ECW) from LBM.

Assays

All samples for any individual were measured in the same assay run for each analyte. Serum SHBG, total testosterone

and PSA were measured by an electrochemiluminescence immunoassay (ECLIA). The inter-assay CVs for SHBG at 45.7 nmol/L was 2.1%; total testosterone at 0.087 nmol/L, 2.8%; and for total PSA at 1.12 ng/mL, 3.2%. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and urea were measured photometrically. The CV for ALT at 17.2 U/L was 4.8% and AST at 18 U/L was 6.8%. The CV for urea at 7.2 nmol/L was 1.2%. Total cholesterol was analyzed using a calorimetric assay and its CV at 1.97 mmol/L was 1.4%.

Statistical analysis

Sample size calculation was based on changes in hepatic urea production. Our previous study looking at testosterone supplementation in eight hypogonadal men showed a significant reduction in hepatic urea production by 21±5% after 2 weeks. Considering that a longer time period than 2 weeks would be required to achieve a change in testosterone level with ADT, 11 patients in each group would be required to achieve 80% power at α level of 0.05.

The treatment effects of ADT on hepatic urea production and protein synthesis were assessed using paired *t* tests. Differences between PRT and UC groups were assessed using a two-sample *t* test. Results are expressed as mean±S.E.M., and a *P* value <0.05 was considered

significant. A linear mixed effects model was used to determine the effect of PRT on hepatic urea production and protein turnover. Statistical analysis was undertaken using the statistical software package SPSS statistics v22 (IBM Corporation), RStudio (Boston, MA, USA) and R (20).

Results

The baseline characteristics of the study patients are listed in Table 1. The mean age was 70.4±1.5 years, and most had localized prostate cancer (*n*=16). There were no significant differences between the UC and PRT groups in terms of age, prostate cancer grading or staging. At baseline, patients in the UC group had higher levels of testosterone (*P*<0.05) and SHBG (*P*<0.01; Table 1), although calculated free testosterone was not significantly different between the groups (*P*=0.1). Otherwise they were well matched in terms of weight, renal and hepatic function. Baseline physical activity as measured by weekly step count was not significantly different between the groups. Out of the 13 patients in the PRT group, 11 patients had an 100% adherence rate as documented in their training log books. Two patients violated study protocol by not filling out their log books as required, but verbally expressed a 100% adherence rate.

Table 1 Baseline clinical characteristics.

Variable	All patients (*n* = 24)	UC (*n* = 11)	PRT (*n* = 13)	*P* value
Age (years)	70.4 ± 1.5	71.7 ± 1.9	69.3 ± 2.3	0.43
Weight (kg)	82.7 ± 2.8	80.4 ± 2.9	84.7 ± 4.7	0.46
BMI kg/m^2	29.3 ± 0.9	28.9 ± 1.1	29.7 ± 1.3	0.63
Gleason score	7.9 ± 0.2	7.5 ± 0.3	8.2 ± 0.2	0.05
Cancer staging				
Localized (*n*)	16	8	8	0.40
Biochemical recurrence (*n*)	6	3	3	
Metastatic (*n*)	2	0	2	
Previous radiotherapy (*n*)	8	3	5	0.28
Previous ADT (*n*)	2	0	2	0.17
Lean body mass (kg)	54.0 ± 1.3	53.4 ± 1.3	54.5 ± 2.2	0.68
LBM (% body weight)	64.8 ± 1.5	66.7 ± 2.0	63.2 ± 2.0	0.24
Fat mass (kg)	27.3 ± 2.1	24.2 ± 2.4	29.9 ± 3.2	0.18
Extracellular water (L)	19.4 ± 0.6	19.1 ± 0.6	19.8 ± 0.9	0.53
Body cell mass (kg)	34.5 ± 0.9	34.4 ± 1.1	34.7 ± 1.3	0.84
Testosterone (nmol/L)	14.2 ± 0.8	16.3 ± 1.2	12.5 ± 0.8	0.02
LH (mIU/mL)	6.6 ± 0.5	7.0 ± 0.8	6.3 ± 0.7	0.51
SHBG (nmol/L)	46.6 ± 2.9	55.9 ± 3.9	38.7 ± 2.7	<0.01
Urea (mmol/L)	5.9 ± 0.3	6.2 ± 0.5	5.7 ± 0.3	0.30
PSA (ng/mL)	9.8 ± 1.5	12.4 ± 2.8	7.7 ± 1.3	0.11
Step count (number)	34538 ± 5180	41274 ± 9235	28838 ± 5377	0.24

Data are presented as mean ± S.E.M.; *P* value is for UC vs PRT group.
BMI, body mass index; LBM, lean body mass; LH, luteinizing hormone; PSA, prostate-specific antigen; SHBG, sex hormone binding globulin.

Table 2 Effect of ADT on whole-body protein turnover and urea synthesis.

	Baseline	Post ADT	*P* value
LRa (µmol/min)	168 ± 58	165 ± 54	0.52
Lox (µmol/min)	25.1 ± 1.6	27.3 ± 1.9	0.11
Lox (% from Ra)	15.1 ± 0.5	16.8 ± 0.6	0.02
LIP (µmol/min)	143 ± 11	137 ± 10	0.25
LIP (% from Ra)	84.9 ± 0.5	83.2 ± 0.6	0.02
Urea production rate (µmol/min)	428 ± 19	487 ± 21	<0.01
Urea production rate/BCM (µmol/min/kg)	12.4 ± 0.5	14.3 ± 0.6	<0.01

Data are presented as mean ± s.e.m.
BCM, body cell mass; LIP, leucine incorporation into protein; Lox, leucine oxidation (a measure of irreversible loss of protein); LRa, leucine rate of appearance (a measure of protein breakdown).

Change in protein turnover and urea production

Table 2 shows the rates of protein turnover and urea production in all study participants before and after ADT. After 6 weeks of ADT, there was no significant change in LRa, a measure of protein turnover. There was a 12.6±4.9% ($P<0.05$) (Fig. 1) increase in leucine oxidized as a proportion of LRa (percent Lox/LRa), which represents the proportion of amino acids irreversibly lost when adjusted for changes in protein turnover. Conversely, the rate of leucine incorporation into protein (when adjusted for protein turnover) significantly decreased after ADT (Table 2).

The baseline rate of urea production for all study participants was 427.6±18.8 µmol/L. Following 6 weeks of ADT, there was a 14.8±4.1% increase in hepatic urea production to 486.5±21.3 µmol/min (Fig. 1; $P<0.01$). Significance was retained (p<0.01) when corrected for changes in BCM, a functional component of LBM (Table 2).

Effect of PRT

Table 3 shows change from baseline in protein and urea data for participants in both the UC and PRT groups. Overall, there were no significant differences between groups in terms of baseline values or changes in protein turnover or hepatic urea production. Percent Lox/LRa increased in both groups, although this reached statistical significance only in the UC group ($P<0.05$). There was a significant increase in urea production in both the UC and PRT groups ($P<0.05$), which was retained when corrected for BCM ($P<0.05$).

Other endpoint measures

Table 4 shows changes in anthropometric and biochemical characteristics in all study participants before and after 6 weeks of ADT. As expected, ADT caused a profound reduction in serum testosterone (by 93.5±5.1% from 14.2±0.8 nmol/L to 0.8±0.6 nmol/L, $P<0.001$). There were also decreases in luteinizing hormone (LH) and PSA and a rise in SHBG ($P<0.001$). There was no change in BCM, but a significant reduction in LBM (% body weight) and increase in FM ($P<0.05$). ADT resulted in a rise in serum urea concentration by 12.4±21.2% ($P<0.05$). Transaminases, including both alanine transaminase (ALT) and aspartate transaminase (AST), increased ($P<0.01$) as did total cholesterol ($P=0.02$), high-density lipoprotein (HDL) ($P<0.01$) and triglycerides ($P<0.05$). There were no significant changes in other endocrine parameters such as glucose or energy expenditure.

Effect of PRT

Table 5 shows the comparison between UC and PRT groups following 6 weeks of ADT. LBM was significantly reduced in the UC group, as opposed to no change in the PRT group, and the difference between groups was statistically significant ($P<0.01$). The functionally active muscle mass (BCM) was reduced only in the UC group
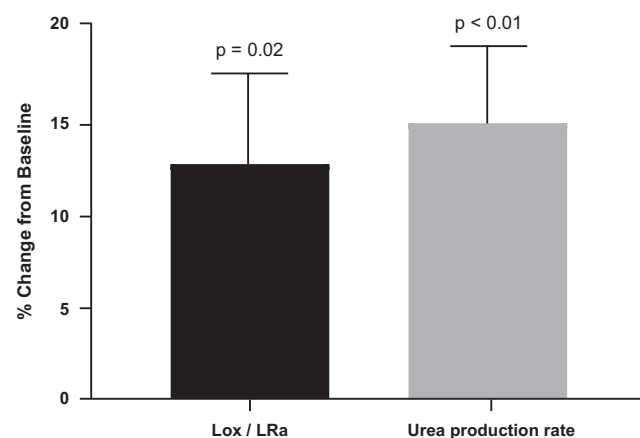
Figure 1
Percentage change in Lox/LRa and urea production rate after 6 weeks of ADT. Data expressed as mean ± s.e.m.

Table 3 Protein turnover and urea synthesis in usual care and exercise groups before and after ADT.

	UC		PRT		
	Δ from baseline (%)	*P* value	Δ from baseline (%)	*P* value	***P* value**
LRa	−1.1 ± 3.5	0.65	−1.5 ± 5.1	0.64	0.94
Lox	13.0 ± 6.9	0.08	8.1 ± 8.9	0.45	0.66
Lox (% from Ra)	14.6 ± 6.6	0.03	10.6 ± 7.6	0.23	0.69
LIP	−3.3 ± 3.7	0.33	−2.9 ± 5.3	0.64	0.96
LIP (% from Ra)	−2.3 ± 0.9	0.03	−1.5 ± 1.0	0.23	0.63
Urea production rate	12.0 ± 5.3	0.03	17.2 ± 6.2	0.02	0.53
Urea production rate/BCM	14.9 ± 5.8	0.02	16.9 ± 7.2	0.03	0.83

Data are presented as mean ± s.e.m.; *P* value is for UC vs PRT group.
BCM, body cell mass; LIP, leucine incorporation into protein; Lox, leucine oxidation (a measure of irreversible loss of protein); LRa, leucine rate of appearance (a measure of protein breakdown).

($P<0.05$). Testosterone levels fell in both groups, but there was a significantly greater reduction in the UC compared to the PRT group ($P<0.05$), which reflected the difference in baseline testosterone between groups. There was a significant increase in fasting glucose levels and carbohydrate oxidation rate in the UC group ($P<0.05$) but not in the PRT group. HDL cholesterol and triglycerides increased in both groups but reached significance only in the UC group. There were no other differences between groups in terms of endocrine or metabolic parameters during ADT.

Discussion

It has long been known that testosterone induces an anabolic effect on muscle mass. However, it is a fairly recent postulate that this may occur due to an action of testosterone on the liver, influencing whole-body protein metabolism and that this effect is separate and additional to any direct action of testosterone on muscle. In this study we utilized a model of profound testosterone withdrawal during ADT for prostate cancer to investigate the actions of testosterone on the hepatic urea cycle and whole-body protein metabolism. We also investigated whether PRT can offset ADT-induced changes in protein metabolism through preventing the rapid loss of muscle mass. We showed that ADT results in increased hepatic urea production and raised serum urea. This was associated with a whole-body catabolic effect, as represented by a rise in protein oxidation and a reduction in protein synthesis. However, PRT was unable to offset ADT-induced adverse effects on the urea cycle and protein metabolism, although there was a preservation of LBM in the PRT group. Thus, this study provides biochemical evidence of the potent effect of testosterone withdrawal on the hepatic urea cycle, resulting in whole-body protein

loss which could not be mitigated by co-administration of short-term, home-based PRT.

This study builds on earlier data in which we demonstrated that selective exposure of the liver to orally administered testosterone reduced protein oxidation to the same extent as systemic (transdermal) testosterone administration (8), which was indicative of a hepatic site of testosterone action. We have further biochemical evidence that the intra-hepatic pathway mediating this process is the urea cycle, as testosterone reduces hepatic urea cycle activity in a model of physiological testosterone replacement in hypogonadal men (10). Importantly, we showed in this study that the increase in protein oxidation and nitrogenous waste formation associated with ADT does not seem to be a consequence of increased muscle breakdown, as the observed rate of protein turnover (indicated by leucine rate of appearance in our experimental model) was not increased. Thus, the catabolic effect of testosterone withdrawal is mediated through accelerated loss of amino acid nitrogen via the hepatic urea cycle, causing irreversible loss of total body protein. As anabolic hormones such as growth hormone downregulates hepatic urea synthesis (21, 22), while catabolic hormones such as glucocorticoids and glucagon upregulate urea cycle enzymes (23, 24, 25), it is conceivable that androgens directly modify hepatic urea cycle activity. Collectively, these findings suggest that a physiological action of testosterone in the liver is to limit oxidative loss from the circulating amino acid pool, thus mediating a whole-body protein anabolic effect. This liver-mediated action of testosterone may be important in the maintenance of muscle mass in aging males, and it identifies a potential therapeutic target for treatment of muscle loss in various disease states.

Aging is associated with a loss of muscle mass. In healthy young adults, LBM comprises approximately 60% of total body mass, but this declines after age 40 years

Table 4 Biochemical and metabolic characteristics at baseline and after 6 weeks of ADT.

	Baseline (n = 24)	**6 weeks post ADT** (n = 24)	**P value**
Weight	82.7 ± 2.8	84.3 ± 2.9	0.27
BMI	29.3 ± 0.9	29.4 ± 4.4	0.93
Lean body mass (kg)	54.0 ± 1.3	53.7 ± 1.2	0.13
LBM (% body weight)	64.8 ± 1.5	64.2 ± 1.4	0.02
Fat mass (kg)	27.3 ± 2.1	27.9 ± 2.1	0.03
Extracellular water (L)	19.5 ± 0.6	19.3 ± 0.6	0.56
Body cell mass (kg)	34.5 ± 0.9	34.3 ± 0.9	0.44
Testosterone (nmol/L)	14.2 ± 0.8	0.8 ± 0.6	<0.001
LH (mIU/mL)	6.6 ± 0.5	0.9 ± 0.5	<0.001
SHBG (nmol/L)	46.6 ± 2.9	50.4 ± 3.7	<0.001
Urea (mmol/L)	5.9 ± 0.3	6.5 ± 0.3	0.03
Creatinine (μmol/L)	86.1 ± 3.0	85.1 ± 2.9	0.45
ALT (IU/L)	25.8 ± 2.8	33.6 ± 3.4	<0.01
AST (IU/L)	26.6 ± 1.5	30.8 ± 1.6	<0.01
GGT (U/L)	30.6 ± 2.9	33.3 ± 3.7	0.23
Glucose (mmol/L)	4.6 ± 0.1	4.7 ± 0.4	0.05
Total cholesterol (mmol/L)	4.3 ± 0.2	4.5 ± 0.2	0.02
LDL cholesterol (mmol/L)	2.6 ± 0.2	2.6 ± 0.2	0.61
HDL cholesterol (mmol/L)	1.2 ± 0.1	1.3 ± 0.1	<0.01
Triglycerides (mmol/L)	1.1 ± 0.1	1.4 ± 0.1	0.03
PSA (ng/mL)	9.8 ± 1.5	3.7 ± 1.0	<0.001
REE (kcal/day)	1398 ± 54	1345 ± 51	0.11
Fox (mg/min)	45.7 ± 4.8	38.9 ± 4.7	0.27
Cox (mg/min)	86.8 ± 54.1	93.8 ± 9.2	0.64

Data are presented as mean ± s.e.m.; *P* value is for UC vs PRT group. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; Cox, carbohydrate oxidation; Fox, fat oxidation; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; LBM, lean body mass; LDL, low-density lipoprotein; LH, luteinizing hormone; PSA, prostate-specific antigen; REE, resting energy expenditure; SHBG, sex hormone-binding globulin.

to 40% at 70 years, resulting in sarcopenia (26). As testosterone levels decrease with age, androgen deficiency likely plays a large role in the initiation and progression of this condition (27). Thus, ADT offers a unique accelerated model for studying male aging and frailty. ADT is associated with rapid changes in body composition, with a 2–4% reduction in LBM and 10–20% increase in FM after just 12 months of androgen deprivation (28, 29). However, its specific effects on muscle have not been fully elucidated (30). Much of our knowledge is derived from animal models which provide insight into the role of the androgen receptor (AR) in muscle regulation. In these models, experimental androgen deprivation typically results in a rapid loss of muscle mass (30). However, differences have been observed between myocyte-specific AR-knockout (mARKO) mice versus global AR-knockouts or orchidectomized mice. While muscle loss in the mARKO mice was limited to the highly sensitive levator-ani muscle with preservation of limb muscles (31, 32), only global ARKO mice had significant reductions in the mass of hind limb skeletal muscle (31, 33). This indicates that testosterone effects on muscle mass are not mediated entirely by direct action on myocytes. Testosterone is known to interact with other cell types (besides myocytes) and intracellular signaling pathways in muscle, including satellite cells (34), motoneurons (35), myokines, growth hormone and IGF-1 (36). Furthermore, ARs are also known to be present in human liver (37). Thus, our findings provide evidence of an additional novel pathway causing muscle catabolism during ADT- through upregulation of the hepatic urea cycle, increasing whole-body nitrogen losses and reducing the available amino acid pool for muscle synthesis.

We found a significant increase in hepatic transaminases during ADT. GnRH analogs are associated with mild enzyme elevations in 3–5% of patients, the cause of which is unclear (38). However, a potential factor could be the development of non-alcoholic hepatic steatosis from testosterone deficiency, as previous studies show that androgen and androgen signaling is critical for maintaining lipid metabolism in the liver (39). This is in keeping with our findings of a significant increase in FM, total cholesterol and triglycerides after just 6 weeks of ADT.

As a secondary aim, we examined whether the introduction of a predominantly home-based PRT program at the start of ADT could lessen its adverse effects on muscle and protein loss. Exercise is currently recognized as a management strategy to ameliorate many of the adverse effects of ADT (40). Trials of supervised exercise programs in cancer patients report better compliance rates and improved outcomes compared to pure home-based exercise programs (41, 42). However, fully supervised programs may be comparatively less scalable or accessible, and many prostate cancer patients experience difficulty in finding and affording a tailored exercise program. Transitioning to community-based programs can also be confronting for these patients (43, 44). Thus, we adopted a partially supervised program, in that patients were given two supervised training sessions in the first week as well, as additional

Table 5 Biochemical and metabolic characteristics in usual care and exercise groups before and after ADT.

	UC (*n* = 11)		PRT (*n* = 13)		*P* value
	Δ from baseline	*P* value	Δ from baseline	*P* value	
Weight (kg)	−0.2 ± 0.4	0.65	3.0 ± 2.5	0.25	0.26
BMI	−0.04 ± 0.1	0.76	0.05 ± 0.16	0.74	0.66
Lean body mass (kg)	−0.9 ± 0.2	<0.01	0.2 ± 0.3	0.58	<0.01
Fat mass (kg)	0.5 ± 0.3	0.11	0.8 ± 0.5	0.12	0.69
Extracellular water (L)	−0.1 ± 0.3	0.63	−0.1 ± 0.3	0.77	0.89
Body cell mass (kg)	−0.8 ± 0.3	0.04	0.2 ± 0.4	0.58	0.08
Testosterone (nmol/L)	−16.1 ± 1.2	<0.001	−11.2 ± 1.4	<0.001	0.02
LH (mIU/mL)	−6.6 ± 0.7	<0.001	−5.0 ± 1.0	<0.001	0.22
SHBG (nmol/L)	3.3 ± 3.1	0.32	4.3 ± 3.6	0.42	0.83
Urea (mmol/L)	0.2 ± 0.4	0.67	0.9 ± 0.3	<0.01	0.14
Creatinine (µmol/L)	−0.6 ± 1.6	0.74	−1.2 ± 1.8	0.46	0.78
ALT (IU/L)	8.7 ± 3.4	0.03	6.9 ± 3.1	0.01	0.70
AST (IU/L)	5.5 ± 1.8	0.01	3.2 ± 2.0	0.04	0.41
GGT (U/L)	3.0 ± 2.7	0.29	2.5 ± 3.5	0.12	0.91
Glucose (mmol/L)	0.2 ± 0.1	0.04	0.1 ± 0.1	0.38	0.43
Total cholesterol (mmol/L)	0.3 ± 0.1	0.05	0.2 ± 0.2	0.11	0.69
LDL cholesterol (mmol/L)	0.03 ± 0.1	0.82	0.1 ± 0.1	0.65	0.87
HDL cholesterol (mmol/L)	0.1 ± 0.1	0.02	0.07 ± 0.03	0.06	0.23
Triglycerides (mmol/L)	0.3 ± 0.1	0.03	0.2 ± 0.2	0.09	0.74
PSA (ng/mL)	−7.7 ± 2.4	0.01	−4.7 ± 1.5	<0.01	0.29
REE (kcal/day)	−26.0 ± 43.0	0.56	−77.3 ± 45.7	0.12	0.42
Fox (mg/min)	−6.2 ± 6.7	0.37	−7.3 ± 10.0	0.48	0.93
Cox (mg/min)	9.9 ± 14.9	0.03	3.9 ± 22.9	0.87	0.84

Data are presented as mean ± s.e.m.; *P* value is for UC vs PRT group.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; Cox, carbohydrate oxidation; Fox, fat oxidation; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; LBM, lean body mass; LDL, low-density lipoprotein; LH, luteinizing hormone; PSA, prostate-specific antigen; REE, resting energy expenditure; SHBG, sex hormone-binding globulin.

tools including an exercise manual, online videos and weekly phone calls to foster adherence. Despite the short duration of exercise, we found that patients in the PRT group had a significantly smaller loss of LBM compared to those in the UC group. This was similar to findings by Fennichia *et al.* who demonstrated an improvement in LBM after just 6 weeks of a PRT program in a control group of patients and a reduction in FM in the diabetic group (45). Furthermore, although there were no overall differences between groups, a significant increase in protein loss (as reflected by an increase in leucine oxidation) was found only in the UC group. The finding that PRT undertaken at commencement of ADT may, to some degree, offset protein loss is consistent with that of Hanson *et al.* (46) who showed that acute resistance exercise in ADT-treated prostate cancer patients increased muscle protein synthesis. Similarly, in elderly sarcopenic patients, 2 weeks of resistance training increased protein synthesis measured by incorporation of ^{13}C-leucine into vastus lateralis muscle protein (47). Thus, the trend toward an increase in the proportion of amino acids shuttled for protein synthesis in our study during PRT may allow preservation of muscle mass during ADT.

We did not find any effect of PRT on the rate of hepatic urea production in our study. The rate of urea production increased in both UC and PRT groups equally after 6 weeks of ADT. Previous studies using [15]N2-labeled urea show that there are no changes in urea production during low- or high-intensity exercise (48, 49). Following an endurance training program, an initial increase in urinary nitrogen excretion was found to return to pre-training levels within 2 weeks (50). Therefore, the persistent increase in urea production regardless of a PRT component in our study most likely reflects the loss of testosterone inhibition on the hepatic urea cycle during ADT. In addition, as PRT did not have any effect on the urea cycle, the preservation of LBM that occurred in the PRT group was a likely consequence of its direct effect on muscle.

This is the first study to examine the relationship between protein turnover and hepatic urea production using isotopic methods in the experimental model of severe testosterone deficiency seen in ADT. Both the leucine and urea turnover techniques are established methods for quantifying whole-body protein metabolism, the integrity of which was validated in our previous study (10). The concordance of these two

methods was again demonstrated in our current study. However, this study has some limitations including the small sample size and lack of standardization of dietary protein intake. Participants were instructed to adhere to their regular diet; however, variations in dietary protein intake can influence urea production. In addition, our exercise program was home based, and greater changes in muscle mass and protein turnover may have been achieved with supervised PRT. Nevertheless, we observed that home-based PRT was effective in offsetting the ADT-induced reduction in muscle mass only after 6 weeks of intervention.

The results of this study build on our previous findings of a testosterone effect on the hepatic urea cycle, thus providing further insight into the physiological actions of testosterone in regulating protein metabolism and body composition in older men. This also has implications for the pathogenesis of sarcopenia. The significance of this study is that it raises the possibility of using liver-targeted therapies in the prevention of protein losses through the hepatic urea cycle. Thus, future directions may involve clinical trials of solely liver-targeted orally administered crystalline testosterone (8) therapy in patients with sarcopenia or prostate cancer patients on ADT to reduce the loss of muscle mass without inducing systemic side effects.

In summary, we discovered that the suppression of testosterone to castrate levels during ADT results in greater nitrogen losses through the urea cycle, thus suggesting a novel pathway of muscle catabolism. This is a significant finding, as it may lead to future therapeutic benefits including the possible use of liver-targeted testosterone in the treatment of sarcopenia. Additionally, the introduction of PRT at the start of ADT may offset the loss of muscle mass, most likely through direct effects on muscle, thus providing further evidence of its beneficial role in cancer treatment.

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