Androgen deprivation therapy for prostate cancer: novel mechanisms of testosterone action and the benefits of home-based progressive resistance training

Teresa Lam

A thesis submitted for the degree of Doctor of Philosophy

School of Medicine

Western Sydney University

Acknowledgements

Statement of Authentication

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material in full or in part, for a degree at this or any other institution.

…………………………..
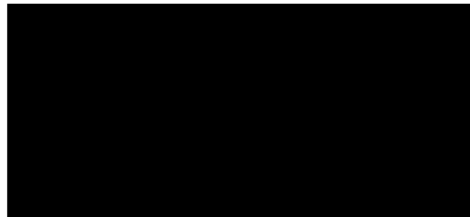
Teresa Lam

Preface

This thesis is presented as a series of manuscripts focusing on a novel aspect of testosterone action on the hepatic urea cycle and the benefits of home-based resistance training in men with prostate cancer. Each chapter presented is self-contained and includes a specific outline of authorship. A general reference list of all the chapters is provided at the end of the thesis. Of the manuscripts presented here, all four chapters have been published in peer review journals. To assist with continuity throughout, an introductory chapter is included at the beginning of the thesis, along with a general summary chapter at the end. Manuscripts are formatted according to the guidelines of the journal they are published in. All manuscripts are jointly authored, but in each case, I am the first author.

Table of Contents

Publications

Journal Articles

Lam T, Poljak A, McLean M, Bahl N, Ho KKY, Birzniece V. Testosterone prevents protein loss via the hepatic urea cycle in humans. European Journal of Endocrinology. 2017; 176:489-496

Lam T, McLean M, Hayden A, Poljak A, Cheema B, Gurney H, Stone G, Bahl N, Reddy N, Shahidipour H, Birzniece V. A potent liver mediated mechanism for loss of muscle mass during androgen deprivation therapy. Endocrine Connections. 2019; 8:605-615

Lam T, Birzniece V, McLean M, Gurney H, Hayden A, Cheema BS. The adverse effects of androgen deprivation in prostate cancer and the benefits and potential anti-oncogenic mechanisms of progressive resistance training. Sports Medicine Open. 2020; 6:13

Lam T, Cheema B, Hayden A, Lord SR, Gurney H, Gounden S, Reddy N, Shahidipour H, Read S, Stone G, McLean M, Birzniece V. Androgen deprivation in prostate cancer: benefits of home-based resistance training. Sports Medicine Open. 2020; 6:59

Conference Articles

Lam T, Cheema B, Hayden A, Gurney H, Gounden S, Reddy N, Stone G, McLean M, Birzniece V. ADT in prostate cancer patients: Prevention of adverse effects using a 6-month home-based progressive resistance training program. Asia-Pacific Journal of Clinical Oncology. 2017; 13 (Supplement 4):125-126

Conference Oral Presentations

Lam T, Cheema B, Hayden A, Lord S, Gurney H, Gounden S, Reddy N, Shahidipour H, Read S, Stone G, McLean M, Birzniece V. ADT in prostate cancer patients: the benefits of a 12-month home-based progressive resistance program. (Bryan Hudson Clinical Award Winner) The Annual Scientific Meeting of the Endocrine Society of Australia and the Society for Reproductive Biology, Sydney 2019

Lam T, McLean M, Hayden A, Poljak A, Cheema B, Gurney H, Bahl N, Reddy N, Shahidipour H, Birzniece V. A liver mediated mechanism for loss of muscle mass during androgen deprivation therapy. (Paul Lee Best Abstract Award session). The Annual Scientific Meeting of the Endocrine Society of Australia and the Society for Reproductive Biology, Adelaide 2018

Lam T, Cheema B, Hayden A, Gurney H, Gounden S, Reddy N, Stone G, McLean M, Birzniece V. ADT in prostate cancer patients: prevention of adverse effects using a 6-month home based progressive resistance training program. The Annual Scientific Meeting of the Endocrine Society of Australia and the Society for Reproductive Biology, Perth 2017

Lam T, McLean M, Bahl N, Poljak A, Ho KKY, Birzniece, V. Testosterone prevents protein loss via the hepatic urea cycle. The Annual Scientific Meeting of the Endocrine Society of Australia and the Society for Reproductive Biology, Gold Coast 2016

Awards and Grants

2019- Bryan Hudson Clinical Endocrinology Award- Endocrine Society of Australia best clinical research presentation at the Annual Scientific Meeting

Glossary of Terms

ADT- androgen deprivation therapy

ALT- alanine aminotransferase

AMP- adenosine monophosphate

AMPK- adenosine monophosphate-activated protein kinase

AST- aspartate aminotransferase

ATP- adenosine triphosphate

BDNF- brain-derived neurotrophic factor

BMD- bone mineral density

Cox- carbohydrate oxidation

CRPC- castrate resistant prostate cancer

FM- fat mass

Fox- fat oxidation

GnRH- gonadotrophin releasing hormone

GH- growth hormone

HbA1c- glycosylated haemoglobin

HDL- high-density lipoprotein

HRQOL- health related quality of life

IGFBP- IGF binding protein

IGF-1- insulin-like growth factor-1

IL-1β- interleukin 1 beta

IL-6- interleukin 6

IL-8- interleukin 8

LBM- lean body mass

LDL- low-density lipoprotein

LIP- leucine incorporation into protein

Lox- leucine oxidation

LRa- leucine rate of appearance

MiRNA- microRNA

mTOR- mammalian target of rapamycin

Nrf-2- nuclear factor erythroid 2-related factor 2

PCSM- prostate cancer specific mortality

PGC-1α- peroxisome proliferator-activated receptor gamma coactivator-1-alpha

PI3K- phosphoinositide 3-kinase

PRT- progressive resistance training

PSA- prostate-specific antigen

REE- resting energy expenditure

SHBG- sex hormone binding globulin

TNF-α- tumour necrosis factor alpha

TUGT- timed up and go test

List of Tables

List of Figures

Supplemental Tables

Appendices

Appendix 1	Resistance training logbook	Page 157-174
Appendix 2	Exercise logbooks	Page 172-174
Appendix 3	Physical activity diary	Page 175-177

Abstract

Testosterone is a key anabolic hormone which has a major role in the regulation of muscle mass and function, glucose and fat metabolism, mental well-being and general health. Androgen deprivation therapy (ADT) is a common treatment modality in men with prostate cancer. In reducing testosterone to castrate levels, it offers a unique model to study the physiological effects of testosterone.

Testosterone is known to act on multiple pathways which regulates muscle mass, fibre hypertrophy and protein turnover. Protein is hydrolysed into amino acids which are then available for synthesis of new protein, or alternatively can be eliminated as urea by the hepatic urea cycle; a rate limiting step in the irreversible loss of protein nitrogen. Although testosterone deficiency is well-known to be associated with loss of muscle mass and other protein depots, the effect of testosterone on the hepatic urea cycle has not been studied in humans. ADT also leads to a substantial increase in fat mass, worsening glucose tolerance, impairment in muscle function, and deterioration in mental health. In fact, cardiovascular disease accounts for approximately a quarter of deaths amongst men with prostate cancer. Exercise has been used to improve ADT-induced adverse effects, but existing studies focus on reversing established changes with supervised programs which have limitations in terms of access and cost. Home-based progressive resistance training (PRT), if found effective, may provide a viable alternative to supervised training programs.

The work described in this thesis aimed to determine a hepatic site of testosterone action via the urea cycle- firstly in a cohort of hypogonadal men, followed by a population of men with prostate cancer starting ADT. A home-based PRT program was

also evaluated in this population regarding its efficacy in preventing the adverse effects of ADT when initiated at the start of treatment.

Stable isotope methodology, a state-of-the-art technique, was utilised to measure hepatic urea production. Whole-body protein metabolism was quantified by the leucine turnover technique. Results showed, for the first time, that testosterone regulates whole body metabolism by suppressing the hepatic urea cycle. This was demonstrated through a reduction in protein loss and hepatic urea production following administration of testosterone in hypogonadal men, and conversely, an increase in hepatic urea production and rise in protein oxidative losses following commencement of ADT in men with prostate cancer. This is of clinical importance, as it raises the possibility of using liver-targeted testosterone therapy as a treatment for sarcopenia, thus avoiding the adverse effects of systemic testosterone therapy.

This thesis also demonstrated, using a randomised trial design, that implementation of a 12-month home-based PRT program at the start of ADT had beneficial effects on body composition, physical activity levels and quality of life in men with prostate cancer. These findings are important given that only approximately 12% of men with prostate cancer currently meet exercise-oncology guidelines with the large majority being inactive. It is also known that vigorous physical exercise is associated with reduced prostate cancer mortality. Thus, by increasing physical activity levels, home-based PRT programs may contribute to reductions in prostate cancer morbidity and mortality. Furthermore, weight gain after a prostate cancer diagnosis is associated with poorer outcomes, and obesity correlates with higher rates of biochemical recurrence after a prostatectomy. The rates of depression and anxiety in the prostate cancer population are also high, resulting in reduced survival. This thesis showed that a home-

based PRT program was able to offset the detrimental gains in fat mass following treatment with ADT and was able to provide improvements in quality of life, particularly in the mental health and vitality domains, which were sustained at 12 months. Given the impact of these factors on prostate cancer survivorship, these benefits may translate into improvements in treatment outcomes and mortality.

In summary, the work described in this thesis demonstrates, for the first time, a hepatic site of testosterone action via the urea cycle in humans. Additionally, it shows the benefits of a home-based PRT program in preventing ADT-induced adverse effects. These findings may contribute to the discovery of a potential, novel treatment method for sarcopenia, and a viable alternative to supervised exercise programs during prostate cancer treatment.

Chapter 1: Introduction

1.1 Background to the thesis

Androgens are major anabolic hormones which exert a dose-dependent effect on muscle mass and are critical for the maintenance of muscle strength and function, bone mass and body composition in men (1). Testosterone replacement in hypogonadal men increases muscle mass and strength (2,3) while the age-related decline in testosterone levels is associated with sarcopenia, leading to increased falls, disability and loss of functional independence (4). This represents an escalating public health burden with the direct healthcare costs resulting from sarcopenia reported to approximate US$18.5 billion (5). Thus, greater research into the physiological role of testosterone in maintaining muscle mass may contribute to the development of novel treatments which function to improve the management of sarcopenia. This will result in substantial cost savings to the public health system (6).

Androgens act on a multitude of pathways to regulate muscle mass and induce fibre hypertrophy, including those controlling protein synthesis and breakdown, as well as pluripotent stem cell commitment and differentiation (7). It is also known that testosterone is a key regulator of skeletal muscle protein balance, shifting protein balance in favour of net protein accretion, resulting in a gain of muscle mass (8). Thus, the hypogonadal state stimulates protein catabolism. Protein undergoes constant turnover and is hydrolysed into amino acids, with the elimination of nitrogen as urea by the hepatic urea cycle. This represents a rate-limiting step in the irreversible loss of protein (9). It is known that the hepatic urea cycle is regulated by a variety of hormones including glucocorticoids, growth hormone and glucagon (10,11). Although it has long

been proven that testosterone is nitrogen sparing (12), the literature surrounding the action of testosterone on the hepatic urea cycle remains contradictory (13,14), and direct biochemical evidence in humans is lacking. However, recent evidence substantiates the role of a hepatic-mediated mechanism of testosterone action. Thus, this thesis will investigate the action of testosterone on the hepatic urea cycle, in two separate studies involving hypogonadal men, and in a cohort of men with prostate cancer treated with androgen deprivation therapy (ADT).

Prostate cancer has the second highest incidence of all cancers among men worldwide (15) and androgen receptor signaling strongly promotes growth, proliferation and invasiveness of prostate cancer. Androgen deprivation therapy (ADT) with gonadotrophin releasing hormone (GnRH) analogues and anti-androgen therapy is a common form of therapy leading to a decline in prostate-specific antigen (PSA) in about 90% of patients (16). However, in rendering patients hypogonadal, ADT is associated with a rapid loss of muscle mass far exceeding that of normal aging, thereby offering a unique model to study the physiological effects of hypogonadism on muscle. Other adverse effects include an increase in fat mass (FM), development of insulin resistance, lower bone density, reduced sexual function and quality of life (17,18). There have been consistent positive outcomes regarding the benefits of exercise in treating these adverse effects, and for these reasons, the American College of Sports medicine recognises exercise as synergistic medicine during treatment for prostate cancer. In particular, progressive resistance training (PRT), is a key therapy in the treatment of sarcopenia (19), and can also mitigate adverse changes in body composition during ADT (20). However, despite these recommendations, only approximately 12% of men with prostate cancer currently meet exercise-oncology

guidelines with the large majority (~48%) being inactive (21). Thus, supervised exercise programs have the benefit of improving exercise compliance and physiological outcomes but can be constrained by access and expense. Home-based exercise programs may offer an alternative solution, but there is a current paucity in the literature regarding their efficacy. Therefore, the benefits of a 12-month home based PRT program will be investigated in a cohort of patients starting ADT.

1.2 Hypothesis

1.2.1 Testosterone affects the hepatic urea cycle by reducing hepatic urea production, thus decreasing protein oxidative loss. This will increase nitrogen availability for new protein synthesis. ADT will have an opposing effect, with increased hepatic urea production resulting in greater protein oxidative loss. This will reduce nitrogen availability for new protein synthesis, providing a mechanism for loss of muscle mass during ADT.

1.2.2 The introduction of a 12-month home-based PRT program at the start of ADT can prevent the detrimental changes in body composition, physical function, metabolic derangements and mental health in a cohort of men with prostate cancer.

1.3 Aims

This thesis has 3 main aims:

1.3.1 To determine whether testosterone reduces hepatic urea production in humans.

1.3.2 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.

1.3.3 To investigate whether a year long, home-based PRT program, instituted at the start of ADT, would prevent adverse effects of treatment in prostate cancer patients (including metabolic function, body composition, mental health and physical capacity).

1.4 Framework of the thesis

This thesis is divided into six chapters.

1.4.1 **Chapter 1** introduces the thesis, providing a background and overview of the aims and hypothesis of the individual studies

1.4.2 **Chapter 2** provides a critical appraisal of current literature with the first section focused on the known mechanisms of androgen action in muscle, including genomic and non-genomic pathways, regulation of skeletal muscle protein balance, overview of the hepatic urea cycle and hormonal regulation, and discussion of the recent findings of a hepatic site of testosterone action. The second section comprises a review article published in *Sports Medicine- open*

discussing the adverse effects of ADT in men with prostate cancer, current evidence of benefit regarding PRT, and the potential oncological pathways likely to be affected. The last section discusses current evidence surrounding home-based exercise programs and deficiencies in the literature.

1.4.3 **Chapter 3** encompasses a manuscript published in the *European Journal of Endocrinology*. This study involved the measurement of protein oxidation and hepatic urea production using stable isotope technology in hypogonadal men pre- and post- transdermal testosterone supplementation. This is the first study in humans showing that testosterone acts on the liver to reduce hepatic nitrogen loss through the urea cycle.

1.4.4 **Chapter 4** encompasses a manuscript published in *Endocrine Connections*. This study utilises 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. Results from this study adds to earlier findings (chapter 3) and provides further biochemical evidence of a testosterone effect on the urea cycle by showing that ADT increases hepatic urea production.

1.4.5 **Chapter 5** encompasses a manuscript published in *Sports Medicine- open*. A 12-month home based PRT program was instituted at the start of ADT in the cohort described in chapter 4. The results from this study strengthens current literature regarding the benefits of both PRT and a long-term home-based exercise program. A home-based PRT program instituted at the start of ADT is able to counteract detrimental changes in body composition and improve both physical activity and mental health over a 12-month period.

1.4.6   **Chapter 6** incorporates the discussion of the outcomes with respect to the original hypotheses. The significance and indication of these findings will be discussed, as well as its contribution to knowledge, including recommendations for future action and research.

Chapter 2: Literature Review

2.1 Androgens, muscle, and the hepatic urea cycle

2.1.1 The anabolic effects of androgens on skeletal muscle: genomic and non-genomic pathways

Androgens are important endocrine regulators of male sexual development and maintenance of muscle, bone, adipose tissue and body composition. After reaching peak levels in early adulthood, androgen levels decline with age. The loss of endogenous androgens parallels the reduction in muscle mass and function seen with aging (3,22), while testosterone treatment in hypogonadal and eugonadal men can increase skeletal muscle mass, with effect size correlating linearly with the dose of administered testosterone and circulating testosterone levels (1).

Androgens induce muscle fibre hypertrophy by acting at multiple steps along the pathways which regulate muscle protein synthesis and breakdown, and pluripotent stem cell commitment and differentiation (7). Importantly, the androgen receptor (AR) exerts a significant role in this process, as administration of (AR) antagonists to men is associated with loss of muscle mass. Studies in global androgen receptor (AR) knockout (ARKO) mice show a reduction in the mass of all hind limb muscles by 9 weeks of age, with up to a 20% reduction in mass of individual muscles (23).

Sinha-Hikim *et al* showed that the AR is expressed in a variety of cell types in human skeletal muscle including fibroblasts, vascular endothelial, smooth muscle cells, mast cells and satellite cells which are the predominant site of AR expression. In response to androgen administration, the AR receptor is up-regulated, resulting in a dose-response

increase in satellite cell numbers (24). These contribute to new myonuclei which fuse together to form myofibres for muscle growth and regeneration, as well as hypertrophy of both type 1 (slow oxidative) and 2 (fast oxidative) muscle fibres. Mesenchymal-derived pluripotent stem cells are present in both muscle and fat tissue and serve as a reservoir for the generation of new satellite cells. Singh *et al* showed that testosterone promotes commitment of these pluripotent precursor cells into the myogenic lineage and inhibits their differentiation into the adipogenic lineage. This is achieved through an AR-dependant pathway as the effect of testosterone and dihydrotestosterone on myogenesis and adipogenesis was blocked by bicalutamide, an AR antagonist (25). Thus, this finding provides a unifying explanation for the reciprocal effects of androgens on muscle and fat mass (7,25).

Furthermore, Rana *et al* have identified several genes involved in myoblast differentiation and atrophy which are regulated by androgens via the AR receptor. In orchiectomised male mice, the addition of dihydrotestosterone repressed *myogenin*, essential for muscle terminal differentiation, and promotion of myotubule formation. A similar effect occurred with *Tceal*, *Igf2* and *calcineurin,* which are all responsible for myoblast differentiation. These findings suggest that in males, androgens act via the AR in part to promote peak muscle mass by maintaining myoblasts in the proliferative state and delaying the transition to differentiation during muscle growth and development. This allows formation of additional myoblasts prior to fusion into myofibers, resulting in an overall increase in muscle size and mass. In contrast, *myogenin* controls neurogenic atrophy by upregulating the ubiquitin ligases *Fbxo32* and *MuRF1* that promote muscle protein degradation. Thus, androgens can preserve

muscle mass in adult muscle by suppression of the ubiquitin ligase-mediated atrophy pathways (26).

Skeletal muscle is innervated by motor neurons which contain AR and is also a site of androgen action. Degenerative disorders of motor neurons, such as Kennedy's disease (27) which results in muscle wasting, reflects the important role of androgens in motor neurons. Furthermore, dihydrotestosterone treatment of rats with amyotrophic lateral sclerosis (ALS) attenuated neuromuscular junction and axonal and motor neuron loss resulting in amelioration of muscle atrophy (28). Androgens also affects skeletal muscle cells through non-genomic pathways, via an increase in intracellular calcium (29).

In summary, androgens exert an anabolic effect on skeletal muscle through the AR receptor located on numerous cell types including pluripotent stem cells, satellite cells, myocytes and motor neurons, as well as through non-genomic mechanisms. In the next section, the anabolic effect of androgens on skeletal muscle protein balance will be discussed.

2.1.2 Androgen regulation of skeletal muscle protein balance

Skeletal muscle mass is regulated in part by the coordinated balance between rates of muscle protein synthesis and muscle protein breakdown. Thus, a long-term shift in this balance favouring net protein synthesis results in muscle hypertrophy while net protein breakdown results in muscle atrophy (8).

Androgens exert potent protein anabolic effects in human skeletal muscle. In healthy, eugonadal males, fasted net protein balance measured by stable isotopes is

improved following administration of testosterone (30,31) and oxandrolone, a synthetic analogue of testosterone (32). Similarly, in hypogonadal males, testosterone replacement increases protein synthesis and skeletal muscle mass (33,34). This process is achieved, in part, by improving synthetic efficiency which refers to the rate of protein synthesis relative to availability of amino acid precursors (35). Following testosterone administration, and in the presence of hyperaminoacidemia, there is greater inward amino acid transport, intracellular amino acid appearance and thus, subsequent protein synthesis (35).

The prominent molecular mechanisms behind the androgen-mediated increase in protein synthesis remains undefined. This may be mediated through direct interaction with the activated AR as Sheffield-Moore *et al* (32) found that short-term (5 days) exposure to oxandrolone resulted in an increase in skeletal muscle AR messenger RNA (mRNA) concentrations and Ferrando *et al* (36) showed an increase in AR protein expression after one month of treatment with testosterone enanthate. Alterations in muscle protein kinetics also require multiple events such as changes in translation, inhibition of catabolic signalling and activation of anabolic signalling pathways to occur (8). This is hypothesized to be due to increased anabolic signalling through mammalian target of rapamycin complex 1 (mTORC1) via upstream effectors such as insulin growth factor-1 (IGF-1)/Akt and/or extracellular signal-regulated kinase (ERK) 1/2 (36-38). In humans, IGF-1 mRNA and protein content is increased in skeletal muscle following androgen administration (34).

Testosterone also mediates a reduction in protein catabolism (8). In older men, administration of testosterone enanthate decreased protein breakdown in the fasted state which was associated with an increase in lean body mass (LBM) (36,39). Androgen-

mediated changes in markers of protein breakdown are also observed in animal models (8). In mice, castration increased proteasomal enzymatic activity in the levator ani muscle, and higher levels of autophagy markers and lysosome enzymatic activity in both the levator ani and triceps muscle. Administration of testosterone inhibited both lysosome and proteasome pathways in a dose dependent manner (40). The ubiquitin ligases, transforming growth factor beta (TGFβ)/myostatin/activin/Smad signalling and autophagy have all been demonstrated to be involved in testosterone-mediated decreases in protein catabolism (8).

In summary, testosterone plays a key role in the regulation of skeletal muscle protein balance, and significantly alters muscle mass, in part, by shifting protein balance in favour of net protein accretion. Thus, the hypogonadal state stimulates protein catabolism, resulting in the hydrolysis of protein into amino acids. The next section will focus on the function of the hepatic urea cycle in the elimination of excess amino acids, its known regulation by a variety of hormones including the potential role testosterone.

2.1.3 Amino acids and the hepatic urea cycle

Protein mass is constantly turning over in a dynamic process of breakdown and synthesis (41). Approximately 0.6% of total body nitrogen is replenished each day, indicating that total body protein is replaced every 160 days (42). Amino acids derived from proteolysis are either oxidised or resynthesized into protein. Oxidation of amino acids represents an irreversible loss of protein, a hallmark of catabolism. The liver is a major site of protein metabolism and degradation of amino acids which is controlled by the urea cycle. Surplus α-amino nitrogen derived from degradation of amino acids

enters the urea cycle as ammonia and is converted to urea in the mitochondria and cytosol of hepatocytes. Urea is then eliminated, representing a rate-limiting step in the irreversible loss of protein nitrogen (9). This is illustrated in Figure 1.

Figure 1: The hepatic urea cycle and elimination of surplus amino acids (43)
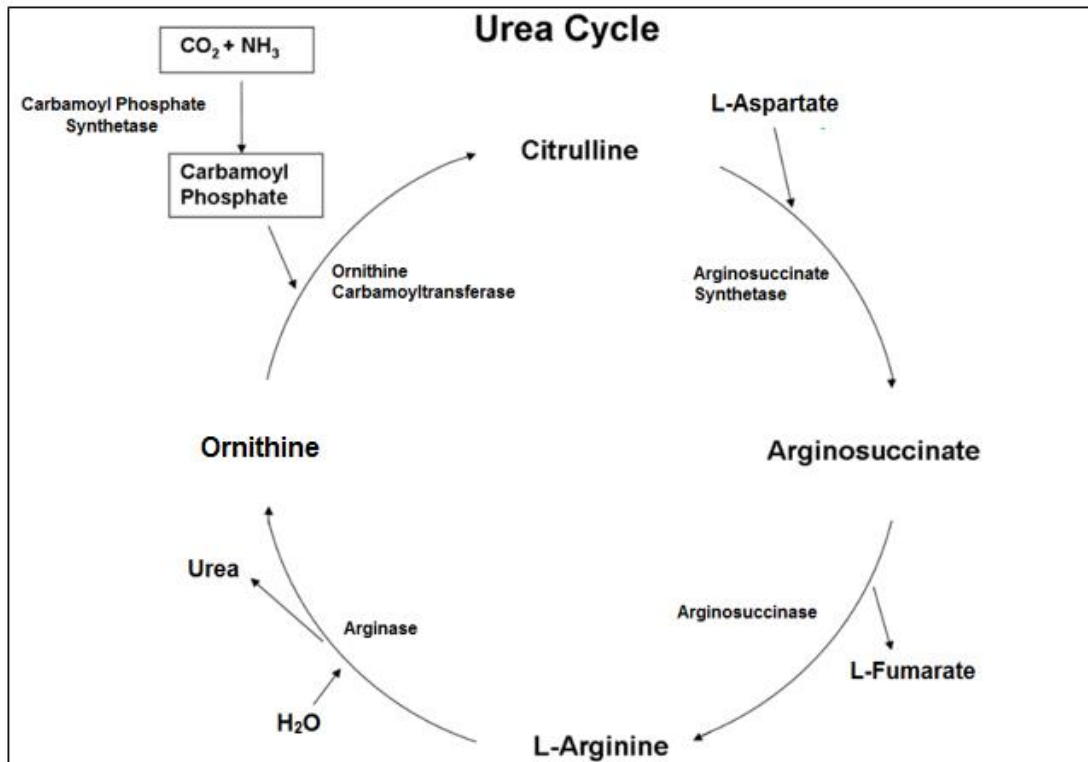
2.1.3.1 Hormonal regulation of the hepatic urea cycle

It is known that protein turnover and urea synthesis are under hormonal control. In animal models, the catabolic effects of glucocorticoids and anabolic effects of growth hormone are accompanied by an enhancement and suppression of urea synthesis, respectively (44). In humans, Grofte et al found that glucagon released during hypoglycaemia results in a doubling of functional hepatic nitrogen clearance (FHNC), a validated substrate-independent measure of hepatic urea synthesis. This lead to an accelerated, irreversible loss of nitrogen from the body, paralleled by a decrease in

amino acid uptake in muscle (45). Similarly, administration of glucocorticoids, well known to cause protein catabolism, resulted in increased FHNC (46). *In-vitro* studies have also shown glucocorticoids, glucagon and growth hormone to influence urea cycle enzymes in the liver (11). Glucocorticoids increase gene expression of enzymes promoting urea synthesis (47). Hepatic glucocorticoid receptors control urea cycle function via transcriptional activation of the Arginase 1 gene (48). Similarly, in cultured foetal rat hepatocytes, treatment with glucagon upregulated urea cycle enzymes including carbamoyl phosphate synthetase (CPS), arginosuccinase (ASL), and arginase (47). The reverse was noted when normal rats were treated with growth hormone, an anabolic hormone (11).

2.1.3.2 Whole-body protein anabolic effects of testosterone are mediated through the liver

It is long-established that testosterone is nitrogen sparing (12). However, despite the known effects of multiple hormones on the urea cycle, biochemical evidence of the direct role of testosterone on the hepatic urea cycle is currently lacking and evidence from animal studies is contradictory. An early study by Riggs et al (49) in ovariectomised female rats showed that treatment with testosterone propionate daily for ten days significantly reduced arginine synthetase activity by one fifth compared to controls (13). In contrast to this, De Angelis et al reported that the intraperitoneal injection of 1 mg/day of testosterone propionate into female rats increased liver arginase activity by 82% (14). Thus, early work in female rats report discordant effects of testosterone on urea cycle enzyme activity.

More recently, Birzniece *et al* showed that the protein anabolic effects of testosterone are mediated through the liver (50,51) after discovery that the inhibition of whole-body protein loss by testosterone was equivalent for both oral (hepatic) and transdermal (systemic) delivery (51,52). Oral delivery of non-esterified testosterone exposes the liver to high portal levels of testosterone which undergoes first-pass hepatic metabolism, preventing its appearance in the systemic circulation. This differs from transdermal administration of testosterone which enters the systemic circulation reaching both hepatic and extra-hepatic tissues (52). It was found that the administration of low dose (40mg/day) of oral crystalline unconjugated testosterone (exposing only the liver to testosterone) reduced whole-body protein loss equivalent to that seen with transdermal testosterone (51). Given that the liver is also known to possess AR (53,54), these findings are consistent with a hepatic mediated anti-catabolic effect of testosterone.

Although there is some, albeit contradictory, evidence based on animal studies that the intrahepatic mechanism may be the hepatic urea cycle, there is currently no precise biochemical evidence to support this in humans.

2.1.4 Summary

Androgens are the major anabolic hormones that exert a dose-dependent effect on muscle mass and strength. The anabolic effects are achieved through multiple pathways. These include the formation and hypertrophy of muscle fibres, as well as differentiation of pluripotent stem cells and myoblasts. Androgens also regulate skeletal muscle protein balance, shifting it favour of net protein accretion resulting in

anabolism. Protein is continuously turning over in a dynamic process of breakdown and synthesis. Surplus amino acids derived from proteolysis is converted to urea and eliminated via the hepatic urea cycle, which forms a rate-limiting step in the irreversible loss of protein nitrogen. Recent evidence suggests that there is an intrahepatic mechanism for the whole-body anabolic effect of testosterone but there is currently no direct biochemical evidence that this effect is mediated by the hepatic urea cycle.

The following section represents a review article focusing on the adverse effects of androgen deprivation therapy (ADT) in men with prostate cancer, and the use of progressive resistance training (PRT) in the alleviation of these effects. ADT used in the treatment of prostate cancer suppresses testosterone to castrate levels resulting in a rapid loss of muscle mass far exceeding that of normal aging. Thus, ADT offers a unique model to study the physiological effects of hypogonadism on muscle, providing insight into the physiological actions of testosterone in maintaining body composition.

2.2 The adverse effects of androgen deprivation therapy in prostate cancer and the benefits and potential anti-oncogenic mechanisms of progressive resistance training

Publication

2.2.1 Abstract

Prostate cancer has the second highest incidence of all cancers amongst men worldwide. Androgen deprivation therapy (ADT) remains a common form of treatment. However, in reducing serum testosterone to castrate levels and rendering men hypogonadal, ADT contributes to a myriad of adverse effects which can affect prostate cancer prognosis. Physical activity is currently recommended as synergistic medicine in prostate cancer patients to alleviate the adverse effects of treatment. Progressive resistance training (PRT) is an anabolic exercise modality which may be of benefit in prostate cancer patients given its potency in maintaining and positively adapting skeletal muscle. However, currently, there is a scarcity of RCTs which have evaluated the use of isolated PRT in counteracting the adverse effects of prostate cancer treatment. Moreover, although physical activity in general has been found to reduce relapse rates and improve survival in prostate cancer, the precise anti-oncogenic effects of specific exercise modalities, including PRT, have not been fully established. Thus, the overall objective of this article is to provide a rationale for the in-depth investigation of PRT and its biological effects in men with prostate cancer on ADT. This will be achieved by (1) summarising the metabolic effects of ADT in patients with prostate cancer and its effect on prostate cancer progression and prognosis, (2) reviewing the existing evidence regarding the metabolic benefits of PRT in this cohort, (3) exploring the possible oncological pathways by which PRT can affect prostate cancer prognosis and progression, and (4) outlining avenues for future research.

2.2.2 Introduction

Prostate cancer has the second highest incidence of all cancers amongst men worldwide and is the fifth leading cause of cancer death in men. In 2018, an estimated 1,276,106 new cases of prostate cancer was reported worldwide, with higher prevalence in the developed countries (15). The mechanisms of prostate carcinogenesis has marked heterogeneity and consists of both genetic and environmental factors, with risk of the disease increasing with age and positive family history (55). Although androgens (including testosterone and dihydrotestosterone) affect proliferation and differentiation of prostate luminal epithelium and drive prostate cancer cell growth, there is conflicting data on the role of endogenous testosterone in human prostate cancer pathogenesis *de novo.* A pooled analysis of 18 prospective studies showed no association between the risk of prostate cancer and testosterone levels (56). Yet, positive associations have been found between mutations in genes involved in the biosynthesis and degradation of testosterone, and higher prostate cancer risk (57,58). Although similar controversies occur regarding the link between obesity, diabetes and risk of prostate cancer development (59-63), there is now strong evidence that being overweight or obese increases the risk of advanced prostate cancer (64).

Androgen receptor signalling strongly promotes growth, proliferation and invasiveness of prostate cancer. Thus, androgen deprivation therapy (ADT), using gonadotrophin releasing hormone (GnRH) analogues and/or anti-androgen agents are a common and effective therapy for patients with locally advanced and metastatic prostate cancer. Long-acting GnRH analogues, such as leuprolide, causes down-regulation of the pituitary-gonadal axis, resulting in 'chemical castration' due to suppression of testicular testosterone production. ADT leads to a decline of prostate-

specific antigen (PSA) in about 90% of patients (16). However, in rendering the patient severely hypogonadal, ADT is associated with significant adverse metabolic effects. Consequences of ADT include the development of insulin resistance, reduced muscle and bone mineral density (BMD), increased fat mass, sexual dysfunction and reduced quality of life (17,18).Thus, there is a need for secondary treatment methods to combat the adverse effects of ADT.

Progressive resistance training (PRT) is an anabolic form of exercise that involves challenging the skeletal muscles with unaccustomed loads through use of free weights (e.g. barbells, dumbbells, medicine balls, sandbags), machine weights (e.g. leg press) and/or body weight (e.g. push-ups, pull-ups) and impact loading/plyometric exercises such as jumping. To facilitate continued muscular anabolic adaptation over the long-term, training variables including intensity and volume must be manipulated over time (65). It is well established that PRT can treat sarcopenia in older men and women (over 50 years) (66) and muscle wasting in some chronic diseases, including patients affected with end-stage renal disease (67) and AIDS-related muscle wasting (68). The myogenic effect of PRT has been associated with many other beneficial physiological, functional and psychological adaptations across a range of healthy and chronically diseased populations. The benefits are likely to extend to patients with cancer. In fact, the Clinical Oncology Society of Australia has recently endorsed the use of PRT as standard practice in cancer care (69).

To date, only a few robust studies have investigated the efficacy of PRT in patients receiving ADT for prostate cancer and the specific biological effects of this exercise modality are not completely understood in this cohort. Therefore, the overall objective of this review paper is to provide a rationale for the in-depth investigation of PRT and

its biological effects in men with prostate cancer on ADT. This will be achieved by (1) summarising the adverse consequences of ADT in patients with prostate cancer and its effect on prostate cancer progression and prognosis, (2) summarising the existing evidence regarding the benefits of PRT in this cohort, (3) exploring the possible oncological pathways by which PRT can affect prostate cancer prognosis and progression, and (4) outlining avenues for future research.

2.2.3 Adverse consequences of androgen deprivation therapy and their potential effects on prostate cancer progression and prognosis

Androgens play a vital role in the regulation of body composition, insulin and glucose sensitivity, growth factors and inflammation. Thus, the development of hypogonadism following ADT is associated with multiple adverse effects which have potential negative effects on prostate cancer prognosis.

2.2.3.1 Body composition

ADT is associated with a decrease in lean body mass (LBM) and increase in fat mass (FM), resulting in sarcopenic obesity (70). These changes occur rapidly, starting after just 3 months of ADT (71,72), with the average duration of therapy in high risk prostate cancer being 18 months (72). After 1 year, FM has been shown to increase by $7 - 10\%$, while LBM has been shown to decrease by $2 - 4\%$ (73), ten times the annual loss occurring in aging (74). These changes are sustained up to two years after initiating ADT (75). Hamilton *et al* found that ADT results in accumulation of both visceral

(22%) and subcutaneous (13%) fat, with increased insulin resistance likely arising from visceral fat accumulation (76).

The consequences of sarcopaenic obesity in men with prostate cancer are significant. Cheung *et al* reported that long-term ADT was associated with a reduction in lower-limb muscle function. The muscle groups most affected are those involved in generating body-weight support and regulating gait and balance (77). This leads to increased frailty, with a cross-sectional study showing that between 22% - 24% of current and past ADT users were recurrent fallers, compared to 5% of men not on ADT (78). These falls were also more likely to result in injuries including haematomas and fractures (78).

Weight gain after a prostate cancer diagnosis is associated with poorer outcomes (79). A higher baseline BMI correlates with greater prostate cancer specific mortality (PCSM) (80,81) and obesity is associated with higher rates of biochemical recurrence after prostatectomy for early stage prostate cancer (82). Furthermore, a meta-analysis of prospective cohort studies reported a 15% higher risk of PCSM per 5kg/m^2 increase in BMI (83).

2.2.3.2 Insulin resistance and diabetes mellitus

Insulin resistance and type 2 diabetes mellitus are known complications of ADT. Multiple prospective studies have shown decreased insulin sensitivity during ADT with a 25.9% increase in fasting plasma insulin levels and 12.8% reduction in insulin sensitivity after just three months (84). After one year of ADT, insulin resistance as measured by HOMA-IR increased by 39% (85).

Multiple studies have also consistently reported a significant link between ADT and subsequent diagnosis of diabetes (86-88). In a retrospective study of 12,191 men with

prostate cancer, ADT was associated with a 60% increased risk of diabetes (89). These changes in glucose metabolism occur before any changes in body composition are apparent, highlighting the direct effect of ADT on glucose metabolism.

Higher insulin and glucose levels are associated with a worse prostate cancer prognosis (90). Higher c-peptide levels (surrogate marker for endogenous insulin production) are associated with increased risk of PCSM as well as high-risk prostate cancer (Gleason ≥ 7) (91,92). Similarly, a meta-analysis of 17 cohort studies showed that pre-existing diabetes was associated with a 29% increase in PCSM and 37% increase in all-cause mortality in prostate cancer patients (93).

2.2.3.3 Growth factors and IGF-binding proteins

There are many alterations in hormonal, metabolic and inflammatory pathways in response to ADT that may contribute to the development of diabetes and insulin resistance. Insulin-like growth factor-1 (IGF-1) is a peptide produced by the liver and is involved in regulation of cell proliferation and differentiation. IGF-1 exerts multiple effects on glucose, fat and protein metabolism. The production of IGF-1 is stimulated by growth hormone (GH) secretion from the anterior pituitary gland which is potentiated by testosterone (51). ADT has been shown to have either no effect on circulating IGF-1, or a 10% increase after 6 months of combined anti-androgen and GnRH therapy (94,95). Higher serum levels of IGF-1 are associated with increased all-cause mortality and PCSM in men with advanced prostate cancer (96). These detrimental effects are also seen in studies of prostate cancer xenografts, where increased expression of IGF-1 and its receptor by prostate cancer cells results in tumour progression to castrate resistant prostate cancer (CRPC) (97).

The actions of the IGFs are modulated by a family of high-affinity IGF binding proteins (IGFBPs 1 – 6) which function to regulate IGF-1 and IGF-2 bioactivity (98). IGFBP-2 is the main IGFBP produced by prostate epithelial cells, and is increased in patients with prostate cancer, correlating with tumour stage and grade (99). Following androgen withdrawal, higher IGFBP-2 mRNA expression promotes androgen-independent tumour growth, and also correlates with a higher Gleason score (100,101). Conversely, higher serum IGFBP-3 is associated with a lower risk of developing advanced-stage prostate cancer (100,102), and in-vitro studies show an increase in IGFBP-3 beginning within months of androgen withdrawal (103). As IGFBP-3 is the principal binding protein for IGF-1, an increase in IGFBP-3 is expected to reduce IGF-1 bioavailability. Thus, higher circulating IGFBP-3 would be of great advantage in cancer patients, exerting direct effects on cancer cells as well as reducing IGF bioactivity.

2.2.3.4 Lipid profile

ADT is associated with altered lipid metabolism. After 12 months of ADT, Smith *et al* (104) found a 9.0% increase in total cholesterol, 11.3% increase in high-density lipoprotein (HDL), 26.5% increase in triglycerides and 7.3% increase in low-density lipoprotein (LDL). Like changes in body composition, these changes are rapid and can occur as early as 3 months following initiation of ADT (105).

Current evidence strongly suggests that lipid availability to cancer cells, whether newly synthesized or exogenously acquired, likely promotes prostate cancer growth and progression (106). Elevated serum triglycerides are associated with increased risk of prostate cancer recurrence after a radical prostatectomy (107). Similarly, high total

cholesterol correlates with increased risk of lymph node metastases and high LDL levels is predictive of high Gleason scores (108). Furthermore, elevated lipids, along with the aforementioned metabolic changes, also results in high cardiovascular mortality amongst prostate cancer patients receiving ADT (109).

2.2.3.5 Cardiovascular disease

Cardiovascular disease (CVD) accounts for approximately a quarter of deaths amongst men with prostate cancer (110). ADT may indirectly contribute to development of CVD by inducing metabolic changes that are well-established risk factors for development of atherosclerosis (111). There is clinical evidence which suggests a positive association between ADT and CVD (111). A meta-analysis of 6 observational studies showed that the risk of cardiovascular mortality was 17% higher amongst those receiving ADT than those without (109). O'Farrell *et al* (112) found the highest risk of mortality in those with a history of CVD before cancer diagnosis, and in the first 6 months of ADT. For these reasons, the United States Food and Drug Administration has issued a warning on GnRH agonists for increased risk of diabetes and certain CVDs (heart attack, sudden cardiac death, and stroke) (113).

2.2.3.6 Changes in other hormonal systems, myokines inflammatory cytokines

Circulating adipokines such as adiponectin and leptin are important regulators of insulin sensitivity. Prostate cancer patients undergoing ADT have leptin levels double that of those who have just undergone prostatectomy and/or radiotherapy without ADT (114). Leptin levels increase in proportion to increase in fat mass, especially central adiposity. Studies of leptin levels and prostate cancer aggressiveness have produced mixed results. While some studies show a positive association between leptin levels and Gleason score (115,116), others did not find serum leptin to be a predictive biomarker for advanced stage following radical prostatectomy (117).

Housa *et al* (118) found higher adiponectin levels in locally advanced, compared to organ confined prostate cancer, and proposed that increased serum adiponectin levels may serve as a protective factor against tumour progression. Conversely, other studies found a negative association between plasma adiponectin levels and histological grade and stage (117,119). Levels of adiponectin have been found to increase with ADT (120,121). This is a paradoxical finding, as generally, adiponectin is characterised by a strong inverse correlation with fat mass and insulin resistance (120). However, the increase in adiponectin does not seem sufficient to counteract the adverse effects of ADT on hyperinsulinaemia (122).

Pro-inflammatory cytokines have also been implicated in the development of diabetes and may be modulated by testosterone (123). After 12 weeks of ADT, there is a fall in interleukin 6 (IL-6) levels along with higher levels of interleukin 1 beta (IL-1β) and interleukin-8 (IL-8) (124). Conversely, Maggio *et al* found that 12 months of ADT did not affect plasma cytokine levels in men with prostate cancer (125). Obesity

is associated with a subclinical inflammatory state with higher plasma concentrations of pro-inflammatory mediators such as IL-6, tumour necrosis factor-alpha (TNF-α) and IL-1β (126). Based on epidemiological studies, higher IL-6 levels are associated with prostate cancer biochemical recurrence (127) and poorer overall survival (128). Increased serum IL-6 levels are also found in patients with castrate-resistant and metastatic prostate cancer (128,129). Similarly, higher levels of TNF-α is associated with more aggressive disease, prostate cancer progression, relapse and mortality (124,130,131).

2.2.3.7 Effect on bone mineral density

ADT is associated with a significant reduction in bone mineral density (BMD), with more rapid bone loss compared to normal aging. Post-menopausal women experience an annual average of 3% decline in BMD at the spine (132). Following initiation of ADT, the annual rate of bone loss at the lumbar spine and femoral neck regions have been reported as 4.6% and 3.8%, respectively (133). In a cross-sectional study, men with prostate cancer treated with ADT had a 7.2-7.8% lower lumbar spine BMD, and trends towards a lower hip BMD compared to men not receiving ADT and healthy controls (134). This reduction in BMD is translated into a higher fracture risk. In a large cohort study of 180,000 older men, ADT increased the relative risk of any fracture and hip fracture by 1.4 (135), thus increasing morbidity and mortality.

2.2.3.8 Psychophysiological effects

The prevalence of depression and anxiety in men with prostate cancer, across the treatment spectrum is high (136). In particular, men receiving ADT have clinically

significant decreased quality of life, particularly in the physical and sexual aspects compared to controls (137). These psychological conditions are associated with psychophysiological side effects that encompass poorer treatment outcomes and reduced survival (136,138,139). In turn, depression results in a chronically activated hypothalamo-pituitary-adrenal axis, immune dysfunction, inflammation, oxidative stress and increased cytokine production thus worsening cancer prognosis (140).

2.2.3.9 Summary

In summary, the negative systemic effects of ADT can potentially worsen prostate cancer prognosis. In the next section of this review, we discuss the clinical trials that utilise PRT in the treatment of these effects in prostate cancer.

2.2.4 The benefits of PRT during ADT

2.2.4.1 PRT and physiological adaptations

Muscle hypertrophy induced by PRT is the product of increased muscle fiber cross-sectional area (141) and is accompanied by the enhancement of subcellular structures (e.g. mitochondrial morphology and density) and increased substrate metabolism. This improvement in the metabolic capacity of skeletal muscle underlies a range of beneficial adaptations that may be particularly important to men treated with ADT.

Much of the current evidence regarding muscle adaptation in PRT is drawn from studies involving the elderly population with sarcopenia, a similar cohort to those on ADT (142). In sarcopenia there is a reduction in the number of both slow switch type I and fast twitch type II muscle fibers and specific type 2 muscle fiber atrophy (143),

leading to a decline in muscle strength (144). PRT in this population has been shown to increase type IIa muscle fiber cross-sectional area (143,145). Thus, this physiological adaptation may improve physical function and contribute to improved glucose metabolism due to increased GLUT4 activity and enhanced insulin response via skeletal muscle (146). Furthermore, PRT also has beneficial effects on mitochondrial function and proteostasis, the loss of which is implicated in the pathophysiology of muscle loss in sarcopenia (147).

2.2.4.2 PRT in the treatment of ADT-induced adverse effects

The benefits of isolated PRT in the treatment of ADT-induced adverse effects have been shown in 5 randomized controlled trials to date (148-152). The details of each trial, including sample size, duration, type of intervention and findings are summarized in tables 1 and 2.

2.2.4.2.1 Effect on body composition and muscular strength

PRT has been shown to be beneficial in the maintenance of LBM during ADT. Alberga *et al* (151) found that patients randomized to the PRT group was able to maintain total LBM as versus the control group. Similarly, Nilsen *et al* (150) documented a site-specific increase in LBM of the lower limb, upper limb and appendicular region in patients receiving 16 weeks of PRT versus control. Skeletal muscle biopsies were collected in one trial (150). Patients in the PRT group had a significant increase in total muscle fiber cross sectional area, with the greatest effect noted in type II muscle fibers, as versus those in the control group, who had an overall

reduction (153). The number of myonuclei per type 1 fiber also increased in the PRT group.

There was a consistent improvement in both upper and lower arm strength across 4 trials following three to twelve months of PRT (148,150-152). Taafe *et al* (152) also reported an improvement in cardiorespiratory fitness in the PRT group, as reflected by an increase in the 400m walk test.

PRT also has positive effects on FM. Alberga *et al* (151) reported that percent body fat significantly increased in the control group versus the PRT group after 24 weeks. Likewise, Winters-Stone *et al* reported a reduction in FM in patients undergoing PRT, as opposed to the control group who gained fat mass (154).

2.2.4.2.2 Insulin resistance and type 2 diabetes

The effect of PRT in men on ADT has not been extensively evaluated. Only one trial by Winters-Stone *et al* (154) reported a reduction in serum insulin and IGF-1 levels in the PRT group compared to an increase in both biomarkers in the control group.

2.2.4.2.3 Bone mineral density

Only two trials investigated bone mineral density (BMD) changes following 16 (150) and 52 (149) weeks of PRT. No differences in BMD outcomes were noted except preservation of BMD at the L4 site in patients in the PRT group versus the control group (149). Bone turnover markers including osteocalcin and urinary deoxypyrodinoline did not change (149) .

2.2.4.2.4 Psychological effects

Physical exercise is recognised as a powerful modulator of neuroplasticity and immune response with immunosurveillance-enhancing properties (155). Health related quality of life (HRQOL) was assessed in three studies (148,150,152). Segal *et al* (148) reported an improvement in HRQOL following PRT while no differences were found by Nilsen *et al* (150). Taafe *et al* (152) found improvement in fatigue and vitality after 6 and 12 months of PRT.

Table 1: Characteristics of randomized controlled trials of isolated PRT in men receiving ADT for prostate cancer

Study identification	Population		Sample size (N) Mean age (y)	PRT intervention	Control condition	Duration (weeks)
	Major inclusion criteria	Major exclusion criteria				
Alberga *et al*, 2012 Canada	Histologically documented prostate cancer; scheduled to receive radiotherapy with or without ADT; consent of treating oncologist (Note: this article reported a subgroup analysis limited to patients on ADT)	Severe cardiac disease (New York Heart Association class III or IV); uncontrolled hypertension, pain, psychiatric illness; residence >1 hour from the study center	N=49 66y	PRT supervised by a certified fitness consultant, 3 sessions/week, 24 weeks Ten exercises (i e leg extension, leg curl, seated chest fly, latissimus pulldown, overhead press, triceps extension, biceps curls, calf raises, low back extension, and modified curl-ups) using 60-70% 1RM load, 8-12 reps per set, 2 sets per exercise Load increased by 5lb (2 3kg) when able to complete >12 reps/set	Usual care (no exercise)	24
Nilsen *et al*, 2015, 2016 Norway	Intermediate or high-risk profile based on PSA and histology and extent of the primary tumour; referred to high-dose radiotherapy 2–6 months after initiation of neo-adjuvant ADT; adjuvant ADT continuing for 9-36 mo ; age ≤75 years;	Strength training ≥1 session/wk; osteoporosis medication usage; medical conditions that could complicate participation	N=58 66y	Two supervised (high intensity) plus one unsupervised (moderate intensity) PRT session/wk, 16 weeks Nine exercises (i e Smith machine half squat, leg press, Smith machine standing calf raises, knee flexion, knee extension, chest press, seated row, seated shoulder press, and biceps curl), 1-3 sets/exercise, 6-10 RM, loading increased with strength adaptation	Usual care (no exercise)	16
Segal *et al* , 2003 Canada	Histologically documented prostate cancer; scheduled to receive >3mo ADT; consent of treating oncologist	Severe cardiac disease (New York Heart Association class III or IV); uncontrolled hypertension (>160/95 mmHg); uncontrolled pain; unstable bone lesions; residence >1 hour from the study center	N=135 68y	PRT supervised by a certified fitness consultant, 3 sessions/week, 12 weeks Nine exercises (i e leg extension, calf raises, leg curl, chest press, lat pulldown, overhead press, triceps extension, biceps curls, and modified curl-ups) using 60-70% 1RM load, 8-12 reps per set, 2 sets per exercise Load increased by 5lb (2 3kg) when able to complete >12 reps/set	Usual care (no exercise)	12
Taafe *et al*, 2017 Australia	Histologically confirmed prostate cancer; >2mo exposure to and anticipated to receive 12 mo additional ADT; without PSA evidence of disease activity; medical clearance	Bone metastatic disease; evidence of PSA disease activity; chronic conditions that could inhibit exercising; non-ambulatory; structured exercise in the previous 3 mo;	N=105 68y	Two supervised PRT group-based sessions (n<10), 2 sessions/wk, 52 weeks Impact-loading: bounding, skipping, drop jumping, hopping, and leaping activities that produced ground reaction forces of 3 4–5 2 times body weight and progressive in nature Resistance training: six principal exercises (i e chest press, seated row, shoulder press, leg press, leg extension, and leg curl) and supplementary exercises, 2-4 sets per exercise at an intensity of 6–12 RM Participants also performed impact loading routine 2 sessions/wk at home	Usual care (no exercise)	52
Winters-Stone *et al*, 2014, 2015 USA	Histologic evidence of prostate cancer; currently receiving ADT; BMD T-score -2 5 or higher; medical clearance from physician	Currently receiving chemotherapy; bone metastases in the hip or spine, bone-active medications other than ADT (e g , bisphosphonates); participation in 30 min or more of moderate–vigorous PRT per week	N=51 70y	Two supervised plus one home-based PRT session/week, 52 weeks PRT exercises (i e wall-sits, squats, bent-knee deadlifts, lunges, one-arm row, chest press, lateral raise, and push-ups) and impact loading (i e two-footed jump) using free weights or weighted vest PRT performed for 1-2 sets x 6-14 reps/exercise Jumping progressed from 1-10 sets x 10 reps All loading progressed with strength gains	Placebo control (stretching and relaxation)	52

Table 2: Key results of the five included RCTs

Study identification	Physiological outcomes (assessments, units)	Adherence to PRT intervention	Key Results
Alberga *et al*, 2012 Canada (103)	Body weight (kg); BMI (kg/m^2); DEXA (total lean mass (kg) and percent body fat (%))	Not reported	Percent body fat increased in the control group versus PRT group (p=0.005); total lean mass was maintained in the PRT group versus a loss in the control group (p=0.005)
Nilsen *et al*, 2015, 2016 Norway	Body composition via DEXA (lean body mass: total, trunk, lower extremities, upper extremities, appendicular; fat mass: total and trunk in kg, and percent body fat (%)); body mass (kg); BMI (kg/m^2); BMD (total body, total lumbar, total hip, trochanter, femoral neck); skeletal muscle biopsy (total muscle CSA, type I and II muscle CSA, μm^2; number of myonuclei, nuclei/fibre; myonuclear domain, cytoplasm:nuclei; number of satellite cells; androgen receptors, myostatin, mitochondrial proteins (i.e. citrate synthase, cytochrome c oxidase subunit IV (COXIV), HSP60); indicators of muscle cellular stress (HSP70, alpha B-crystallin, HSP27, free ubiquitin, and total ubiquitinated proteins)	84% and 88% upper and lower body exercises completed, respectively	Lower extremity (p=0.01), upper extremity (p=0.05) and appendicular (p=0.001) lean body mass significantly increased in PRT versus control. No change in BMD outcomes. Significant increase in total muscle fiber CSA in PRT versus control (p=0.04) showing a larger effect in type II (p=0.03) than type I fibers (p=0.11). Significant increase in number of myonuclei per type I fibers (+17%, p=0.01) but not type II fibers in PRT versus control. Significant reduction in myonuclear domain in type I fibers but not type II fibers in PRT versus control (p=0.05). No change androgen receptor or myostatin content, or any mitochondrial protein or indicator of muscle cellular stress. Post hoc: within-group analysis revealed that HSP70 was reduced in the PRT group and a trend towards a reduction in citrate synthase in the control group
Segal et al., 2003 Canada	Body weight (kg); BMI (kg/m^2); waist circumference (cm), sum of four skinfolds (mm)	79%	No change in body weight, BMI, waist circumference, or subcutaneous skinfolds
Taafe *et al*, 2017 Australia	PSA (ng/ml), total testosterone (ng/dl)	69%	No significant change in PSA or testosterone
Winters-Stone *et al*, 2014, 2015 USA	BMD of proximal femur (total hip, greater trochanter, and femoral neck) and lumbar spine (L1-L4) via DEXA; bone turnover via serum osteocalcin (ng/mL) and urinary deoxypyrodinoline (nmol/L); Body composition via DEXA (total lean mass, total fat mass, and trunk fat mass in kg; percent body fat (%)); insulin (mclU/ml); IGF-1 (ng/ml); SHBG (nmol/ml); total testosterone (ng/dl); body weight (kg)	84% and 43% for supervised and home-based sessions, respectively	PRT had a significant effect on preservation of BMD (-0.4%) at the L4 vertebrae compared with losses (-3.1%) in the placebo control group (p=0.03). Adjusting for patients who completed the study (i.e. follow-up assessments) the PRT program significantly reduced total fat mass (p=0.02) with a trend toward reduced body fat percentage (p=0.06) and trunk fat mass (p=0.07) versus control; and deoxypyrodinoline decreased in the control group versus the PRT group (p=0.03). Reduction of total fat mass (p=0.04) and trunk fat mass (p=0.03) associated with reductions in fasting insulin

2.2.4.3 Summary

In summary, PRT is beneficial in the treatment of ADT-induced adverse effects, with positive effects on body composition, muscle strength and cardiorespiratory fitness and QOL. However, there is currently inconclusive evidence establishing the relationship between PRT and prostate cancer progression and recurrence.

2.2.5 The effect of PRT on cancer growth pathways

In a prospective cohort study following over 2000 men with prostate cancer, it was found that men who were physically active lived significantly longer. Three or more hours per week of vigorous exercise was associated with a 61% decreased risk of dying from prostate cancer (156). Although this association does not necessarily indicate causation, it has led to interest in exploring mechanisms by which exercise might favourably influence the biology of cancer cell growth. While it is known that exercise can lower the risk of developing cancer, and is associated with lower relapse rates and increased survival, its precise anti-cancer effects have not been fully established (138). In the above, the important role of PRT in the treatment of the adverse effects of ADT was discussed. The next section provides a summary of current literature regarding the potential benefits of PRT on oncogenic pathways in prostate cancer. As there is currently a paucity of studies in this area, evidence derived from studies of other pathologies and cancer types will be incorporated. This is outlined in Figure 2.

Figure 2: The potential inhibitory effects of PRT on the prostate cancer growth pathway
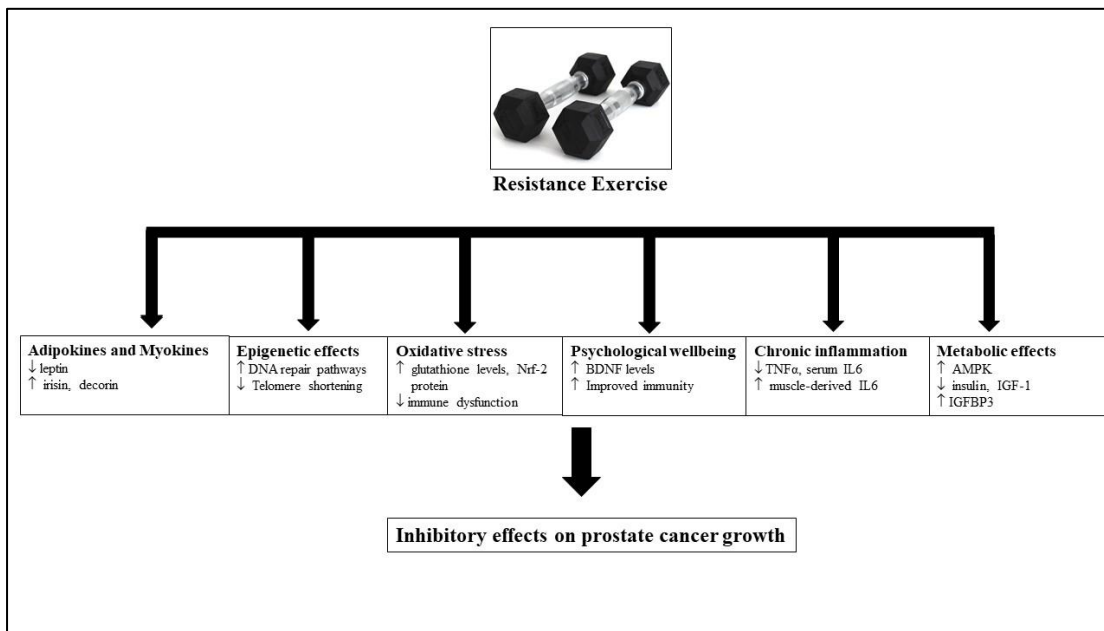
2.2.5.1 Metabolic effects

Muscle tissue produces many factors that are associated with cancer progression and metastatic potential. When muscle contraction occurs during exercise, adenosine triphosphate (ATP) is consumed for energy derivation, reducing the ATP/AMP (adenosine monophosphate) ratio. This results in cellular activation of the liver kinase B1- (LKB1-) adenosine monophosphate-activated protein kinase (AMPK) pathway. AMPK inhibits the mammalian target of rapamycin (mTOR) protein, which has been implicated in prostate cancer progression (157). Powerful muscle contraction results in potent stimulation of AMPK (158) which also results in translocation of the GLUT-4 membrane transporter in myocytes (159), leading to glucose influx and lowering of serum glucose levels, which has a favourable impact on prostate cancer prognosis (160). Stimulation of AMPK also suppresses tumour growth, uptake of glucose and aerobic glycolysis of tumour cells, known as the Warburg effect (161).

Both insulin and IGF-1 regulate cell proliferation, differentiation, survival and apoptosis. These molecules bind to their tyrosine kinase receptors and activate several signalling

pathways including phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mTOR resulting in inhibition of apoptosis and promotion of cell growth and angiogenesis (162). The principal binding protein of IGF-1, IGFBP-3, can reduce IGF-1 bioactivity further inhibiting cancer growth (163). IGFBPs not only modulate the bioavailability and signalling of IGFs, but also have independent actions on cell growth and survival (98). In-vitro studies have shown IGFBP-3 to inhibit proliferation, adhesion, invasion and metastasis of prostate cancer, independent of IGF-1 (164,165). IGFBP-3 is also a potent inhibitor of MAPK signalling, which is implicated in the development of castrate-resistant prostate cancer (166). Higher serum IGFBP-3 is associated with a lower risk of developing advanced-stage prostate cancer (102). However, while PRT has been shown to reduce plasma IGF-1(154) and increase IGFBP-3 (163) levels in prostate cancer, current epidemiological studies in cancer populations show significant heterogeneity in the response of the systemic IGF axis to exercise (167). This discrepancy may be attributed to baseline concentrations of the IGF ligands, as Nishida *et al* (168) showed that participants with elevated baseline IGF-1 experienced the greatest decrease in response to exercise. Furthermore, there are current limitations in oncological research regarding the exercise response of autocrine, as compared to systemic IGF-1 (167). In older adults with rheumatoid arthritis, PRT increased total lean and appendicular muscle mass, which was associated with increases in muscular IGF-1 and IGFBP-3 with no changes in systemic levels (169). Thus, more studies are required in the specific tissue response of the IGF-1 axis to PRT in prostate cancer.

2.2.5.2 Chronic inflammation and antioxidant pathways

It is known that chronic inflammation in prostate cancer is associated with prostate cancer progression and poorer overall survival (130). Stimulation of muscle contraction during PRT

releases myokines that lower systemic inflammation (170), with 4-8 weeks of PRT reducing serum IL-6 and TNFα in prostate cancer patients (171). While IL-6 derived from macrophages and adipocytes has pro-inflammatory effects (170), muscle-derived IL-6 can counteract TNF-α, which as previously discussed, is associated with significantly worse outcomes in men with prostate cancer (130). Muscle-derived IL-6 acts as an energy sensor, and improves overall metabolic function by increasing insulin-stimulated glucose uptake and whole-body fatty acid oxidation (170). Thus, modulation of systemic inflammation may represent one of the pathways in which PRT may inhibit prostate cancer progression.

Skeletal muscle is a major source of reactive oxygen species (ROS) which is balanced by antioxidant enzymes such as catalase, glutathione peroxidase and glutathione reductase (172). ROS increases oxidative stress on DNA, which can contribute to the initiation and progression of prostate cancer (173). In healthy young men, resistance exercise performed regularly for 6 weeks decreased oxidative stress and increased glutathione levels (174). Prostate cancer patients who participated in vigorous activity had greater expression of the nuclear factor erythroid 2-related factor 2 (Nrf-2) in their normal prostate tissue compared to those who were more sedentary. The Nrf-2 protein stimulates the production of anti-oxidant enzymes, and studies in mice show that loss of Nrf-2 correlate with increased ROS and DNA damage leading to neoplastic transformation of normal prostate tissue (175).

2.2.5.3 Adipokines and myokines

Leptin is an adipokine which is a key regulator of appetite control and body weight. It also has a role in energy homeostasis, insulin secretion, angiogenesis and modulation of innate and adaptive immune responses (176). High circulating levels of leptin enhances growth of prostate cancer cells in vitro (177) and PRT has been found to significantly reduce serum leptin levels

in obese men (178). Resistin is an adipokine known to upregulate pro-inflammatory cytokines (179), and induce prostate cancer cell proliferation (180) while adiponectin has anti-inflammatory properties (181). PRT has been found to reduce serum resistin levels in post-menopausal women (182) while increasing serum adiponectin in obese young men (183), but has not been extensively studied in the cancer population

Irisin is a myokine generated in the presence of exercise-induced upregulation of peroxisome proliferator-activated receptor gamma coactivator-1-alpha (PGC-1α). It has a role in the regulation of energy metabolism, browning of white adipocytes and improving insulin sensitivity (184). Irisin has been shown to significantly reduce cancer cell proliferation, migration and viability in malignant cancer cell lines without affecting non-malignant cells. Specifically, irisin has cytotoxic effects on prostate cancer cells (185). It has been suggested that endurance training can increase circulating irisin levels in human subjects (186). Similarly, Zhao *et al* (187) found that 12 weeks of PRT significantly increased serum irisin in older adults. Another potential pathway may involve decorin, which is a proteoglycan and a myokine, stimulated by resistance training (188). Recent discoveries show that decorin reduces cancer growth and dissemination (189). In prostate cancer cell models, decorin prevents androgen receptor nuclear translocation and inhibits the production of PSA (190). In an animal model, systemic administration of decorin significantly reduced prostate cancer bone metastasis (191). Thus, myokines released during muscle contraction may have a direct effect reducing cancer growth and spread.

2.2.5.4 Neurotrophic pathway

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family of growth factors which supports differentiation, maturation and survival of neurons in the nervous

system and a reduction in BDNF is implicated in the development of depression (192). BDNF is also secreted by prostate cancer cells and have mitogenic effects on the prostatic epithelium (193). A 12-week resistance training program in older male subjects was found to increase circulating plasma BDNF levels which returned to baseline after de-training (194). Thus, the BDNF signaling pathway may represent one of the modalities by which resistance exercise inhibits prostate cancer cell growth.

2.2.5.5 Epigenetic effects

Exercise has epigenetic effects on the phenotypic expression of various genes involved in cancer (138). In men with low risk prostate cancer, cell cycling and DNA repair pathways were upregulated in those who participated in ≥ 3 hours/week of vigorous activity compared to those who did not (195). MicroRNAs (MiRNA) are small, endogenous non-coding RNA which can modify protein expression through cleavage of specific target mRNAs or through inhibition of their translation. In prostate cancer the presence of oncogenic miRNAs such as miR-21 is predictive of cancer recurrence following radical prostatectomy (196), and serum levels of miR-21 have been found to decrease immediately after resistance exercise in healthy young men (197). Dimauro *et al* (198) found that 12 weeks of moderate intensity, explosive-type resistance training in an elderly cohort counteracts shortening of telomeres, which are nucleotides at the end of chromosomes that protect their integrity. Telomere shortening is one of the earliest molecular genomic events in prostate tumorigenesis and can generate genomic instability (199). In men with early prostate cancer, those who followed a comprehensive lifestyle program, including regular exercise, had increases in telomere length after 5 years (200).

2.2.6 Future Research Directions and Conclusion

To conclude, as the population ages and the number of prostate cancer diagnoses increases across the population, we are likely to encounter more of the deleterious effects of ADT. There is now an endorsement by various oncological societies to incorporate the use of physical activity as synergistic medicine during prostate cancer treatment. PRT is an exercise modality that has been shown to be of benefit in the maintenance of body composition and muscle function during ADT, although it is important to note that current evidence exhibits major heterogeneity within and between studies in terms of patient characteristics and type of PRT intervention. Despite compelling evidence for the application of PRT as standard of care in patients with prostate cancer, there is still paucity in the literature regarding its use in this population. This includes its benefits on specific muscle groups, and its impact on physiological endpoints such as glucose and insulin metabolism, bone turnover, and the adipokines, myokines and inflammatory cytokines affected during ADT, thus providing scope for future research.

Furthermore, this review summarises the current body of evidence on the potential signalling pathways modified either directly or indirectly by PRT, and its positive effects on cancer growth and progression. As many of these pathways are also implicated in the development and progression of prostate cancer, more clinical studies are required in this area to obtain a better understanding of the mechanisms of benefit of PRT.

In summary, PRT is an exercise modality with great potential in the treatment of ADT-induced adverse effects. However, more research is required regarding its impact on the physiological and biochemical pathways involved in prostate cancer progression.

2.3 Evidence of benefit for home-based exercise programs in the prostate cancer population

As aforementioned, there are consistent positive outcomes regarding the benefits of exercise in reducing treatment related adverse effects and improving symptoms in men with prostate cancer (21). Due to advancements in the field of exercise oncology, the American Cancer Society and American College of Sports Medicine have implemented exercise guidelines relating to prostate cancer survivors. Survivors are advised to avoid inactivity regardless of cancer stage and treatment, and to undertake 150 min/week of moderate or 75 min/week of vigorous aerobic exercise, and to perform resistance exercise of moderate or high intensity on two or more days a week (201). However, in a population-based sample of Australian men, Galvao *et al* found that only 12% of men with prostate cancer reported meeting current exercise-oncology guidelines with the large majority (~48%) being inactive. Most inactive men were in the contemplative or preparation stage of motivation readiness (21). Thus, for this reason, trials of supervised exercise programs in the cancer population report better compliance rates and improved outcomes compared to home-based exercise programs (152,202). Supervised programs have the advantage of offering participants a safe, secure, well-equipped exercise environment with experienced fitness consultants to provide leadership, build confidence and provide ongoing positive feedback (148). However, supervised programs have limitations in terms of accessibility (particularly for patients living outside of metropolitan areas), expense, and transitioning to community-based programs can often be confronting for patients (203-205). Thus, home-based exercise programs may provide an alternative option.

Currently, there is a paucity of literature relating to the viability and efficacy of home-based exercise (either PRT and/or aerobic) programs in the prostate cancer population. Kim *et al*

(206) compared a 6-month home-based exercise program consisting of weight bearing and resistance exercise to whole body stretching performed 3 to 5 times per week in men with prostate cancer on ADT and found a patient retention rate of 80.4% at 6 months. There were no significant differences between groups in terms of BMD and QOL. However, patients in the exercise group had preservation of muscle strength, as measured by hand grip strength. Attrition was prevented by employing individual exercise prescriptions, goal setting, educational verbal persuasion and frequent monitoring of exercise behaviors (206). In a feasibility study, Alibhai *et al* (207) found that a 6-month, home-based mixed aerobic and PRT program had a probability of > 20% of being inferior to supervised personal training in terms of physical fitness as measured by VO_2 peak and grip strength. In a multicenter, 12-month RCT consisting of a supervised mixed aerobic/PRT program for 6 months followed by a similar home-based program for 6 months, Galvao *et al* (208) found an improvement in cardiorespiratory fitness, lower limb physical function, muscle strength, QOL and physical and emotional functioning at both 6 and 12 months. However, the significant gains in LBM at 6 months were not sustained at 12 months.

Thus, the evidence pertaining to the benefits of a home-based exercise program in men with prostate cancer remain inconsistent. Given that a home-based program may represent a cost-effective intervention to enhance exercise participation in this population, a greater number of studies are required to assess its feasibility and efficacy in the longer-term, as well as the type of exercise (aerobic versus PRT) that would provide maximal benefit and compliance rates.

Chapter 3: Testosterone prevents protein loss via the hepatic urea cycle in humans

Publication

Lam T, Poljak A, McLean M, Bahl N, Ho KKY, Birzniece V. Testosterone prevents protein loss via the hepatic urea cycle in humans. European Journal of Endocrinology. 2017; 176:489-496

3.1 Abstract

Context: The urea cycle is a rate limiting step for amino acid nitrogen elimination. The rate of urea synthesis is a true indicator of whole-body protein catabolism. Testosterone reduces protein and nitrogen loss. The effect of testosterone on hepatic urea synthesis in humans has not been studied.

Objective: To determine whether testosterone reduces hepatic urea production.

Design: an open label study

Patients and Intervention: Eight hypogonadal men were studied at baseline, and after two weeks of transdermal testosterone replacement (Testogel, 100 mg/day).

Main Outcomes Measures: The rate of hepatic urea synthesis was measured by the urea turnover technique using stable isotope methodology, with $^{15}N_2$-Urea as tracer. Whole-body leucine turnover was measured, from which leucine rate of appearance (LRa), an index of protein breakdown and leucine oxidation (Lox), a measure of irreversible protein loss, were calculated.

Results: Testosterone administration significantly reduced the rate of hepatic urea production (from 544.4 ± 71.8 to 431.7 ± 68.3 µmol/min; $p < 0.01$), which was paralleled by a significant reduction in serum urea concentration. Testosterone treatment significantly reduced net protein loss, as measured by percent Lox/LRa, by 19.3 ± 5.8 % ($p < 0.05$). There was a positive association between Lox and hepatic urea production at baseline ($r^2 = 0.60$, $p < 0.05$) and after testosterone administration ($r^2 = 0.59$, $p < 0.05$).

Conclusion: Testosterone replacement reduces protein loss and hepatic urea synthesis. We conclude that testosterone regulates whole body protein metabolism by suppressing the urea cycle.

3.2 Introduction

Testosterone increases muscle mass and strength and improves physical function in men (209). Declining levels of testosterone are associated with the development of sarcopenia and frailty in men, and testosterone replacement in hypogonadal men increases muscle mass and strength (2,210). However, tissue-specific biochemical mechanisms of androgen action are not fully understood. Testosterone regulates amino acid availability, facilitating reutilization and increasing muscle protein accretion (31,211). We have previously shown that the whole body protein anabolic effects of testosterone are mediated through the liver (50,51). In hypogonadal men, selective exposure of the liver to testosterone by oral administration reduces protein oxidation, a hallmark of catabolism. This effect is indistinguishable to that of systemic (transdermal) testosterone administration. Thus, the liver is the primary site underpinning the anti-catabolic effect of testosterone. However, the intrahepatic biochemical pathways mediating the protein anabolic action of testosterone are unclear (53,54).

Protein mass is constantly turning over in a dynamic process of breakdown and synthesis. Amino acids, derived from proteolysis are either oxidized and irreversibly lost, or resynthesized into protein. The liver is a major site of protein metabolism and degradation of amino acids, which is controlled by the urea cycle. Surplus α-amino nitrogen derived from degradation of amino acids enters the urea cycle as ammonia and is converted to urea in hepatocytes, and subsequently eliminated. This represents an irreversible loss of protein nitrogen (212). Hormones such as glucagon, glucocorticoids and growth hormone regulate urea cycle enzymes in the liver (11,47). Early studies in rats also observed an inhibitory effect of testosterone on the arginine synthetase system, a key component of the urea cycle (13). While it has since been demonstrated that testosterone increases nitrogen retention (12), evidence that testosterone suppresses urea production in humans is lacking.

61

The aim of this study was to determine in humans whether testosterone inhibits hepatic urea production. We employed stable isotope studies to investigate the effects of systemic testosterone administration on hepatic urea production in hypogonadal men. The leucine turnover technique was undertaken simultaneously to confirm that testosterone enhanced whole body protein anabolism.

3.3 Subjects and Methods

3.3.1 Subjects

Eight hypogonadal men were recruited from Endocrine Outpatients Clinics in Western Sydney, Australia. Inclusion criteria included males aged between 18 to 75 years with diagnosed primary or secondary hypogonadism, and adequate replacement of other hormones in the case of hypopituitarism. Exclusion criteria included patients with diabetes mellitus, malignancies, chronic renal or hepatic illnesses, or taking medications known to interfere with the endocrine system. Out of eight patients, one had Klinefelter Syndrome, four had pituitary adenomas with hypogonadism, and three had hypogonadism of unknown cause.

This study was approved by the Western Sydney Local Heath District Human Research 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 (ACTRN 1261000577415).

3.3.2 Study design

In an open label study, eight hypogonadal subjects were studied at baseline, and after two weeks of testosterone replacement by the transdermal route (Testogel; Besins Healthcare Australia Pty Ltd, 10 mg/g), administered at a dose of 100 mg/day. 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 protein breakdown and oxidative loss, respectively, 2) energy expenditure, 3) body composition, and 4) other biochemical markers of intervention, including serum levels of testosterone, urea, sex-hormone binding globulin (SHBG) and insulin-like growth factor. All participants were instructed to follow their usual diet and physical activity. Participants were studied after an overnight fast in the Blacktown Clinical School and Research Centre, Australia. At each visit, study blood samples were collected and placed on ice, and plasma and serum were separated and stored at -80ºC until analysis.

3.3.3 Methods

3.3.3.1 Protein turnover

The leucine turnover technique was used to measure whole-body protein metabolism. This method is based on the principal of steady-state kinetics whereby the rate of appearance of a substrate equals its rate of disposal. 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. The LRa and Lox were calculated as previously described (213). A-Ketoisocaproic acid (KIC) is formed when leucine undergoes transamination. It is used as a surrogate marker of leucine as it more accurately reflects the intracellular environment (214).

A 0.014 mg/kg priming dose of NaH^{13}CO$_3$ was given after an overnight fast, followed by a primed constant 3 hour infusion of 1-[^{13}C]leucine (prime 0.5 mg/kg, infusion 0.5 mg/kg/h) (215,216). NaH^{13}CO$_3$ and 1-[^{13}C] leucine were obtained from Cambridge Isotope Laboratories (Woburn, MA). On each visit, blood and breath samples were collected before (-10 and 0 min) and during (140, 160, and 180 min) the leucine infusion. Blood was placed on ice, and plasma was separated and stored at -80°C. As described by Nissen et al, KIC was extracted from plasma (217). Plasma KIC enrichment with ^{13}C was measured by gas chromatography-mass spectrometry (GCMS) (MSD 5971A, model 5890, Hewlett-Packard Co., Palo Alto, CA, USA). CO$_2$ enrichment with ^{13}C in breath samples was measured at the University of Surrey, United Kingdom, on a Delta Plus XP isotope ratio mass spectrometer fitted with a Gas Bench II inlet system (Thermo Fisher Scientific, Hemel Hempstead, UK). Based on previous experiences, our coefficients of variation (CV) for LRa and Lox are 3.5 and 6.1%, respectively (218).

3.3.3.2 Urea synthesis

The rate of urea synthesis was measured by the urea turnover technique using stable isotope methodology, with ^{15}N$_2$-Urea as tracer. Surplus α-amino nitrogen derived from protein degradation enters the hepatic urea cycle as ammonia and is converted to urea. Thus, the rate of urea synthesis is a true indicator of net protein catabolism. Previously functional hepatic nitrogen clearance studies used blood α-amino nitrogen concentrations and urinary urea-N excretion during infusion of amino acids (219). Our technique is based on an earlier protocol which employs stable isotopes in the measurement of urea synthesis (220), offering time and sensitivity advantage.

After an overnight fast, a priming dose of ^{15}N$_2$-Urea (3.4 mg/kg; Cambridge Isotope Laboratories, Andover, MA) was given, followed by a continuous infusion of the tracer at 0.34

mg/kg/h for 4 hours. On each visit, blood samples were collected before (-10 and 0 min) and during (120, 180, 210 and 240 min) the primed infusion, when steady state was reached. Plasma samples were collected into chilled tubes, separated immediately and stored at -80ºC 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 described (221), with modifications. Briefly, plasma samples were treated with ethanol for one hour at -20ºC to precipitate proteins. The labelled and non-labelled urea in the samples was then converted to 2-hydroxypyrimidine using malonaldehyde bis (dimethyl acetal) and HCl during the one-hour incubation. The samples were evaporated to dryness in a Speed Vac concentrator for at least three hours and reacted with MSTFA (N-methyl-N-(trimethyl-silyl) trifluoroacetamide) to form a trimethylsilyl derivative of 2-hydroxypyrimidine. Enrichments of $[^{15}N_2]$ and $[^{15}N_1]$-urea were determined by GC-MS (MSD 5971A, model 5890, Hewlett-Packard Co., Palo Alto, CA, USA). Enrichments were expressed as tracer to tracee molar ratios and calculated from the deconvoluted enrichment peak ratios (m/z 155/153). 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 tracer to tracee ratio. In our hands, day to day variation in urea production is 5.5%, inter-assay CV 3.5%, and intra-assay CV 1.8%, assessed in four healthy men on two occasions one week apart.

3.3.3.3 Energy expenditure

Whole-body energy expenditure and substrate oxidation were measured by indirect calorimetry using an open-circuit ventilated hood system (ParvoMedics, Sandy, UT, USA), calibrated against standard gases before each study. The participants rested in a supine position

for at least 30 minutes and a clear plastic hood was placed over their heads for a period of 20 minutes. Two measurements were taken and averaged.

3.3.3.4 Body composition

Fat mass (FM), lean body mass (LBM), and extracellular water (ECW) were assessed using Bioelectrical Impedance Spectroscopy (BIS; SFB7 analyser, ImpediMed Ltd, Qld, Australia) (222). After 20 minutes of rest, two measurements were taken in the supine position, and the average was taken. Body cell mass (BCM), an estimate of muscle mass, was calculated by subtracting ECW from the LBM.

3.3.4 Assays

All samples for any individual were measured in the same assay run for each analyte. Concentrations of serum SHBG, testosterone and total PSA were measured by an electrochemiluminescence immunoassay (ECLIA) using a commercial assay kit (Roche Diagnostics, USA). The CV for SHBG at 45.7 nmol/L was 2.1% and the CV for total testosterone at 8.7 nmol/L was 2.8%. The CV for total PSA at 0.3 ng/mL was 2.4%. Serum urea and albumin were measured photometrically. The CV for urea at 7.2 mmol/L was 1.2%, and albumin at 49.6 g/L was 0.4%. The concentration of serum insulin was measured by chemiluminescent microparticle immunoassay (CMIA) and the CV at 55.08 µU/mL was 2.5%. Serum creatinine was measured by a kinetic calorimetric assay and the CV at 72.9 µmol/L was 2.3%. Serum IGF-I levels were measured by RIA after acid ethanol extraction, and the CVs for IGF-I were 8.3% at 14.7 nmol/l and 7.4% at 28.6 nmol/l.

3.4 Statistical analysis

The treatment effects of testosterone on urea synthesis and protein turnover were assessed using paired t tests. Results are expressed as mean ± SEM, and a p value <0.05 was significant. Linear regression analysis was used to correlate changes in urea synthesis with other endpoint measures. A linear mixed-effect model was used to determine the effect of changes in serum testosterone, LBM, BCM and IGF-1 on urea synthesis. Statistical analysis was undertaken using the statistical software package SPSS statistics v22 (IBM corporation, Armonk, NY, US) and RStudio (Boston, MA, US).

3.5 Results

The mean age of participants was 49.1 ± 4.9 years. Their clinical, hormonal and metabolic characteristics at baseline, and after two weeks of transdermal testosterone therapy are shown in Table 1. Administration of 100 mg/day of transdermal testosterone for two weeks increased serum levels of testosterone into the normal range (Figure 3).

Figure 3: Serum testosterone levels at baseline and at the end of the two-week treatment period with transdermal testosterone (100 mg/day). Data are presented as mean ± SEM.
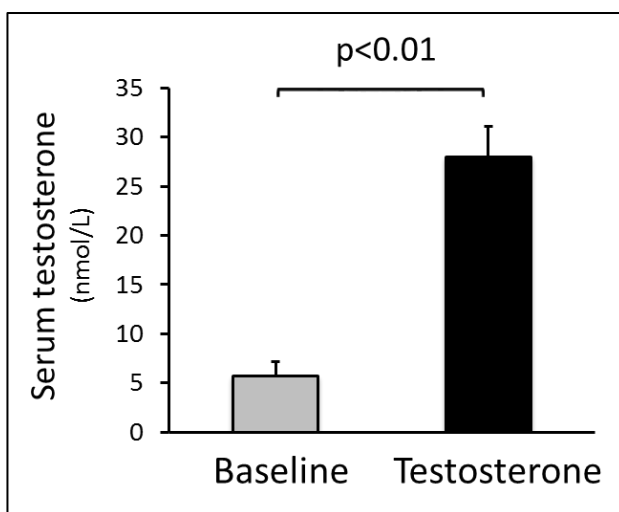
3.5.1 Urea and protein turnover

The baseline rate of hepatic urea synthesis was 544.4 ± 71.8 µmol/min. Following testosterone administration for two weeks, urea production fell significantly by $21 \pm 5\%$ ($p < 0.01$; Figure 4) accompanied by a fall ($p < 0.01$) in serum urea concentration (Table 3).

Figure 4: Urea production rate at baseline and the end of the two-week treatment period with transdermal testosterone (100 mg/day). Data are presented as mean $\pm$ SEM.
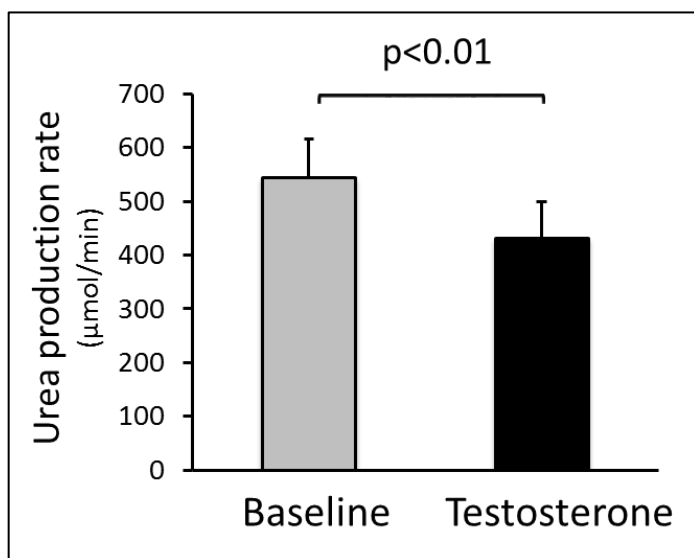
Table 3: Clinical, hormonal, and metabolic measures at baseline and after two weeks of transdermal testosterone treatment

	Baseline	Testosterone	**P value**
Weight (kg)	99.9 ± 14	101 ± 14	**<0.05**
BMI	31.5 ± 3.9	31.8 ± 3.9	**<0.05**
Lean body mass (% weight)	69.1 ± 2.1	69.9 ± 2.1	**0.32**
Body cell mass (kg)	46.0 ± 5.6	46.6 ± 5.1	**0.42**
Extra-cellular water (L)	22.0 ± 3.0	22.5 ± 2.9	**0.19**
SHBG (nmol/L)	29.6 ± 6.4	27.3 ± 5.2	**0.19**
IGF-1 (nmol/L)	17.6 ± 2.9	18.4 ± 2.9	**0.30**
Urea (mmol/L)	6.0 ± 0.5	4.4 ± 0.4	**<0.01**
Creatinine (µmol/l)	77.9 ± 3.3	76.8 ± 2.4	**0.57**
Albumin (g/l)	41.4 ± 0.9	40.9 ± 8.0	**0.41**
ALT (IU/l)	28.8 ± 2.1	27.0 ± 2.1	**0.47**
AST (IU/l)	27.6 ± 2.7	24.6 ± 1.4	**0.37**
Glucose (mmol/L)	5.6 ± 0.3	5.7 ± 0.2	**0.40**
Insulin (mIU/L)	59.9 ± 13.0	77.9 ± 35.2	**0.58**
PSA (ng/mL)	0.80 ± 0.3	0.94 ± 0.2	**0.07**
REE (kcal/day)	1717.6 ± 135.5	1793.8 ± 154.5	**<0.05**
Fox (mg/min)	53.2 ± 5.5	54.0 ± 10.1	**0.93**
Cox (mg/min)	116.6 ± 13.3	128.2 ± 26.9	**0.64**

Data are presented as mean ± S.E.M. BMI, body mass index; SHBG, sex-hormone binding globulin; IGF-1, insulin-like growth factor; ALT, alanine aminotransferase; AST, aspartate aminotransferase; PSA, prostate-specific antigen; REE, resting energy expenditure; Fox, fat oxidation; Cox, carbohydrate oxidation.

Testosterone administration did not significantly affect LRa, a measure of protein turnover (Table 4). Leucine oxidized as a proportion of LRa (percent Lox/LRa), which represents the proportion of amino acids that are irreversibly lost when adjusted for changes in protein turnover, was significantly reduced by 19.3 ± 5.8 % ($p < 0.05$) following testosterone administration.

Table 4: Effects of testosterone on whole body protein turnover

	Baseline	Testosterone	**P value**
LRa (µmol/min)	211.1 ± 22.6	215.8 ± 26.0	**0.57**
Lox (µmol/min)	42.8 ± 5.2	35.9 ± 6.0	**<0.05**
Lox (% from Ra)	20.5 ± 1.5	16.4 ± 1.6	**<0.05**

Data are presented as mean ± SEM. LRa, leucine rate of appearance (a measure of protein breakdown); Lox, leucine oxidation (a measure of irreversible loss of protein).

3.5.2 Other endpoint measures

On average, the weight of participants increased by 1.1 ± 0.4 kg (Table 3). Two weeks of testosterone administration did not significantly change any of the body composition parameters. Following two weeks of testosterone administration, there was a significant increase in resting energy expenditure by 76.2 ± 26.4 kcal/day ($p < 0.05$). There were no significant changes in the rates of fat or carbohydrate oxidation. There were no significant changes in any of the measured endocrine markers, including serum levels of SHBG, IGF-1, glucose or insulin. Similarly, testosterone did not significantly change levels of liver transaminases (AST, ALT), albumin, creatinine or prostate-specific antigen (Table 3).

3.5.3 Association between urea synthesis and other end-point measurements

The rate of hepatic urea production was significantly correlated with net Lox, a maker of irreversible loss of protein before (r^2 =0.60, $p < 0.05$; Figure 5a) and after testosterone replacement ($r^2 = 0.59$, $p < 0.05$). The slopes of the relationship between urea production and Lox were similar before and during the treatment (p=0.34). Hepatic urea production significantly correlated with serum urea concentration at baseline and during testosterone

administration (p<0.05; Figure 5b). The reduction in hepatic urea synthesis could not be predicted by changes in serum testosterone, LBM, BCM or IGF-1 by a linear mixed-effects model.

Figure 5a: Association between hepatic urea production and leucine oxidation (Lox) at baseline (broken line) and at the end of the testosterone administration (solid line)
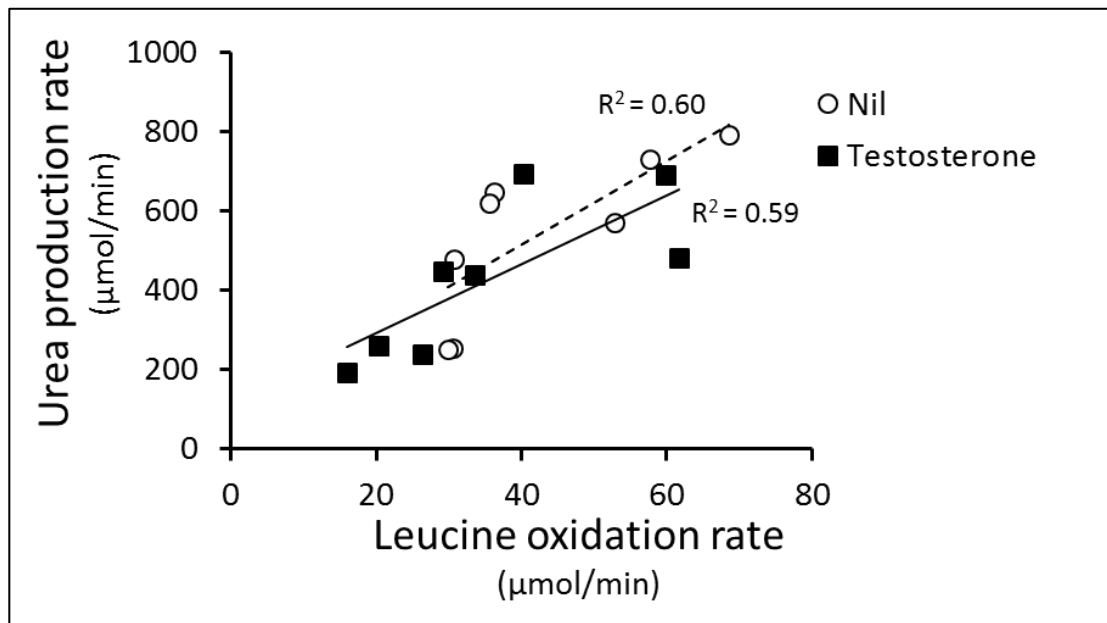
Figure 5b: Association between hepatic urea production and serum urea concentration at baseline (broken line) and at the end of the testosterone administration (solid line).
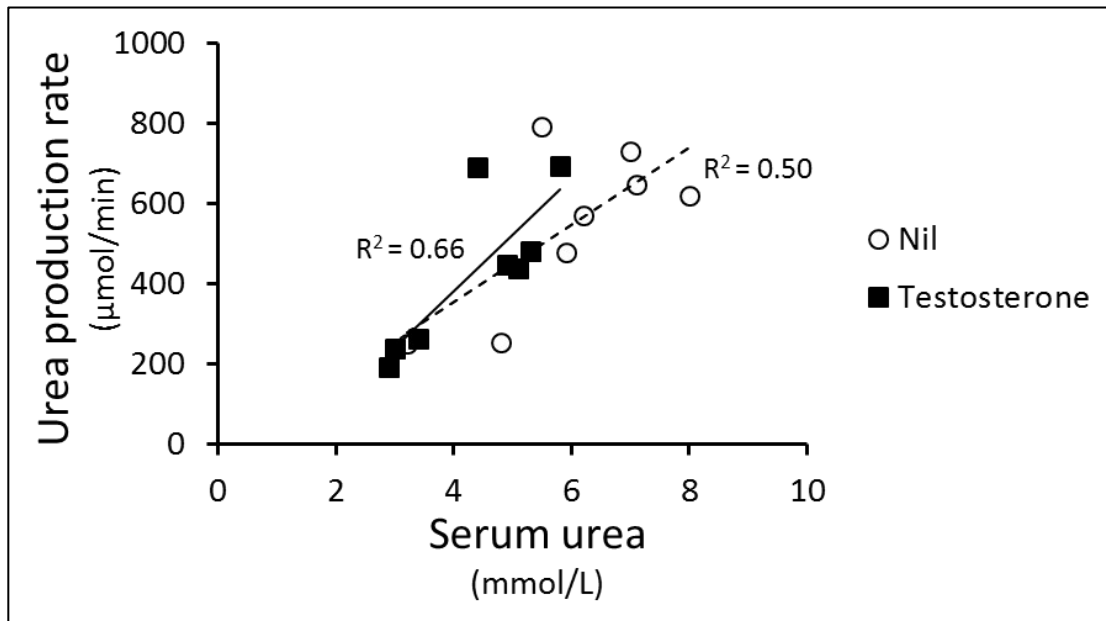
3.6 Discussion

This study is the first to investigate the effects of testosterone on hepatic urea production in humans. We show that testosterone replacement significantly reduces hepatic urea production in hypogonadal men. This occurs in parallel with a significant fall in leucine oxidation, an index of irreversible loss of whole-body protein. We provide evidence that testosterone induction of protein anabolism in humans is mediated by inhibition of the hepatic urea cycle, thereby conserving amino acids for protein synthesis (Figure 6).

Hormones that cause catabolism, such as glucocorticoids and glucagon stimulate urea synthesis, as reflected in an enhancement of functional hepatic nitrogen clearance (44). At the *in vitro* level, glucocorticoids increase gene expression of enzymes promoting urea synthesis (47). Similarly, treatment with glucagon up-regulates enzymes of the urea cycle in cultured rat

hepatocytes such as carbamoyl phosphate synthetase, arginosuccinase and arginase (47). The reverse occurred when rats were treated with growth hormone, an anabolic hormone (11). However, the effect of testosterone is not established, and the evidence from animal studies is contradictory. In ovariectomised female rats testosterone treatment for ten days significantly reduced arginine synthetase activity, a key enzymatic component of the urea cycle (49). However, intraperitoneal injection of testosterone into female rats has been also shown to increase liver arginase activity (14). Thus, early work in female rats report discordant effects of testosterone on urea cycle enzyme activity. Although it is long established that testosterone is nitrogen sparing (12), biochemical evidence that testosterone inhibits the urea cycle has been lacking. We here provide unequivocal evidence for a direct effect of testosterone on the hepatic urea cycle in humans. We found that restoring testosterone levels into the normal range reduced hepatic urea production by approximately 20% in hypogonadal men.

Figure 6: Protein metabolism:
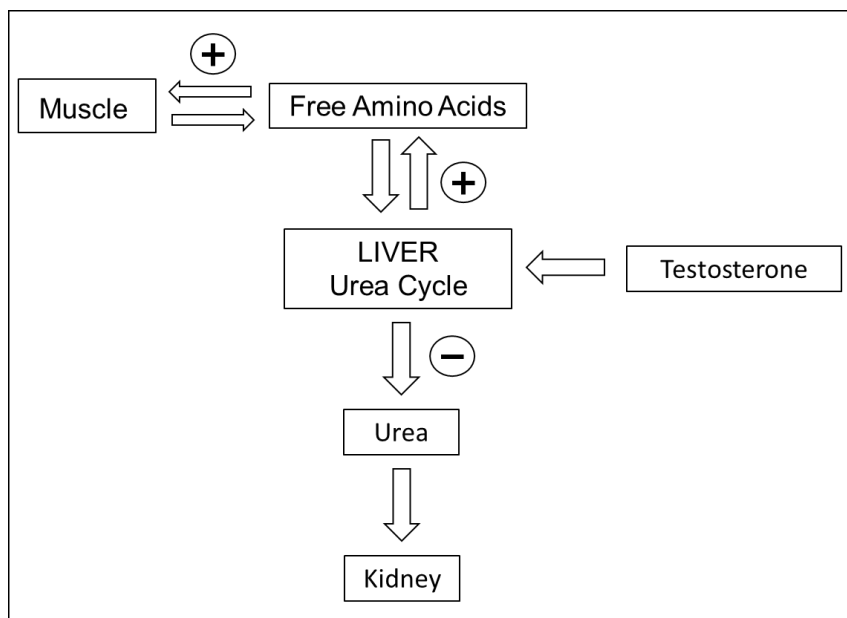
Amino acids derived from proteolysis in muscle partake in a shuttle to and from the liver with a proportion metabolised irreversibly to urea in the liver and excreted by the kidney. We propose that testosterone reduces amino acid loss via inhibition of the hepatic urea cycle.

Androgens increase muscle mass and strength (4). While androgens regulate muscle protein economy indirectly through the urea cycle, they also act directly on muscle stimulating myoblast growth and differentiation, inducing muscle fibre hypertrophy (7). The extent to which the indirect and direct actions of androgens contribute to muscle mass has been a subject of great interest and explored by utilizing muscle specific AR knockout models. These models provide evidence of a cell type-specific effects of AR activation in the regulation of muscle anabolism. Studies in myocyte-specific AR knockout (mARKO) mice show surprising preservation of skeletal muscle mass, with muscle loss restricted only to the highly androgen-sensitive levator ani muscle (223,224). Conversely, studies in mARKO mice on the effects of orchidectomy (223), and global AR knockout mice, show a significant but selective reduction in the mass of hind limb skeletal muscles (23). This indicates that androgen receptor activation purely in myocytes is insufficient to induce muscle anabolism. It has been proposed that androgen effects on muscle may be mediated through other cell types, such as satellite cells, myofibroblasts and motor neurons (225,226), via non-genomic pathways (227), or through testosterone aromatization to oestradiol (228). Thus, the regulation of muscle mass by androgens is complex, involves direct, indirect and paracrine mechanisms. We now add an additional component to this system by demonstrating that testosterone action on the liver reduces loss of amino acid nitrogen, potentially increasing the pool available for muscle anabolism.

There is a linear relationship between the hepatic urea synthesis rate and the blood amino acid concentration. Therefore, reduction in amino acid concentration in the blood (due to e.g. decrease in protein breakdown in the muscle) may reduce the rate of urea production. However, this did not occur, as we provided evidence that protein breakdown (LRa) was unaffected by testosterone replacement. Thus, it is highly unlikely that the mechanism for reduced urea production in our study would have resulted from a reduction in amino acid

availability. Furthermore, previous research reports increase in amino acid plasma concentration during testosterone replacement (229). As amino acid availability in blood is expected to increase with testosterone administration, this would result in an increase in urea production. However, the reverse was found in our study. Therefore, this indicates that testosterone must act on the liver directly to reduce urea production.

The leucine and urea turnover techniques are established methods for quantifying whole-body protein metabolism. Both methods quantify the oxidative metabolism of amino acids by tracking the disposal of labelled molecular constituents of amino acid. The leucine turnover technique tracks the metabolic fate of the carbon moieties of amino acids, providing a measure of whole-body protein oxidative loss. The urea turnover technique tracks nitrogen through the generation of urea in the liver. As these are processes common to amino acid oxidative catabolism, the finding of a strong relationship between rates of leucine oxidation and urea production in the present study, is a predictable outcome. Not only does this association validate the integrity of both methodologies in our hands, it confirms that leucine and urea turnovers provide internally concordant indices of whole-body protein turnover.

We have previously reported that the inhibition of whole-body leucine oxidative loss by testosterone in hypogonadal men is equal between oral and transdermal delivery (51). Because rates of Lox and urea production are measures of amino acid catabolism, this means that testosterone delivered by the oral and transdermal routes is expected to reduce urea production equally. Thus, androgen exposure to the liver underlies the mechanistic prevention of whole-body protein catabolism by testosterone. Strong evidence for the importance of this mechanism comes from our study findings that solely hepatic testosterone exposure delivered by the oral route reduces catabolism in hypopituitary men with hypogonadism and in post-menopausal women (50,51). As the effect of the liver-targeted testosterone was only evident in the presence of growth hormone (GH) (51), full testosterone effect on protein metabolism is expected in the

current study, as these patients had isolated hypogonadism with an intact GH-IGF-1 axis. This indicates that in the presence of adequate hepatic exposure to GH, testosterone reduces whole body protein loss by affecting the hepatic urea cycle. These findings open a novel approach of targeting the hepatic urea cycle to enhance whole body protein anabolism. This liver-targeted approach has an advantage in situations where systemic testosterone treatment poses a health risk in men or virilisation in women. Thus, there is a potential to develop liver-targeted testosterone as a novel, safe and cost-effective treatment for sarcopenia in both men and women.

This study has some limitations, such as the small sample size and that it was not blinded, or placebo controlled. However, as each patient served as its own control, this short-term intervention causing substantial changes in hepatic urea production in the absence of any changes in lifestyle and body composition provides strong evidence of a biological effect. The outcome measures are objective and provide proof of principle that in humans testosterone reduces hepatic urea production in parallel with a reduction in an independent measure of whole-body protein loss. The results are unlikely to occur by chance since reduction in urea synthesis (-21 %) exceeds that of the day-to-day reproducibility of the method (CVs 5.5%).

In summary, we provide the first evidence that testosterone stimulates protein anabolism by acting on the liver to reduce irreversible nitrogen loss through the urea cycle. These findings are highly significant as may lead to the future use of liver-targeted testosterone therapy as a potential intervention in the treatment of sarcopenia, without the adverse effects of systemic testosterone.

Chapter 4: A potent liver mediated mechanism for loss of muscle mass during androgen deprivation therapy

Publication

4.1 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 randomised controlled trial.

Patients and Intervention: Twenty-four prostate cancer patients were studied before and after 6 weeks of ADT. Patients were randomised 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 $12.6\pm4.9\%$ ($p<0.05$). 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.

4.2 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 (4). In men, testosterone is critical for maintaining muscle mass and function, bone mass and body composition (230). Testosterone levels begin to decrease from the fourth decade of life, with up to 1% reduction per year of increasing age (231). This is exacerbated by the increasing prevalence of obesity, burden of disease and medication use with aging, resulting in poorer health outcomes (232). In older men, testosterone replacement increases lean body mass (LBM), reduces fat mass (FM) (236), with some studies also showing an improvement in physical function (237). 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 (74,238). 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 (50,51), with the recent discovery that the urea cycle may be the intra-hepatic pathway mediating this process (239). Administration of testosterone in hypogonadal men significantly reduced the rate of hepatic urea production, paralleled by a reduction in protein loss (239). 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 (240). Similarly, PRT in

prostate cancer patients on ADT can mitigate adverse changes in body composition (20). A supervised exercise program instituted at the start of ADT has been found to preserve appendicular lean mass and prevent gains in fat mass over 3 months (241). 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.

4.3 Subjects and Methods

4.3.1 Subjects

Twenty-four men with prostate cancer scheduled to receive conventional ADT with gonadotrophin releasing hormone (GnRH) analogues 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 to 80 years with histologically confirmed prostate cancer of early or locally advanced stage, or metastatic disease with bone involvement only ($\leq$ 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).

4.3.2 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 (4) other biochemical markers, including serum levels of testosterone, urea, sex-hormone binding globulin (SHBG), 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 8.30 – 9.00 am, 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 usual care (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.

4.3.3 Progressive Resistance Training 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 3 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 (242). One week of exercise supervision (2 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 logbook 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.

4.3.4 Methods

4.3.4.1 Protein turnover

Whole-body protein metabolism was measured using the leucine turnover technique as previously described (239). In brief, after an overnight fast, an intravenous (IV) priming dose of $NaH^{13}CO_3$ was followed by a primed constant 3-hour IV infusion of 1-[^{13}C] leucine (Cambridge Isotope Laboratories; Woburn, MA). 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 (214). KIC was extracted from plasma as described by Nissen *et al* (217) and plasma KIC enrichment

with ^{13}C was measured by gas chromatography-mass spectrometry (GCMS; MSD 5971A, Hewlett-Packard Co., Palo Alto, CA). 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, Hemel Hempstead, UK). 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 (213). Based on our previous experiences, coefficients of variation (CV) for LRa and Lox are 3.5% and 6.1% respectively (218).

4.3.4.2 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 (239). 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 4 hours. 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 (239). Enrichments of [$^{15}N_2$] and [$^{15}N_1$]-urea were determined by GCMS (MSD 5971A, Hewlett-Packard Co., Palo Alto, CA). 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%.

4.3.4.3 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.

4.3.4.4 Body Composition

Lean body mass (LBM) and fat mass (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). (222) Change in body cell mass (BCM), a functional component of lean body mass, was estimated by subtracting extracellular water (ECW) from LBM.

4.3.5 Assays

All samples for any individual were measured in the same assay run for each analyte. Serum sex-hormone binding globulin (SHBG), total testosterone and prostate-specific antigen (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%.

4.3.6 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 two weeks. Considering that a longer time period than 2 weeks would be required to achieve a change in testosterone level with ADT, eleven 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 $\pm$ 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(243).

4.4 Results

The baseline characteristics of the study patients are listed in Table 5. 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 5), 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 logbooks as required, but verbally expressed a 100% adherence rate.

Table 5. 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	
Biochemical recurrence (n)	6	3	3	**0.40**
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; SHBG, sex-hormone binding globulin; PSA, prostate-specific antigen

4.4.1 Change in protein turnover and urea production

Table 6 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 $12.6 \pm 4.9\%$ ($p < 0.05$) (Figure 7) 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 6).

The baseline rate of urea production for all study participants was 427.6 ± 18.8 µmol/L. After 6 weeks of ADT, there was a $14.8 \pm 4.1\%$ increase in hepatic urea production to 486.5 ± 21.3 µmol/min (Figure 7; $p < 0.01$). Significance was retained ($p < 0.01$) when corrected for changes in BCM, a functional component of lean body mass (Table 6).

Table 6. 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. LRa, leucine rate of appearance (a measure of protein breakdown); LIP, leucine incorporation into protein; Lox, leucine oxidation (a measure of irreversible loss of protein); BCM, body cell mass

Figure 7: Percentage change in Lox/LRa and urea production rate after 6 weeks of ADT. Data expressed as mean ± S.E.M.
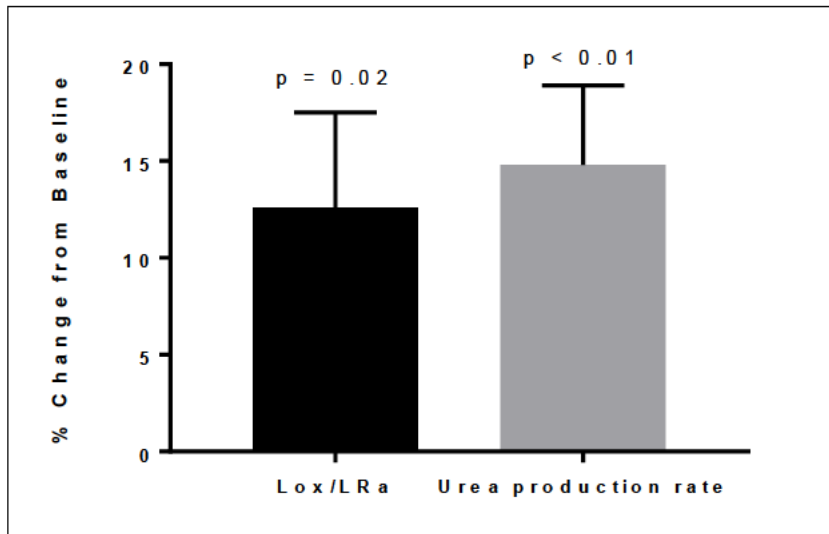
4.4.1.1 Effect of PRT

Table 7 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 ($p < 0.05$).

Table 7. Protein turnover and urea synthesis in usual care and exercise groups before and after ADT

	UC		PRT		P value
	Δ from baseline (%)	P value	Δ from baseline (%)	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; LRa, leucine rate of appearance (a measure of protein breakdown); LIP, leucine incorporation into protein; Lox, leucine oxidation (a measure of irreversible loss of protein); BCM, body cell mass

4.4.2 Other endpoint measures

Table 8 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 $93.5 \pm 5.1\%$ 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 prostate-specific antigen (PSA), and a rise in sex hormone binding globulin (SHBG) ($p < 0.001$). There was no change in BCM, but a significant reduction in LBM (% body weight) and increase in fat mass (FM) ($p < 0.05$). ADT resulted in a rise in serum urea concentration by $12.4 \pm 21.2\%$ ($p < 0.05$). 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.

Table 8. 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; BMI, body mass index; LBM, lean body mass; LH, luteinizing hormone; SHBG, sex-hormone binding globulin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; LDL, low-density lipoprotein; HDL, high-density lipoprotein; PSA, prostate-specific antigen; REE, resting energy expenditure; Fox, fat oxidation; Cox, carbohydrate oxidation.

4.4.2.1 Effect of PRT

Table 9 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 ($p<0.05$). 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.

Table 9. 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; BMI, body mass index; LBM, lean body mass; LH, luteinizing hormone; SHBG, sex-hormone binding globulin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; LDL, low-density lipoprotein; HDL, high-density lipoprotein; PSA, prostate-specific antigen; REE, resting energy expenditure; Fox, fat oxidation; Cox, carbohydrate oxidation.

4.5 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 (51), 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 (239). 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 down-regulates hepatic urea synthesis (10,44) while catabolic hormones such as glucocorticoids and glucagon up-regulate urea cycle enzymes (47,48,244), 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 ageing 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 to 40% at 70 years, resulting in sarcopenia (245). As testosterone levels decrease with age, androgen deficiency likely plays a large role in the initiation and progression of this condition (246). 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 (73,142). However, its specific effects on muscle have not been fully elucidated (247). 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 (247). However, differences have been observed between myocyte specific AR-knockout (mARKO) mice versus global AR-knockouts or orchiectomized mice. While muscle loss in the mARKO mice was limited to the highly sensitive levator-ani muscle with preservation of

limb muscles (223,224), only global ARKO mice had significant reductions in the mass of hind limb skeletal muscle (23,223). 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 (226), motoneurons (225), myokines, growth hormone and IGF-1 (248). Furthermore, androgen receptors are also known to be present in human liver (53). Thus, our findings provide evidence of an additional novel pathway causing muscle catabolism during ADT- through up-regulation 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 analogues are associated with mild enzyme elevations in 3 – 5% of patients, the cause of which is unclear (249). 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(250). This is in keeping with our findings of a significant increase in fat mass, 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 (251). Trials of supervised exercise programs in cancer patients report better compliance rates and improved outcomes compared to pure home-based exercise programs (152,252). 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 (203,204). Thus, we adopted a partially supervised program, in that patients were given two supervised training sessions in the first week as well, as additional 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 (253). 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* (254) who showed that acute resistance exercise in ADT-treated prostate cancer patients increased muscle protein synthesis. Similarly, in elderly sarcopenic patients, two weeks of resistance training increased protein synthesis measured by incorporation of ^{13}C-leucine into vastus lateralis muscle protein (255). Thus, the trend towards 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}N$_2$ labelled urea show that there are no changes in urea production during low or high intensity exercise (220,256). Following an endurance training program, an initial increase in urinary nitrogen excretion was found to return to pre-training levels within two weeks (257). 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 (239). 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. It is also important to note that participants in the PRT group had lower levels of serum testosterone and SHBG at baseline which may potentially impact on the effect of PRT on hepatic urea production. 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 (51) 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.

Chapter 5: Androgen deprivation therapy in prostate cancer: the benefits of home-based resistance exercise

Publication

5.1 Abstract

In men with prostate cancer, androgen deprivation therapy (ADT) has detrimental effects on body composition and health related quality of life (HRQOL), which can be ameliorated with exercise interventions including progressive resistance training (PRT). Existing studies focus on reversing established changes using supervised programs. We investigated whether a home-based PRT program, instituted at the start of ADT, could prevent adverse effects over 12-months. Twenty-five patients scheduled to receive at least 12 months of ADT were randomly assigned to either usual care (UC) (n=12) or PRT (n=13) starting immediately after their first ADT injection. Body composition, body cell mass (BCM; a functional component of lean body mass), insulin sensitivity, QOL and muscle function were measured at 6 weeks, 6- and 12 months. Data were analysed by a linear mixed model. ADT had a negative impact on body composition, bone mineral density, muscle strength, glucose metabolism and HRQOL. Compared to PRT patients, UC patients had greater reductions in BCM by -1.9 ± 0.8 % (p = 0.02) and higher gains in fat mass by 3.1 ± 1.0 % (p = 0.002) at 12 months. There was a significant increase in physical activity levels (p = 0.02), and HRQOL in the mental health (p = 0.01) and vitality (p = 0.02) domains in the PRT compared to the UC group. In conclusion, a home-based PRT program instituted at the start of ADT counteracts detrimental changes in body composition, improves physical activity and mental health over 12 months.

5.2 Introduction

Prostate cancer has the second highest incidence of all cancers amongst men worldwide and is the fifth leading cause of cancer death in men (15). Androgen receptor signalling strongly promotes prostate cancer growth. Thus, androgen deprivation therapy (ADT) with gonadotrophin releasing hormone (GnRH) analogues is a commonly utilised therapy for men with prostate cancer. However, in rendering patients severely hypogonadal, ADT is associated with multiple adverse effects. Changes in body composition occur rapidly, with increases of 7 – 10% in fat mass (FM), and decreases of 2 – 4% in lean body mass (LBM) after 1 year of ADT (73), with these effects persisting up to two years following cessation (258). Patients also experience a reduction in muscle strength and bone mineral density (BMD), development of type 2 diabetes, and a deterioration in health-related quality of life (HRQOL) (18).

ADT also modifies multiple endocrine regulatory pathways comprising those affecting glucose and insulin metabolism, insulin-like growth factors (IGFs) and its binding proteins (IGFBPs), as well as adipokines including leptin and adiponectin (163). Changes in these factors may affect tumour progression. Insulin-like growth factor-1 (IGF-1) is a peptide produced by the liver and is involved in the regulation of cellular proliferation, while IGF-binding proteins (IGFBPs) regulate the bioavailability of IGFs but also have independent actions on cell growth and survival (98). Higher circulating IGF-1 is associated with increased all-cause mortality in men with advanced prostate cancer (96), while there is an inverse relationship between IGFBP-3 and development of advanced-stage prostate cancer (102). Thus, ADT may induce changes in the insulin pathway and IGF axis which in turn modifies metabolism and disease progression.

Physical exercise is currently recognised as an effective strategy to ameliorate many of the adverse effects of ADT (201). Clinical trials have shown both resistance and aerobic exercise improve body composition, metabolic profile, functional capacity, fatigue and HRQOL (122). Progressive resistance training (PRT) is an anabolic form of exercise which involves challenging the skeletal muscles with unaccustomed loads. It is well established that PRT is beneficial in the treatment of sarcopaenia in older men and women (66), and is also efficacious in the treatment of ADT-induced adverse effects (241).

However, there are current limitations relating to exercise intervention studies in prostate cancer. Firstly, much of the evidence is derived from interventional studies occurring in patients on stable ADT after an average treatment time of 14 months (241), while the development of adverse effects is the most pronounced during the initial months of ADT (75,259). Only Cormie *et al.* (241) has examined the use of a supervised exercise program in the prevention of adverse effects when administered at the initiation of ADT. This showed a preservation of appendicular LBM and prevention of gains in whole body FM, when compared to usual care. Secondly, current studies are focused on supervised training programs located in exercise clinics. While compliance rates are superior compared to purely home-based exercise programs (152,252), fully supervised programs are resource intensive, less accessible and transitioning to community-based programs after a period of close supervision can be confronting for some patients (203,204). Thirdly, the benefits of PRT on the IGF axis and adipokines has not been established, despite a growing body of literature documenting their role on prostate cancer development and progression (96,102,163,177). Thus, more evidence is needed regarding the feasibility and efficacy of home-based programs.

We hypothesize that the introduction of a 12-month home-based PRT program at the start of ADT can prevent detrimental changes in body composition, physical function, metabolic

derangements and HRQOL. The primary aim of this study was to investigate the effect of PRT on body composition, physical function, BMD and HRQOL at 12 months. As a secondary analysis, we also investigated the effect of PRT on glucose and insulin indices, serum IGF-1 and its binding proteins, and adipokines.

5.3 Patients and Methods

This was a 12-month randomised controlled study of progressive resistance training (PRT) in patients with prostate cancer patients commencing ADT. The detailed recruitment procedures have been described previously (260). In brief, 25 men with prostate cancer scheduled to receive conventional ADT with GnRH analogues 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 with $\leq$ 5 sites of metastases and Eastern Cooperative Oncology Group (ECOG) 0 performance status. Exclusion criteria included concurrent chemotherapy or anti-androgen therapy, previous ADT within the last 12 months, or any musculoskeletal, cardiovascular and/or neurological disorders that could inhibit them from exercising. This study was approved by the Western Sydney Local Health District Human Research Ethics' Committee. All participants gave written consent. The study was registered with the Australian and New Zealand Clinical Trials Registry (ACTRN12616001311448).

5.3.1 Experimental Design

A two-armed prospective randomised controlled trial design was implemented. The 25 men were randomised into two arms: PRT (13 men), or usual care (UC) (12 men) using a computer random assignment program. As part of routine care, all participants received non-standardised advice from the treating clinician to participate in regular exercise throughout the intervention period.

5.3.1.1 PRT intervention

Participants assigned to the PRT group undertook 52 weeks of a home-based PRT program starting soon after their first ADT injection. The resistance training regimen was designed to stimulate all major skeletal muscle groups and exert systemic physiological effects on all outcome measures investigated. The program was designed and progressed according to standard training principles and adapted to the needs of the cohort and individual participants enrolled in the study. 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 and/or the loading of each exercise was advanced with strength adaptation (242). There were three stages of exercises. Stage one: incline push-up, bent over row, biceps curl, triceps extension, side shoulder raise, dumbbell squat, split squat and straight leg deadlift. Stage two involved addition of shoulder press. Stage three: standard push-up, decline push-up, curl to press, chair dips, lunge, side shoulder raise, dumbbell squat, straight leg deadlift. Patients advanced to the next stage after 12 weeks depending on strength and ability determined by the supervising exercise physiologist.

One week of exercise supervision (two sessions) were provided at baseline to instruct patients in proper lifting techniques and loading progressions. Patients then returned for a supervised session every 12 weeks to learn the next stage of exercises, and to ensure proper techniques were being utilised at home. To maximise adherence, online instructional videos and a printed training manual was provided for each exercise, and patients were given monthly reminder phone calls. Adherence to exercise was recorded in a training logbook by patients. Overall activity levels of all participants were monitored by physical activity questionnaires and total step counts for 1 week (via a pedometer) prior to their study visits.

5.3.1.2 Primary endpoints

Study endpoints were assessed at baseline, 6 weeks, 6 months and 12 months of ADT.

5.3.1.2.2 Body composition and bone mineral density

LBM and total and regional FM were assessed by dual x-ray absorptiometry (DXA; GE Healthcare Lunar Prodigy Pro) and Bioeletrical Impedance Spectroscopy (BIS) using the ImpediMed Ltd SFB7 analyser (ImpediMed Ltd Qld, Australia) (222). Change in body cell mass (BCM), a functional component of LBM, was estimated by subtracting extracellular water (ECW) from LBM. Vertebral and hip bone mineral density was also measured by DXA at each visit.

5.3.1.2.3 Physical activity

A pedometer (G-Sensor 2026) was used to estimate step counts for one week prior to each visit. Patients were asked to complete an exercise diary for one week prior to each visit and to specify the number of hours of light, moderate or intense physical activity. Patients in the PRT

group were instructed not to include the intervention in their documented activity, but to only include other types of physical activity.

5.3.1.2.4 Physical function

A series of standard tests were used to assess physical function. Maximal strength of the upper and lower body was assessed by an isometric dynamometer (i.e. triceps extension and knee extension) (Chatillon CSD200, JLW Instruments, Chicago, USA) hand-grip strength by a dynamometer (Jamar Plus digital dynamometer). The best performance over 3 trials was recorded. Function lower extremity strength was assessed using the sit-to-stand test (261). The participant is encouraged to complete as many full stands from a chair as possible within 30 sec. The timed get-up-and-go test (TUGT) was used to evaluate dynamic balance and physical performance (262). From a seated position, the participants stood, walked 3 metres, turned around, walked back to the chair, returning to a seated position as quickly as possible (best performance out of three recorded). The Lord sway-meter was used to assess co-ordinated stability, which measures participants' ability to adjust balance in a steady and co-ordinated way while placing them near or at the limits of their base of support. This test uses a 40cm rod attached to the participant at waist level by a firm belt. Participants are then asked to adjust balance by bending or rotating their body without moving their feet, so that a pen mounted vertically at the end of the rod remains within a convoluted track. A total error score is calculated (263). The postural sway test was used to assess dynamic balance. Participants stood barefoot on the floor or foam surface with open and closed eyes. The test records sway path, maximal anterior-posterior and lateral sway, measured by the Physiological Profile Assessment (PPA) Sway Path Mobile Applications (NeuRa) on an iPad attached to the top of an adjustable height table (264). Aerobic capacity (Submaximal VO_2) was measured by a cycle ergometer (Lode Corival Recumbent Ergometer) and data analysed using LEM software.

5.3.1.2.5 Quality of life

HRQOL was assessed at each study visit by the EORTC (European Organisation for Research and Treatment of Cancer) quality of life questionnaires including the QLQ-C30 (used to assess the QOL of cancer patients) and QLQ-PR25 (used to measure disease-specific HRQOL of prostate cancer patients undergoing active treatment). In addition, the Short Form 36 version 2.0 (SF-36v2) physical and psychological health survey was also used.

5.3.1.3 Secondary endpoints

5.3.1.3.1 Glucose and insulin indices

Glucose metabolism was assessed using the oral glucose tolerance test (OGTT). Blood glucose and insulin concentrations were measured at baseline and after a 75g glucose load at 30, 60, 90- and 120-min. Hepatic insulin resistance (the product of total area under the curve for glucose and insulin during the first 30 min) and muscle insulin sensitivity (the rate of decay of plasma glucose concentration from its peak value to its nadir divided by the mean insulin concentration) were calculated (265). Using the OGTT results, calculations were made of the HOMA-IR, the oral disposition index (266), Matsuda index (index of insulin sensitivity) (267) and overall glucose metabolism was estimated as the incremental glucose area under the curve above fasting over 120 min (266).

5.3.1.3.2 Blood biomarkers: Serum IGF-1, IGFBPs and adipokines

At each visit, fasting blood samples were taken and stored at -80°C for analysis. All samples for any individual were measured in the same assay run for each analyte. Serum IGFBP-3, leptin and adiponectin were measured by Abcam's human enzyme-linked immunosorbent assay (ELISA). The inter-assay CVs for IGFBP-3 was 5.7%, and for leptin and adiponectin, < 12%. An electrochemiluminescence immunoassay (ECLIA) was used to measure total testosterone and PSA. The inter-assay CVs for total testosterone at 0.087 nmol/L was 2.8% and for total PSA at 1.12 ng/mL, 3.2%.

5.4 Statistical analysis

Sample size calculation was based on change in body composition as indicated by LBM. Previous research involving PRT in men receiving ADT indicated a difference in LBM of 0.8 ± 0.4 kg between groups after a 12 week PRT intervention (268). Nine participants in each group were required to achieve 80% power at an α level of 0.05. Based on past studies involving supervised exercise programs, an attrition rate of at least 20 % is expected. Therefore, to ensure we had sufficient participant numbers at the end of the intervention, 25 participants were randomised to the study arms (UC group 12 men; PRT group 13 men).

The statistical analysis consisted of a *linear mixed effects model*, using a random patient effect to account for baseline and fixed effects for the visit and intervention arm. In this analysis, a difference in time course behaviour between study arms corresponded to an interaction between visit and intervention. Comparisons at specific time points were made using contrasts extracted from the mixed effects model and separately using two-sample t-tests of change in endpoint. Results are expressed as mean ± S.E.M and a *P* value <0.05 was

considered significant. All analysis was conducted in R version 3.6.0 (Vienna, Australia), RStudio IDE (Boston, MA) and SPSS statistics v22 (IBM corporation).

5.5 Results

5.5.1 Baseline patient characteristics and retention

At baseline, 12 were enrolled in the UC arm and 13 participants in the PRT arm. At 6 months, one participant in the UC arm was unable to attend follow-up due to hospitalisation (but remained in the study) and one participant in the PRT arm discontinued the study. At 12 months, two participants discontinued the study (one from each arm) and two patients from each arm were excluded from final analysis as ADT was discontinued for more than 3 months prior to their 12-month visit (one from each arm). Thus, at 12 months, 10 participants remained in each arm (Figure 10).

Adherence to the home-based PRT program was determined by logbooks. Out of the 13 participants, one consistently did not comply with filling in his logbook citing poor literacy as the reason, although he reported full compliance with the PRT program. Logbook analysis showed that from baseline to 6 weeks, participants completed a mean of 3.0 sessions/week; 6 weeks to 6 months, 2.3 sessions/week, and from 6 to 12 months, 2.2 sessions/week. At 12 months, 50% of patients managed to comply with stage 3 exercises; 30% continued with stage 2 and 20% continued with stage 1.

At baseline (Table 10), UC and PRT participants were well matched in terms of age, blood pressure, number of co-morbidities and medications, body composition, PSA levels, as well as prostate cancer grade and stage. Pre-treatment testosterone levels were significant higher in the

UC group (p = 0.02), but both groups had similar suppressed testosterone levels during ADT. There were no significant differences between groups in terms of baseline activity level as assessed by pedometer step counts and hours of light, moderate or intense physical activity.

Figure 8. Consort Diagram
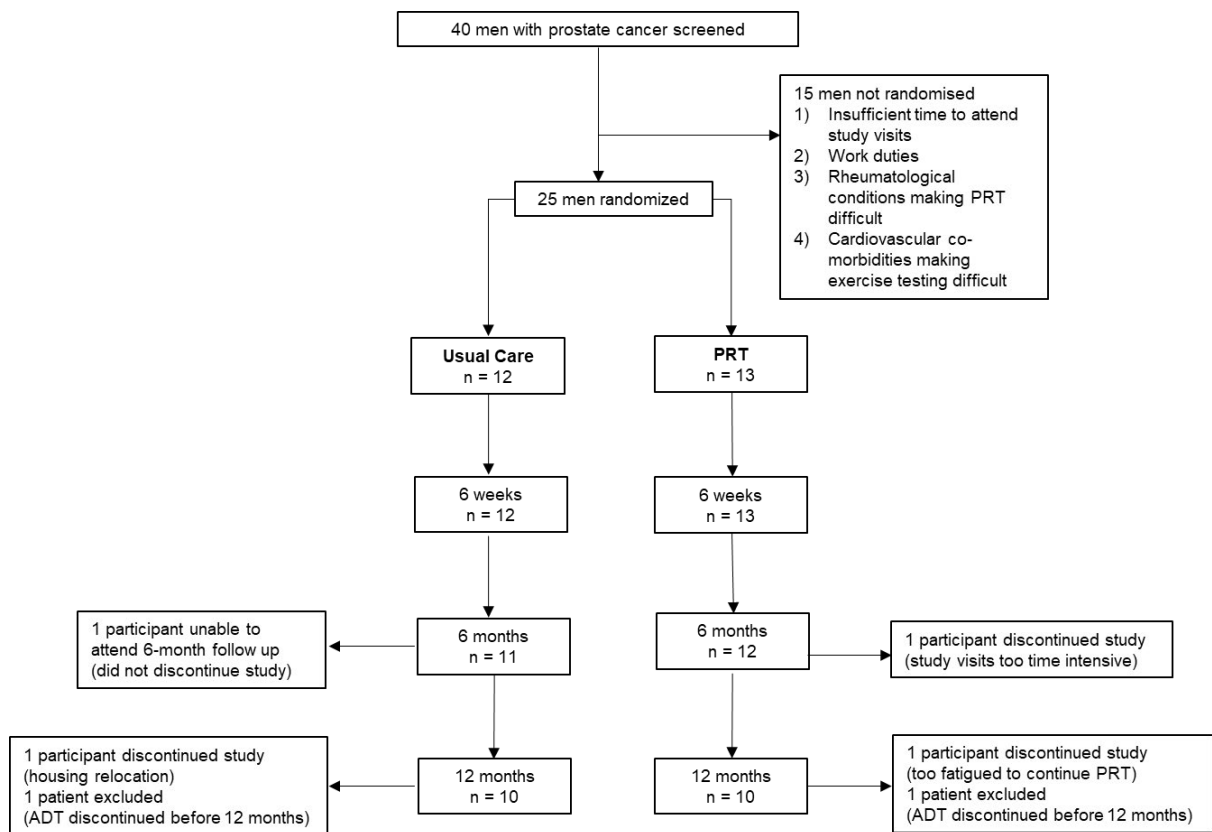
Table 10. Baseline clinical characteristics

Variable	UC (n =12)	PRT (n = 13)	P value
Age (years)	71.8 ± 1.8	69.3 ± 2.3	**0.14**
Weight (kg)	79.9 ± 2.7	87.2 ± 4.7	**0.03**
BMI kg/m^2	28.8 ± 1.1	29.7 ± 1.3	**0.36**
Number of co-morbidities[*]	0.9 ± 0.2	1.5 ± 0.2	**0.81**
Number of medications	1.1 ± 0.3	1.5 ± 1.1	**0.91**
SBP (mmHg)	137 ± 2	139 ± 4	**0.18**
DBP (mmHg)	74 ± 3	74 ± 1	**0.11**
Gleason score	7.6 ± 0.3	8.2 ± 0.2	**0.42**
Cancer staging			
Localized (n)	9	8	
Biochemical recurrence (n)	3	3	**0.36**
Metastatic (n)	0	2	
Previous radiotherapy (n)	8	5	**0.39**
Previous ADT (n)	0	2	**0.26**
Lean body mass (kg)	53.1 ± 1.2	54.5 ± 2.2	**0.16**
LBM (% body weight)	66.5 ± 1.8	63.2 ± 2.0	**0.68**
Fat mass (kg)	24.2 ± 2.1	29.9 ± 3.2	**0.12**
Extracellular water (L)	19.0 ± 0.6	19.8 ± 0.9	**0.05**
BCM (kg)	34.1 ± 1.0	34.7 ± 1.3	**0.58**
Testosterone (nmol/L)	16.1 ± 1.1	12.5 ± 0.8	**0.02**
LH (mIU/mL)	7.0 ± 0.7	6.3 ± 0.7	**0.70**
PSA (ng/mL)	11.9 ± 2.6	7.7 ± 1.3	**0.08**
Step count (number)	40172 ± 8502	28838 ± 5377	**0.14**
Light physical activity (hours)	5.9 ± 1.6	7.9 ± 2.5	**0.09**
Moderate physical activity (hours)	3.9 ± 1.8	3.3 ± 1.4	**0.93**
High physical activity (hours)	0.1 ± 0.1	0.5 ± 0.3	**0.05**

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

5.5.2 Response to ADT in the UC group

Following the administration of ADT, as expected, serum testosterone levels significantly decreased (p < 0.0001) by 6 weeks, remaining at castrate levels at 12 months (p < 0.0001) (Figure 9A). Similarly, serum PSA levels fell from 9.7 ± 1.5 ng/mL to 0.4 ± 0.1 ng/mL at 12 months (Figure 9B).

Figure 9. Response to ADT in the UC group as measured by serum testosterone and PSA

A.   **B**
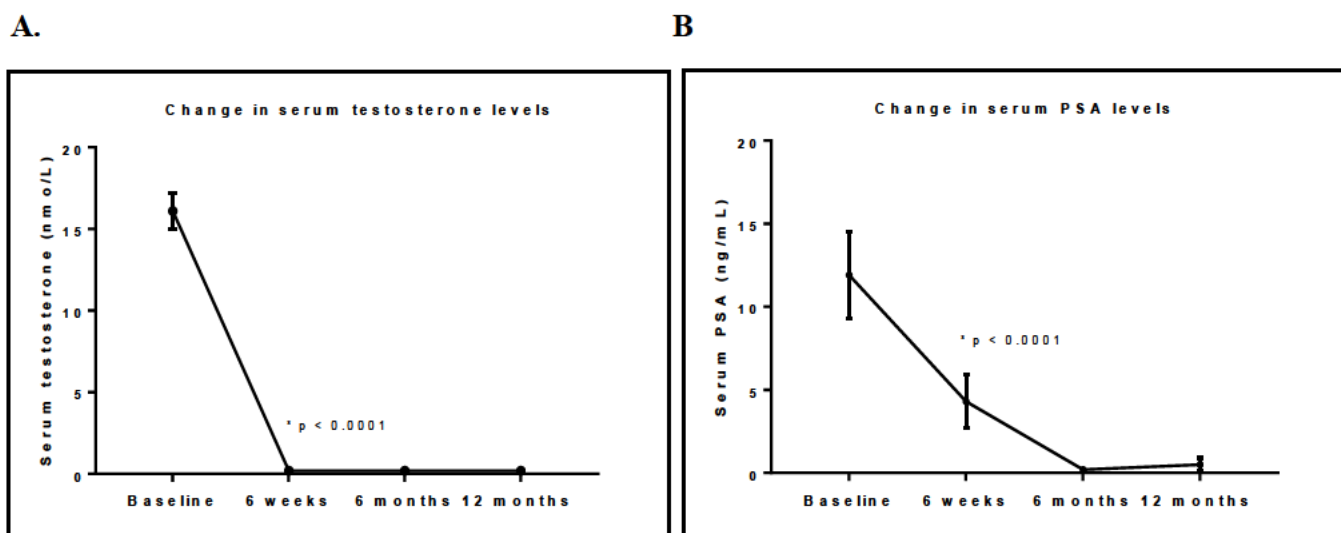
Response to ADT in the UC group as measured by serum testosterone and PSA. (A) Change in serum testosterone at 6 weeks, 6- and 12 months post-ADT (* *p* < 0.001). (B) Change in serum PSA at 6 weeks, 6- and 12 months post-ADT (* *p* < 0.001).

The effect of ADT on measured indices in the UC group are described in detail in Supplementary Table 1. At 12 months, there was a significant reduction in BCM (BCM %) and increase in FM (FM %) as a percentage of total body mass (TBM) by 4.6 ± 0.6 % and 5.5 ± 0.6 % (p < 0.001), respectively (Table 11). Similarly, there were significant increases in fasting glucose by 0.7 ± 0.1 mmol/L (p < 0.001) and hepatic insulin resistance by 31.9 ± 11.3 (p <

0.01). There was a significant reduction in insulin sensitivity as represented by the Matsuda Index by 1.8 ± 0.5 (p < 0.01). BMD also significantly decreased at the lumbar spine by 0.01 ± 0.02 (p < 0.01) as did muscle strength, with a reduction in right hand grip by 4.2 ± 1.0 N (p <0.001), left hand grip by 3.4 ± 1.1 N (p < 0.01) and lower limb strength by 50.9 ± 20.4 N (p < 0.05). There was a reduction in physical function as reflected by a prolongation in the TUGT by 0.3 ± 0.2 sec (p = 0.04). Quality of life also fell with a significant reduction in the QLQ-C30 summary score by 3.2 ± 1.4 (p < 0.05). There were significant increases in IGFBP-3 (< 0.001), leptin (p = 0.01) and adiponectin (p < 0.01) (Table 9 and Supplementary Table 1). There was a significant reduction in the IGF-1: IGFBP3 ratio (p = 0.02) following ADT in the UC group (Figure 11).

Figure 10. Change in IGF1:IGFBP3 ratio in the UC group at 12 months

Change in IGF1:IGFBP-3 ratio
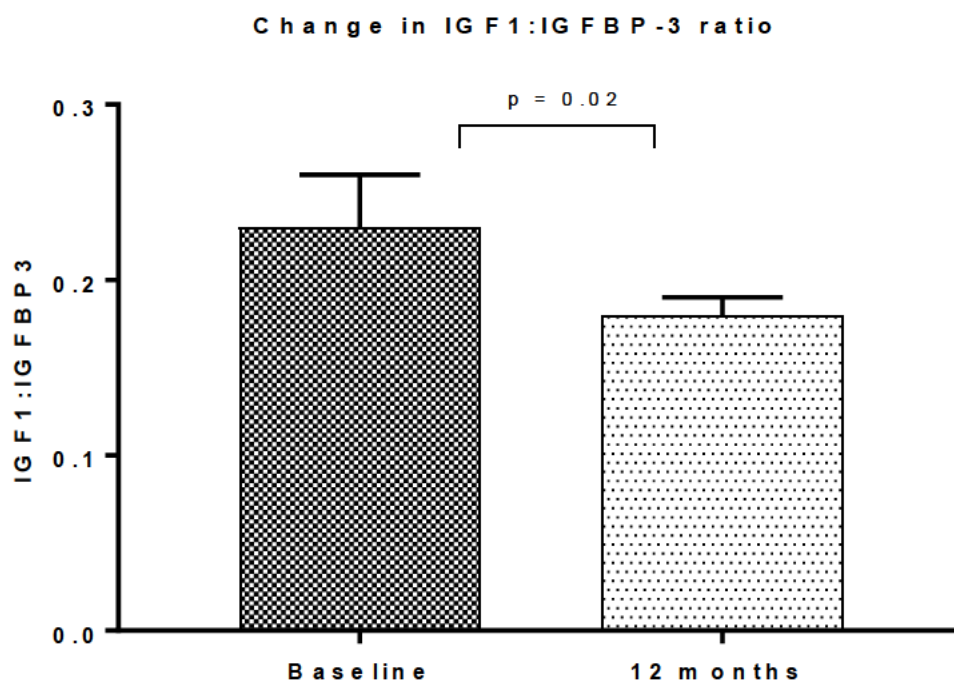
Table 11. Effect of ADT on body composition, metabolism and physical function in the UC group at 12 months

	Baseline	12 months	P value
Total mass (kg)	80.3 (2.6)	82.0 (2.7)	**0.002**
BCM (%)	42.8 (1.7)	39.0 (1.4)	**<0.001**
FM (%)	30.5 (1.9)	34.9 (1.5)	**<0.001**
Neck of femur BMD (left total)	1.002 (0.04)	1.001 (0.04)	**0.28**
Neck of femur BMD (right total)	1.003 (0.05)	0.99 (0.04)	**0.13**
Lumbar spine BMD	1.43 (0.05)	1.36 (0.07)	**0.005**
Fasting glucose (mmol/L)	4.7 (0.1)	5.4 (0.2)	**<0.001**
Fasting insulin (IU/L)	7.2 (1.9)	11.7 (2.3)	**0.22**
Matsuda index	6.2 (0.8)	4.7 (0.8)	**0.002**
HOMA-IR	1.5 (0.5)	2.9 (0.7)	**0.11**
Hepatic insulin resistance	40.7 (11.3)	74.0 (22.7)	**0.008**
Muscle insulin resistance	0.9 (0.4)	1.3 (0.6)	**0.38**
Co-ordinated stability (corners)	0.3 (0.2)	0.8 (0.3)	**0.05**
Right hand grip (N)	37.9 (1.5)	33.2 (1.7)	**<0.001**
Left hand grip (N)	34.4 (1.4)	30.9 (1.6)	**0.004**
Upper limb strength (N)	156.7 (12.4)	144.6 (11.3)	**0.35**
Lower limb strength (N)	294.2 (25.6)	239.5 (15.6)	**0.02**
Sit-to-stand test (number)	17 (1.1)	18 (1.5)	**0.17**
TUGT (sec)	5.6 (0.2)	6.0 (0.3)	**0.04**
QLQ-C30 summary score	82.0 (3.7)	79.2 (4.2)	**0.03**
IGF-1 (ng/mL)	19.9 (2.0)	20.9 (1.4)	**0.39**
IGF-1/IGFBP3	0.23 (0.02)	0.18 (0.01)	**0.02**
IGFBP-3 (ng/mL)	2555.7 (273.5)	3318.1 (209.7)	**<0.001**
Leptin (pg/mL)	3712.2 (731.8)	10492.2 (3040.5)	**0.01**
Adiponectin (x 10^7 pg/mL)	5.1 (0.6)	8.8 (2.3)	**0.007**

Data are presented as mean ± S.E.M; *P* value represents change compared to baseline in the UC group at 12 months; BCM, body cell mass; FM, fat mass; BMD, bone mineral density; TUGT, timed get-up-and-go test; IGF-1, insulin-like growth factor; IGFBP-3, insulin-like growth factor binding protein 3

5.5.3 The effect of PRT on primary endpoints

5.5.3.1 Body composition

The effect of PRT on changes in body composition is shown in Figure 11 and described in detail in Supplementary Table 2. At 6 weeks and 6 months, there was a trend towards a greater reduction in LBM % and BCM % in the UC versus the PRT group but this did not reach statistical significance. However, at 12 months PRT was associated with significant preservation of both LBM % by 2.7 ± 0.8 % (p = 0.001) and BCM % by 1.9 ± 0.8 % (p = 0.02) (Figure 11A) in the PRT compared to the UC group. Similarly, patients in the PRT group experienced less of a gain in fat mass, with a -3.1 ± 1.0 % (p = 0.002) difference between the two groups at 12 months (Figure 11B). When assessing regional fat mass, the biggest difference between groups was observed in truncal fat mass (p = 0.02), with the PRT group gaining 1.8 ± 0.8 kg less than the UC group at 12 months (Table 12).

Figure 11. The effect of PRT on body composition

A.
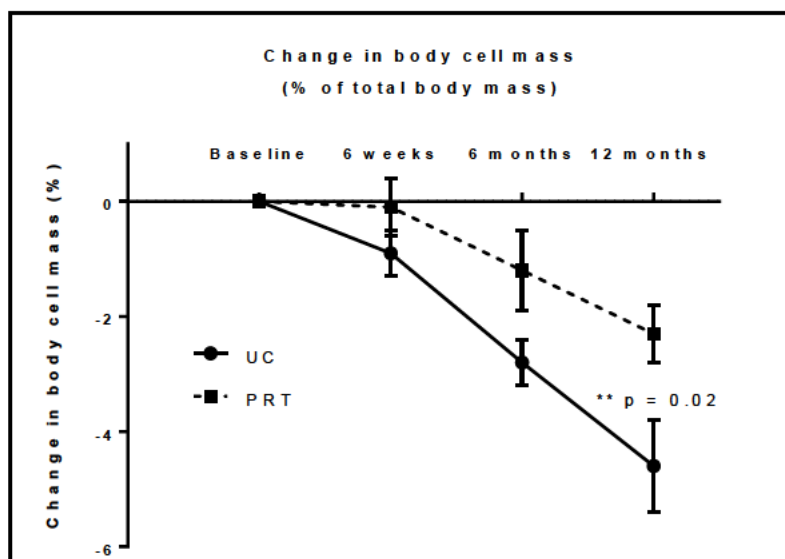
B.
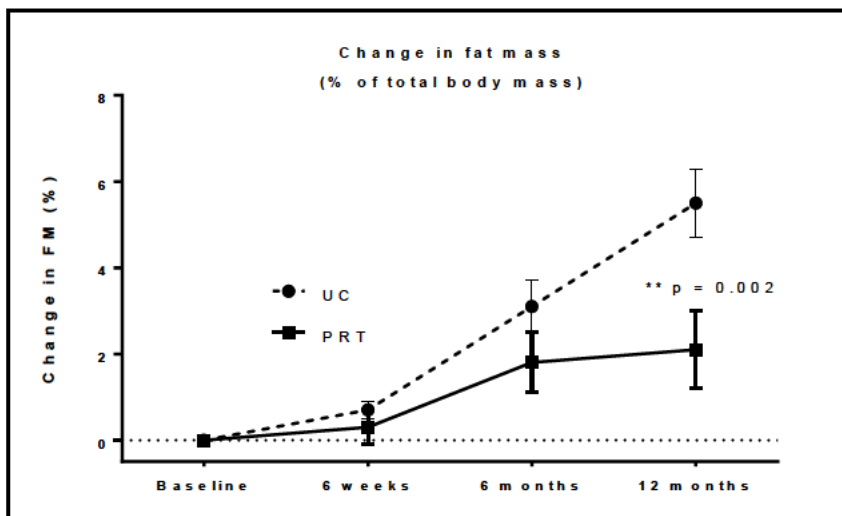
The effect of PRT on body composition. (A) Change in body-cell mass (% total body mass) in the UC and PRT groups at 6 weeks, 6- and 12 months (** $p = 0.01$ at 12 months). (B) Change in fat mass (% total body mass) in the UC and PRT groups at 6 weeks, 6- and 12 months (** $p = 0.02$ at 12 months).

5.5.3.2 Physical function and BMD

A detail description of the changes in physical function and BMD is provided in Supplementary Table 10. There were no differences in BMD, muscle strength, physical function (balance and co-ordinated stability) or submaximal VO_2 between the UC and PRT groups. However, there was a significant increase in physical activity levels as measured by step count in the PRT compared to the UC group at 12 months ($p = 0.02$) (Table 12).

116

Table 12. Group differences in the primary endpoints (body composition, physical activity, HRQOL, glucose and insulin metabolism) at 6 weeks, 6 months and 12 months, reflecting the effect of PRT

Variables	6 weeks	**P value**	6 months	**P value**	12 months	**P value**
Body composition						
Total mass (kg)	1.5 (0.9)	**0.09**	0.5 (0.9)	**0.60**	-1.0 (0.9)	**0.30**
Total LBM (% total mass)	0.5 (0.8)	**0.47**	1.3 (0.8)	**0.11**	2.7 (0.8)	**0.001**
Total BCM (% total mass)	1.0 (0.8)	**0.21**	1.3 (0.8)	**0.10**	1.9 (0.8)	**0.02**
Total FM (% total mass)	-0.4 (0.9)	**0.65**	-1.1 (0.9)	**0.25**	-3.1 (1.0)	**0.002**
FM trunk (kg)	-0.1 (0.7)	**0.94**	-0.8 (0.7)	**0.39**	-1.8 (0.8)	**0.02**
Physical activity						
Step count	12864 (7102)	**0.08**	7719 (7308)	**0.30**	19188 (7805)	**0.02**
SF36v2 health survey						
Physical functioning	0.3 (2.6)	**0.89**	-2.1 (2.7)	**0.45**	1.3 (2.8)	**0.63**
Role- physical	0.002 (2.1)	**0.99**	1.5 (2.2)	**0.51**	-0.6 (2.3)	**0.81**
Bodily pain	5.0 (2.3)	**0.03**	0.3 (2.4)	**0.91**	2.6 (2.5)	**0.30**
General health	5.0 (2.4)	**0.04**	1.0 (2.6)	**0.69**	2.7 (2.6)	**0.32**
Vitality	3.8 (2.4)	**0.11**	5.8 (2.5)	**0.02**	6.0 (2.5)	**0.02**
Social functioning	2.7 (1.9)	**0.15**	4.2 (1.9)	**0.03**	2.1 (2.0)	**0.31**
Role- emotional	1.8 (2.2)	**0.43**	2.6 (2.3)	**0.28**	1.9 (2.4)	**0.43**
Mental health	2.8 (2.2)	**0.21**	3.7 (2.3)	**0.12**	4.9 (2.4)	**0.04**
Physical component summary	1.6 (1.8)	**0.40**	-1.7 (1.9)	**0.37**	-0.1 (2.0)	**0.97**
Mental component summary	3.4 (1.9)	**0.08**	5.7 (2.0)	**0.006**	5.5 (2.1)	**0.01**
Glucose/insulin indices						
Glucose (mmol/L) Baseline	-0.1 (0.2)	**0.73**	0.2 (0.2)	**0.34**	0.01 (0.2)	**0.94**
Insulin (IU/L) Baseline	-5.2 (3.8)	**0.18**	-1.6 (3.9)	**0.68**	3.2 (4.1)	**0.44**
Matsuda Index	2.5 (0.8)	**0.004**	0.4 (0.8)	**0.64**	-0.04 (0.9)	**0.96**

Data are presented as mean ± S.E.M; *P* value represents group differences in mean changes between the UC and PRT groups at 6 weeks, 6 months and 12 months; BCM, body cell mass; FM, fat mass

5.5.3.3 Quality of life

The effect of PRT on HRQOL is summarised in Table 12 and described in detail in Supplementary Table 4. At 6 weeks, there was a reduction in the SF36v2 general health score in the UC group and an increase in the PRT group, with a significant difference of 5.0 ± 2.4 (p = 0.04) between the two groups. At 6 months, there were significantly greater reductions in the EORTC QLQ-C30 score for global health status (p = 0.02), SF36v2 scores for vitality (p = 0.02) and social functioning (p = 0.03) in the UC compared to the PRT group. In addition, the EORTC QLQ-C30 score for pain was significantly higher in the UC compared to the PRT group (p = 0.02). At 12 months, the SF36v2 scores for vitality and mental health both improved in the PRT group, as opposed to a reduction in the UC group (Figure 12A and 12B).

Figure 12. Improvements across SF-36v2 domains after 12 months of PRT
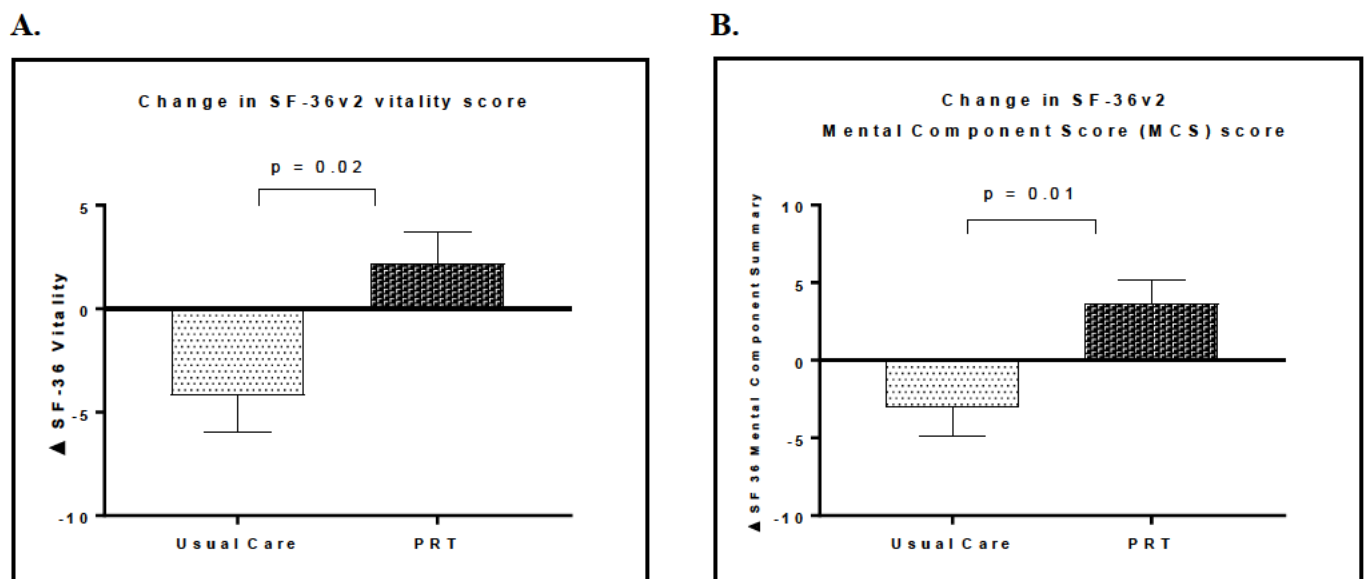
Improvements across SF-36v2 domains after 12 months of PRT. (A) Change in the SF-36v2 vitality score in the UC and PRT groups at 12 months compared to baseline (*p* = 0.02). (B) Change in SF-36v2 mental component score in the UC and PRT groups at 12 months compared to baseline (*p* = 0.01).

118

5.5.4 The effect of PRT on secondary endpoints

5.5.4.1 Glucose and insulin indices

At 6 weeks, there was a decrease in the Matsuda Index in the UC group by -0.3 ± 0.5 (p = 0.47) and a significant increase in the PRT group by 2.2 ± 0.7 (p = 0.009) with an overall difference between groups of 2.5 ± 0.8 (p = 0.004) (Table 10) (Supplementary Table 5). However, this significant early difference was not maintained at 6 and 12 months. There were no significant differences between groups in terms of plasma insulin and glucose levels, HOMA-IR, disposition index, or liver and muscle insulin resistance.

5.5.4.2 Serum IGF-1, IGFBPs and adipokines

IGF-1 did not significantly change throughout the study in either group (Figure 13A). IGF-1: IGFBP-3 ratio fell throughout the study, however there was no significant difference between the groups (Supplementary Table 5). There were significant increases in serum IGFBP-3 and leptin levels in both groups at 12 months but no significant differences between the PRT and UC groups were noted (Figure 13B and 6C). At 12 months, there was a greater increase in adiponectin levels in the UC group compared to the PRT group by 3.2 ± 1.5 pg/mL (p = 0.04) (Figure 13D).

5.5.5 Intervention safety and adherence

In terms of adverse events, one patient in the PRT group developed right shoulder pain (rotator cuff tendonitis) at the completion of the study, requiring physiotherapy.

Figure 13. Change in serum growth factors and adipokines in the UC and PRT groups post-ADT

A.

B.

C.

D.
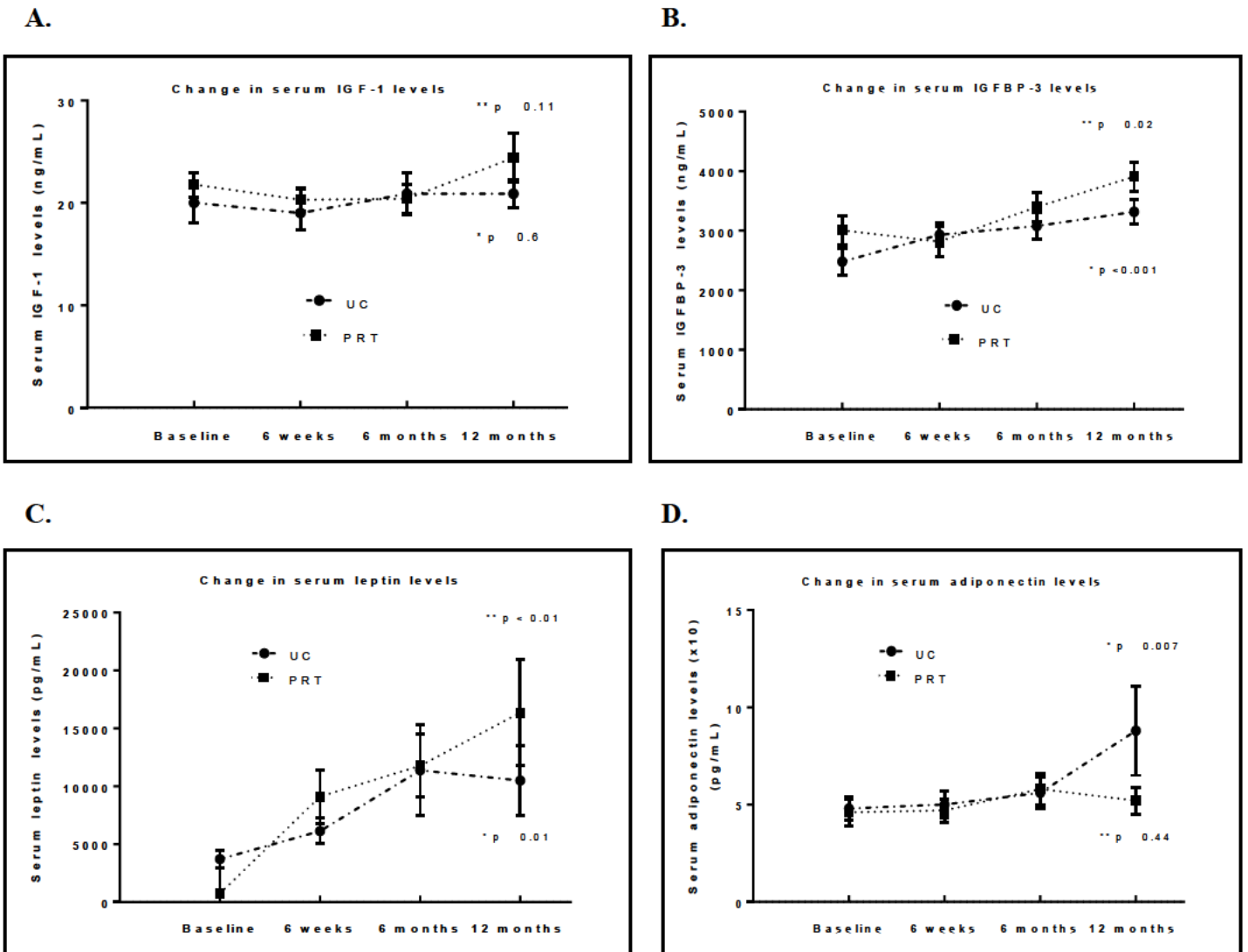
Change in serum growth factors and adipokines across in the UC and PRT groups post-ADT (A) Change in serum IGF-1 levels at 6 weeks, 6- and 12 months in the UC and PRT groups (* reflects change in the UC group at 12 months ** reflects change in the PRT group at 12 months) (B) Change in serum IGFBP-3 levels at 6 weeks, 6- and 12 months in the UC and PRT groups (* reflects change in the UC group at 12 months ** reflects change in the PRT group at 12 months) (C) Change in leptin levels at 6 weeks, 6- and 12 months in the UC and PRT groups (* reflects change in the UC group at 12 months ** reflects change in the PRT group at 12 months) (D) Change in adiponectin levels at 6 weeks, 6- and 12 months in the UC and PRT groups (* reflects change in the UC group at 12 months ** reflects change in the PRT group at 12 months)

5.7 Discussion

This randomised controlled trial examined the efficacy of a 12-month home-based PRT program in the prevention of the adverse effects of ADT. Our study was able to demonstrate the detrimental effects of ADT on body composition, insulin resistance, BMD, physical function and HRQOL. We showed that the early implementation of a 12-month home-based PRT program at the start of ADT resulted in beneficial effects on body composition and physical activity levels, and improvements in HRQOL.

After 12 months of ADT, we found a significant reduction in BCM (a functional component of LBM) and a significant increase in FM in the UC group. This was accompanied by a significant increase in insulin resistance and reduction in insulin sensitivity, as measured by the HOMA-IR and Matsuda index, respectively, at 6 and 12 months. As expected, there were reductions in BMD, muscle strength, physical function and quality of life following 12 months of ADT.

This study showed that a home-based PRT program was able to significantly counteract ADT-induced changes in body composition. PRT was able to offset reductions in LBM by 1.2 ± 0.2 kg at 12 months. Importantly, a similar effect of PRT was also seen with BCM, a functional component of LBM which has not been examined previously. Using a supervised PRT program, Nilsen *et al.* (150) found a preservation of LBM in the PRT group, while a 12-month study conducted by Winters-Stone *et al.* (154) did not show any differences in LBM between the PRT and control group. However, participants in this trial were undergoing ADT two to three times longer than that of Nilsen *et al*, indicating that the benefit of exercise on LBM may diminish with longer duration of ADT (154). Despite a lower intensity, home-based program, we were able to demonstrate a significant effect of PRT on LBM and BCM. This

highlights the importance of PRT as a powerful stimulant of muscle hypertrophy, and the need for early implementation of PRT before detrimental changes in body composition occur.

Our study showed an increase in FM in both the UC and PRT groups but PRT was able to offset a gain in FM by 3.1 ± 1.0 % (2.3 ± 0.8 kg). This is similar to findings by Winters-Stone *et al.* (154) who found a 1.9 kg difference in FM between the UC and PRT groups after 52 weeks of supervised PRT. Importantly, we found that PRT had the greatest effect on truncal fat mass. Dickerman *et al.* (269) found that higher visceral fat and waist circumference, a surrogate of central adiposity, were associated with advanced and fatal prostate cancer. Thus, these results provide evidence on the benefits of home-based PRT programs in counteracting ADT-induced negative effects on body composition in the prostate cancer population.

This study showed significant reductions in muscle strength and physical function with long-term ADT. However, we were unable to demonstrate significant differences in muscle strength or physical function between the UC and PRT groups. Previous studies involving supervised PRT showed gains in both upper and lower limb strength in prostate cancer patients (150-152) and Taafe *et al.* (152) reported an improvement in physical function. It is known that home-based programs may have lower adherence, training volume and loading compared to supervised programs (270), which may have reduced the potential for strength adaptation or maintenance following initiation of ADT in our study.

Our study showed that overall physical activity levels were higher in the PRT compared to UC group at 12 months. Physical activity is important in prostate cancer as Phillips *et al.* found that a higher duration of total, non-vigorous walking activity in prostate cancer survivors was associated with improved HRQOL (271). There is also a correlation between physical activity and reduced mortality in prostate cancer patients (272). Thus, the finding that a home-based PRT program can increase physical activity levels are encouraging, given the insufficient levels

of physical activity in the population of men with prostate cancer, with a study by Silva *et al.* showing that 56.5% of a cohort of men with prostate cancer were inactive (273).

ADT results in a significant decline in bone mass and an increase in fracture risk (274,275). This study demonstrated a significant decline in BMD at the lumbar spine as early as 6 months of ADT, which was not attenuated by PRT. However, other parameters which would influence bone health, such as vitamin D levels, were not examined in this study and may have affected results. Our PRT program may also have been limited in terms of duration and degree of impact loading which is necessary to promote improvements in BMD (149). Only Winters-Stone *et al.* (154) has reported preservation of BMD at the L4 site in patients in the PRT group versus the control group and thus, the effect of PRT on BMD during ADT warrants further investigation.

Psychological distress and anxiety is prevalent amongst men with prostate cancer ranging from 15 – 27%, and highest in those who have yet to undergo treatment (136). This was reflected in our findings, which showed a significant decline in HRQOL following ADT in the UC group. We showed that the use of a concurrent home-based PRT program at the start of ADT was associated with significant improvements in HRQOL. This is in line with the effects of supervised PRT programs which showed improvements in fatigue, vitality and mental health (148,152). These results provide evidence that the mental health benefits of PRT are seen even when implemented in an unsupervised setting. There are multiple reasons why exercise can improve mental wellbeing. Exercise leads to improved self-esteem (276) and also induces physiological effects which impact mood and cognitive function (276). However, it is important to note that men in the PRT group had more regular contact with study co-ordinators. This increases the participant's social support network which can improve HRQOL (277).

Regarding the secondary endpoints, we found an improvement in insulin sensitivity as measured by the Matsuda Index in the PRT group at 6 weeks, although this difference was not maintained at 6 and 12 months. In an RCT involving a supervised PRT program in patients on long term ADT, Winters-Stone *et al.* (154) found a non-significant reduction in insulin levels (a surrogate marker of insulin resistance) in the PRT group compared to an increase in the control group. In a population of patients with type 2 diabetes, a home-based PRT program was also unable to maintain the glycaemic benefits obtained from supervised gymnasium based PRT (270). Thus, PRT may have initial benefits on glucose metabolism that is difficult to sustain during long-term ADT.

Serum IGFBP-3 increased in both the UC and PRT groups, suggestive of an ADT effect. This is consistent with in-vitro studies showing an increase in IGFBP-3 mRNA levels following androgen deprivation, which facilitates cellular apoptosis and inhibits prostate cancer growth (101). IGF-1: IGFBP-3 ratio decreased in the UC group. This may indicate that ADT, through an increase in IGFBP-3, reduces IGF-1 bioactivity, thereby exerting an inhibitory effect on prostate cancer growth. However, we were unable to demonstrate an effect of PRT on the IGF axis. Santa-Mina *et al* (163) previously showed a reduction in the IGF-1:IGFBP-3 ratio and an increase in IGFBP-3 in response to PRT. The absence of significant findings may reflect the heterogeneity in the response of the systemic IGF axis to exercise (167).

PRT did not affect leptin levels but differences between the two groups may have been observed if PRT had exerted a greater beneficial effect on FM (163). There were more substantial increases in adiponectin levels in the UC versus the PRT group at 12 months, likely related to greater gains in subcutaneous adipose tissue (278). There is some discrepancy in the literature related to adiponectin and prostate cancer. While Housa *et al* (118) found higher adiponectin levels in locally advanced, compared to organ confined prostate cancer, Burton *et*

al showed an inverse correlation between adiponectin and histological grade and stage (119). Recently, Lee *et al* reported an 'adiponectin paradox' whereby higher serum adiponectin concentrations were independently associated with incident cancer in type 2 diabetes (279) but the underlying pathophysiology remain unknown (279).

There are limitations to this study. The small sample size would have affected the ability to observe differences, particularly in terms of muscle strength, physical function, and the biomarkers assessed. The large number of outcome measures in relation to the small sample size also raises the possibility of chance findings. Men in the PRT group were also relatively well functioning individuals with minimal co-morbidities, who were highly motivated to undertake home-based exercise. Therefore, this group of participants may not be representative of men with prostate cancer at large. Furthermore, it is recommended that men with prostate cancer should engage in regular exercise (280). Men in the UC group received advice from the treating clinician regarding exercise recommendations, but this was non-standardised. Study outcomes may have differed had a protocolised approach to exercise recommendations in the UC group been adopted. Home-based programs also have limitations- in terms of long-term adherence, achievement of maximal intensity and adequate loading for muscle adaptation.

This study is unique in that it explores the long-term use of isolated PRT at the start of ADT in a home-based setting which has not been investigated previously. Although there is robust data supporting the use of supervised PRT in prostate cancer patients, it is resource intensive and may not be feasible to implement across the whole prostate cancer population. We were able to demonstrate long-term positive outcomes with a more cost-effective alternative, estimated to be less than a third of the total cost of supervised programs. The positive outcomes from this study suggests that guidelines can be modified to maximise exercise participation in the prostate cancer population. This involves the early involvement of an exercise physiologist

to ensure proper conduct of exercises for maximal effectiveness, and for periodic monitoring of exercise compliance.

In conclusion, this is the first randomised controlled trial to show that a home-based PRT program instituted at the start of ADT was able to counteract detrimental changes in body composition and improve both physical activity and mental health over a 12-month period. Thus, a home-based PRT program offers clinicians a viable alternative to more resource-intensive supervised programs when instituted at the commencement of ADT and should be recommended following diagnosis of prostate cancer.

Supplementary Table 1. Effect of ADT on body composition, metabolism and physical function in the UC group

Variables	Baseline (n = 12)	6 weeks (n = 12)	Change from baseline (6 weeks)		6 months (n = 11)	Change from baseline (6 months)		12 months (n = 10)	Change from baseline (12 months)	
				P value			P value			P value
Total mass (kg)	80.3 (2.6)	80.0 (2.5)	-0.3 (0.5)	**0.45**	80.7 (2.6)	0.6 (0 5)	**0.24**	82.0 (2.7)	1.8 (0.5)	**0.002**
BCM (%)	42.8 (1.7)	41.9 (1.6)	-0.9 (0.6)	**0.12**	40.7 (1.6)	-2.6 (0.6)	**<0.001**	39.0 (1.4)	-4.6 (0.6)	**<0.001**
FM (%)	30.5 (1.9)	31.1 (1.9)	0.7 (0.6)	**0.27**	32.9 (1.9)	3.0 (0.6)	**<0.001**	34.9 (1.5)	5.5 (0.6)	**<0.001**
Neck of femur BMD (left total)	1.002 (0.04)	1.01 (0.04)	0.01 (0.01)	**0.21**	1.0002 (0.04)	0.01 (0.01)	**0.42**	1.001 (0.04)	-0.01 (0.01)	**0.28**
Neck of femur BMD (right total)	1.003 (0.05)	1.015 (0.04)	0.01 (0.01)	**0.34**	0.9997 (0.05)	-0.0001 (0.01)	**0.95**	0.99 (0.04)	-0.02 (0.01)	**0.13**
Lumbar spine BMD	1.43 (0.05)	1.42 (0.005)	-0.01 (0.02)	**0.53**	1.39 (0.06)	-0.04 (0.02)	**0.03**	1.36 (0.07)	-0.006 (0.02)	**0.005**
Fasting glucose (mmol/L)	4.7 (0.1)	4.8 (0.1)	0.2 (0.1)	**0.19**	5.2 (0.2)	0.6 (0 1)	**<0.001**	5.4 (0 2)	0.7 (0.1)	**<0.001**
Fasting insulin (IU/L)	7.2 (1.9)	11.3 (5.6)	4.1 (3.4)	**0.23**	12.2 (2.3)	4.8 (3 5)	**0.18**	11.7 (2.3)	4.5 (3.6)	**0.22**
HOMA-IR	1.5 (0.5)	2.5 (1.2)	0.9 (0.8)	**0.25**	1.3 (0.8)	2.9 (0.6)	**0.12**	1.4 (0.8)	2.9 (0.7)	**0.11**
Matsuda index	6.2 (0.8)	5.8 (0.8)	-0.3 (0.5)	**0.50**	4.3 (0.8)	-1.8 (0.5)	**0.001**	4.7 (0.8) `	-1.8 (0.5)	**0.002**
Hepatic insulin resistance	40.7 (11.3)	39.2 (9.3)	-1.5 (10.6)	**0.89**	67.9 (15.4)	27.8 (10.9)	**0.02**	74.0 (22.7)	31.9 (11.3)	**0.008**
Muscle insulin resistance	0.9 (0.4)	0.6 (0.3)	-0.3 (0.3)	**0.36**	1.4 (0.6)	0.4 (0.4)	**0.24**	1.3 (0.6)	0.3 (0.4)	**0.38**
Co-ordinated stability (corners)	0.3 (0.2)	0.08 (0.08)	-0.2 (0.3)	**0.55**	0.7 (0.3)	0.5 (0 3)	**0.09**	0.8 (0 3)	0.6 (0.3)	**0.05**
Right hand grip (N)	37.9 (1.5)	36.8 (1.8)	-1.1 (0.9)	**0.25**	35.6 (1.7)	-2.6 (1.0)	**0.01**	33.2 (1.7)	-4.2 (1.0)	**<0.001**
Left hand grip (N)	34.4 (1.4)	34.5 (1.2)	0.1 (1.0)	**0.92**	33.1 (1.5)	-1.5 (1.0)	**0.16**	30.9 (1.6)	-3.4 (1.1)	**0.004**
Upper limb strength (N)	156.7 (12.4)	158.3 (11.7)	1.6 (7.6)	**0.84**	151.3 (9.6)	-7.2 (7.9)	**0.37**	144.6 (11.3) `	-7.7 (8.1)	**0.35**
Lower limb strength (N)	294.2 (25.6)	263.9 (20.8)	-30.3 (19.2)	**0.13**	247.3 (19.2)	-46.6 (19.7)	**0.03**	239.5 (15.6)	-50.9 (20.4)	**0.02**
Sit-to-stand test (number)	17 (1.1)	18 (1.2)	0.8 (0.7)	**0.25**	19 (1.7)	1.5 (0.7)	**0.04**	18 (1.5)	1.0 (0.7)	**0.17**
TUGT (sec)	5.6 (0.2)	5.4 (0.2)	-0.4 (0.2)	**0.08**	6.2 (0.3)	0.4 (0 2)	**0.04**	6.0 (0 3)	0.3 (0.2)	**0.04**
QLQ-C30 summary score	82.0 (3.7)	78.5 (3.9)	-0.3 (1.3)	**0.85**	75.0 (5.8)	-2.5 (1.4)	**0.09**	79 2 (4 2)	-3.2 (1.4)	**0.03**
IGF-1 (ng/mL)	19.9 (2.0)	19.0 (1.7)	-0.2 (1.3)	**0.9**	20.9 (2.1)	1.2 (1.4)	**0.37**	20.9 (1.4)	1.2 (1.4)	**0.39**
IGF-1/IGFBP3	0.23 (0.02)	0.18 (0.01)	-0.05 (0.02)	**0.02**	0.18 (0.01)	-0.04 (0.02)	**0.03**	0.18 (0.01)	-0.05 (0.03)	**0.02**
IGFBP-3 (ng/mL)	2555.7 (273.5)	2932.7 (194.9)	449.8 (187.9)	**0.02**	3081 (227.1)	602.2 (193.2)	**0.004**	3318.1 (209.7)	741.9 (199.5)	**<0.001**
Leptin (pg/mL)	3712.2 (731.8)	6132.5 (1122.4)	2344.4 (2534.1)	**0.36**	11375.5 (3944.6)	8113.5 (2604.1)	**0.004**	10492.2 (3040.5)	6943.2 (2685.6)	**0.01**
Adiponectin ⁻(x 10⁷ pg/mL)	5.1 (0.6)	5.0 (0.7)	1.9 (1.2)	**0.88**	5.6 (0.8)	1.2 (1 3)	**0.35**	8.8 (2 3)	3.8 (1.3)	**0.007**

Data are presented as mean ± S.E.M; *P* value represents change compared to baseline across the cohort at 6 weeks, 6 months and 12 months; BCM, body cell mass; FM, fat mass; BMD, bone mineral density; IGF-1, insulin-like growth factor; IGFBP-3, insulin-like growth factor binding protein 3

Supplementary Table 2: Effect of PRT on body composition: absolute values and changes over 6 weeks, 6 months and 12 months

Variables	Baseline		6 weeks		Group difference in mean change over 6 weeks PRT vs UC		6 months		Group difference in mean change over 6 mo PRT vs UC		12 months		Group difference in mean change over 12 mo PRT vs UC	
	UC (n = 12)	PRT (n = 13)	UC (n = 12)	PRT (n = 13)		**P value**	UC (n = 11)	PRT (n = 12)		**P value**	UC (n = 10)	PRT (n = 10)		**P value**
Total mass (kg)	80.3 (2.6)	87.3 (4.6)	80.0 (2.5)	88.4 (4.7)	1.5 (0.9)	**0.09**	80.7 (2.6)	89.3 (5.1)	0.5 (0.9)	**0.60**	82.0 (2.7)	88.7 (5.5)	-1.0 (0.9)	**0.30**
Total LBM (kg)	53.1 (1.2)	54.5 (2.2)	52.1 (1.1)	54.7 (2.1)	1.1 (0.7)	**0.09**	51.4 (1.0)	53.9 (2.2)	1.0 (0.7)	**0.14**	50.4 (1.2)	52.8 (2.5)	1.2 (0.7)	**0.10**
Total LBM (% total mass)	66.5 (1.8)	63.2 (2.0)	65.6 (1.7)	62.8 (2.2)	0.5 (0.8)	**0.47**	64.1 (1.7)	61.4 (2.2)	1.3 (0.8)	**0.11**	61.7 (1.4)	60.4 (2.1)	2.7 (0.8)	**0.001**
Total BCM (kg)	34.1 (1.0)	34.7 (1.3)	33.3 (1.0)	34.9 (1.3)	1.0 (0.6)	**0.11**	32.6 (1.0)	34.1 (1.4)	1.0 (0.7)	**0.12**	31.8 (0.9)	33.1 (1.6)	0.8 (0.7)	**0.28**
Total BCM (% total mass)	42.8 (1.7)	40.3 (1.7)	41.9 (1.6)	40.4 (1.8)	1.0 (0.8)	**0.21**	40.7 (1.6)	38.8 (1.9)	1.3 (0.8)	**0.10**	39.0 (1.4)	37.7 (2.0)	1.9 (0.8)	**0.02**
Total FM (kg)	24.1 (2.1)	29.9 (3.2)	24.7 (2.0)	30.6 (3.4)	0.2 (0.8)	**0.78**	26.2 (2.2)	32.3 (3.6)	-0.7 (0.8)	**0.35**	28.5 (2.1)	32.9 (3.6)	-2.3 (0.8)	**0.006**
Total FM (% total mass)	30.5 (1.9)	33.9 (2.2)	31.1 (1.9)	34.1 (2.2)	-0.4 (0.9)	**0.65**	32.9 (1.9)	35.9 (2.3)	-1.1 (0.9)	**0.25**	34.9 (1.5)	36.1 (1.9)	-3.1 (1.0)	**0.002**
FM trunk (kg)	14.8 (1.7)	18.2 (1.9)	15.4 (1.7)	18.7 (2.1)	-0.1 (0.7)	**0.94**	15.8 (1.8)	19.1 (2.2)	-0.8 (0.7)	**0.39**	16.8 (1.8)	19.0 (1.9)	-1.8 (0.8)	**0.02**
FM android (kg)	2.9 (0.3)	3.4 (0.4)	2.9 (0.4)	3.6 (0.4)	0.1 (0.2)	**0.67**	3.0 (0.4)	3.6 (0.4)	-0.1 (0.2)	**0.58**	3.1 (0.4)	3.5 (0.3)	-0.3 (0.2)	**0.15**
FM gynoid (kg)	3.5 (0.2)	4.5 (0.4)	3.6 (0.2)	4.6 (0.5)	0.1 (0.2)	**0.68**	4.0 (0.2)	4.9 (0.5)	-0.1 (0.2)	**0.51**	4.2 (0.3)	5.2 (0.6)	-0.03 (0.2)	**0.87**

Data are presented as mean ± S.E.M; P value represents group differences in mean changes between PRT and UC at 6 weeks, 6 months and 12 months; LBM, lean body mass; BCM, body cell mass; FM, fat mass.

Supplementary Table 3. Effect of PRT on BMD, activity levels and physical function: absolute values and changes over 6 weeks, 6 months and 12 months

Variables	Baseline UC (n = 12)	Baseline PRT (n = 13)	6 weeks UC (n = 12)	6 weeks PRT (n = 13)	Group difference in mean change at 6 weeks PRT vs UC	P value	6 months UC (n = 11)	6 months PRT (n = 12)	Group difference in mean change at 6 mo PRT vs UC	P value	12 months UC (n = 10)	12 months PRT (n = 10)	Group difference in mean change at 12 mo PRT vs UC	P value
Step count	40173 (8502)	28838 (5377)	35166 (5647)	36695 (6885)	12864 (7102)	**0.08**	35661 (7464)	30927 (5818)	7719 (7308)	**0.30**	27343 (8424)	30303 (6309)	19188 (7805)	**0.02**
Physical activity (hours)														
Light	5 9 (1 6)	7 9 (2 5)	10 2 (2 7)	9 0 (3 1)	-3 1 (2 9)	**0.28**	7 8 (1 8)	9 0 (2 2)	-0 6 (2 9)	**0.84**	7 7 (2 5)	9 9 (2 7)	-0 1 (3 2)	**0.98**
Moderate	3 9 (1 8)	3 3 (1 4)	5 6 (2 3)	4 2 (1 5)	-0 8 (2 0)	**0.69**	2 7 (1 7)	3 8 (1 5)	1 5 (2 0)	**0.46**	5 7 (2 3)	2 8 (1 7)	-0 7 (2 2)	**0.74**
Heavy	0 1 (0 1)	0 5 (0 3)	0 4 (0 3)	1 5 (1 2)	0 8 (0 8)	**0.32**	0 3 (0 3)	2 0 (1 0)	1 1 (0 8)	**0.17**	0 9 (0 6)	1 4 (1 0)	0 3 (0 9)	**0.69**
BMD (DXA)														
Neck of femur (left total)	1 002 (0 04)	1 03 (0 04)	1 01 (0 04)	1 04 (0 04)	-0 004 (0 02)	**0.83**	1 0 (0 04)	1 02 (0 04)	-0 004 (0 02)	**0.84**	1 0 (0 04)	0 992 (0 04)	0 01 (0 02)	**0.55**
Neck of femur (right total)	1 003 (0 05)	1 003 (0 04)	1 02 (0 04)	1 03 (0 04)	0 01 (0 02)	**0.45**	1 0 (0 05)	1 02 (0 04)	0 02 (0 02)	**0.25**	0 99 (0 04)	0 995 (0 04)	0 04 (0 02)	**0.10**
Spine (total)	1 428 (0 05)	1 22 (0 05)	1 42 (0 05)	1 23 (0 05)	0 02 (0 03)	**0.58**	1 39 (0 06)	1 21 (0 06)	0 03 (0 03)	**0.36**	1 36 (0 07)	1 18 (0 06)	0 03 (0 03)	**0.34**
Physical Function														
Balance														
Total sway floor (EO)	102 1 (15 0)	96 4 (12 3)	100 5 (18 7)	103 0 (11 7)	8 2 (19 2)	**0.67**	82 5 (8 2)	81 8 (7 8)	0 8 (19 7)	**0.97**	102 9 (14 7)	100 0 (7 6)	3 4 (20 6)	**0.87**
Total sway Floor (EC)	123 7 (10 3)	152 6 (13 5)	137 2 (24 7)	146 5 (11 8)	-19 6 (22 5)	**0.39**	135 2 (10 6)	157 6 (15 1)	-12 2 (23 1)	**0.60**	129 9 (19 4)	171 8 (17 3)	7 1 (24 1)	**0.77**
Co-ordinated stability														
Corners	0 3 (0 2)	0 3 (0 2)	0 08 (0 08)	0 5 (0 2)	0 4 (0 5)	**0.42**	0 7 (0 3)	0 6 (0 4)	-0 2 (0 5)	**0.67**	0 8 (0 3)	1 7 (0 5)	0 8 (0 5)	**0.13**
Sides	1 5 (0 8)	2 2 (0 5)	1 3 (0 4)	2 2 (0 6)	0 2 (0 8)	**0.84**	1 9 (0 5)	1 8 (0 6)	-0 8 (1 3)	**0.37**	1 9 (0 8)	1 0 (0 3)	-1 7 (0 9)	**0.06**
Strength														
Right hand (N)	37 9 (1 5)	36 3 (2 6)	36 8 (1 8)	36 3 (2 5)	1 2 (1 3)	**0.37**	35 6 (1 7)	34 4 (2 6)	1 1 (1 3)	**0.41**	33 2 (1 7)	32 2 (2 7)	1 8 (1 4)	**0.2**
Left hand (N)	34 4 (1 4)	37 1 (2 3)	34 5 (1 2)	36 9 (2 5)	-0 3 (1 3)	**0.84**	33 1 (1 5)	34 8 (2 6)	-0 8 (1 3)	**0.57**	30 9 (1 6)	32 7 (3 0)	0 2 (1 4)	**0.91**
Upper limb (N)	156 7 (12 4)	148 8 (9 0)	158 3 (11 7)	157 2 (8 3)	6 8 (11 8)	**0.57**	151 3 (9 6)	146 3 (12 5)	4 9 (12 2)	**0.69**	144 6 (11 3)	138 4 (10 0)	1 8 (12 8)	**0.89**
Lower limb (N)	294 2 (25 6)	294 2 (19 3)	263 9 (20 8)	306 5 (15 3)	42 6 (28 2)	**0.14**	247 3 (19 2)	258 3 (19 8)	15 6 (28 9)	**0.59**	239 5 (15 6)	222 0 (23 3 3)	-11 0 (30 3)	**0.72**
Sit-to-Stand	17 (1 1)	19 (1 4)	18 (1 2)	21 (1 4)	0 9 (1 0)	**0.40**	19 (1 7)	19 (1 2)	-0 6 (1 0)	**0.56**	18 (1 5)	18 (1 5)	-1 3 (1 1)	**0.25**
TUGT (s)	5 6 (0 2)	5 5 (0 3)	5 4 (0 2)	5 3 (0 2)	0 2 (0 3)	**0.52**	6 2 (0 3)	5 5 (0 2)	-0 5 (0 3)	**0.11**	6 0 (0 3)	5 9 (0 2)	0 1 (0 3)	**0.72**
VO_2 max (mL/kg/min)	26 3 (2 4)	27 3 (1 1)	29 1 (2 7)	27 8 (1 8)	-2 3 (2 2)	**0.33**	28 0 (1 7)	25 2 (1 4)	-1 6 (2 3)	**0.60**	29 2 (1 8)	29 1 (1 9)	0 9 (2 5)	**0.91**

Data are presented as mean ± S.E.M; *P* value is for group differences in mean changes between PRT and UC at 6 weeks, 6 months and 12 months; BMD, bone-mineral density; EO, eyes opened; EC, eyes closed; TUGT, timed get-up-and-go test

Supplementary Table 4. Effect of PRT on quality of life: absolute values and changes over 6 weeks, 6 months and 12 months

Variables	Baseline		6 weeks		Group difference in mean change at 6 weeks PRT vs UC	P value	6 months		Group difference in mean change at 6 mo PRT vs UC	P value	12 months		Group difference in mean change at 12 mo PRT vs UC	P value
	UC (n = 12)	PRT (n = 13)	UC (n = 12)	PRT (n = 13)			UC (n = 11)	PRT (n = 12)			UC (n = 10)	PRT (n = 10)		
EORTC QLQ-C30														
Physical functioning	91 7 (4 4)	95 9 (1 4)	91 7 (3 3)	95 9 (1 8)	0 01 (3 35)	0.99	88 7 (5 5)	93 3 (2 0)	-0 19 (3 51)	0.96	83 3 (7 5)	92 0 (2 4)	2 83 (3 62)	0.43
Role functioning	90 3 (5 2)	93 6 (4 4)	88 9 (6 3)	96 2 (2 8)	4 0 (6 8)	0.56	86 7 (9 2)	91 7 (3 9)	1 9 (7 1)	0.79	83 3 (6 1)	92 6 (4 0)	4 2 (7 5)	0.57
Emotional functioning	91 0 (6 4)	85 9 (4 4)	93 7 (4 2)	85 9 (3 7)	-2 8 (4 6)	0.55	93 3 (4 1)	87 5 (4 5)	-0 8 (4 8)	0.88	89 2 (5 0)	82 5 (5 9)	-3 0 (4 9)	0.55
Cognitive functioning	86 1 (3 5)	84 0 (4 3)	88 9 (3 1)	85 9 (3 7)	-0 9 (5 7)	0.88	86 7 (4 8)	82 6 (6 0)	-1 1 (6 0)	0.86	85 0 (4 6)	85 0 (2 9)	2 6 (6 1)	0.68
Social functioning	88 9 (5 9)	89 7 (4 0)	93 1 (4 8)	83 3 (5 7)	-10 6 (6 3)	0.10	86 7 (4 8)	91 7 (3 8)	3 2 (6 6)	0.63	85 0 (4 6)	91 7 (4 5)	5 4 (6 8)	0.43
Global health status and quality of life (QOL)	82 0 (3 7)	76 6 (4 3)	78 5 (3 9)	82 0 (4 2)	8 9 (5 0)	0.08	75 0 (5 8)	83 3 (4 2)	12 8 (5 2)	0.02	79 2 (4 2)	80 0 (4 3)	8 5 (5 4)	0.12
EORTC QLQ-C30 symptom scales														
Fatigue	11 1 (3 6)	13 7 (3 6)	18 5 (5 7)	13 7 (2 9)	-7 4 (5 4)	0.17	24 4 (6 6)	22 2 (5 1)	-4 5 (5 6)	0.43	17 8 (4 4)	20 0 (6 2)	-1 2 (5 8)	0.83
Pain	5 6 (3 1)	14 1 (2 6)	8 3 (3 8)	12 8 (4 3)	-4 1 (5 8)	0.48	13 3 (5 4)	6 9 (3 8)	-15 1 (6 0)	0.02	15 0 (4 6)	15 0 (5 2)	-6 5 (6 2)	0.30
Insomnia	11 1 (6 3)	17 9 (4 8)	13 9 (4 9)	20 5 (4 7)	-0 2 (7 4)	0.98	23 3 (10 0)	19 4 (6 4)	-10 6 (7 7)	0.17	13 3 (7 4)	23 3 (5 1)	5 2 (7 9)	0.52
EORTC QLQ-PR25														
Sexual activity	70 8 (6 5)	68 0 (8 1)	79 2 (5 5)	71 8 (6 9)	-4 5 (10 2)	0.66	85 0 (6 3)	87 5 (5 5)	5 6 (10 6)	0.60	91 7 (3 7)	80 0 (6 0)	-10 5 (10 9)	0.34
Urinary symptoms	24 3 (5 9)	19 5 (6 5)	21 5 (4 7)	12 4 (2 9)	-3 3 (7 9)	0.68	30 0 (5 2)	20 5 (3 0)	-3 3 (8 3)	0.69	32 5 (5 1)	16 3 (3 9)	-11 1 (8 5)	0.20
Bowel symptoms	2 1 (1 5)	2 9 (1 6)	2 5 (1 9)	4 5 (1 8)	1 1 (2 7)	0.67	2 5 (1 3)	3 5 (1 2)	0 05 92 8)	0.99	4 2 (1 9)	2 5 (1 8)	-2 3 (2 9)	0.43
Hormone-related symptoms	5 6 (2 5)	5 6 (1 8)	10 7 (2 3)	10 3 (2 7)	-0 4 (3 8)	0.92	17 8 (2 2)	15 8 (2 4)	-1 2 (4 0)	0.77	14 5 (2 8)	15 0 (3 5)	0 2 (4 1)	0.96
SF36v2 health survey														
Physical functioning	50 7 (2 4)	52 3 (1 1)	50 0 (2 6)	52 1 (1 6)	0 3 (2 6)	0.89	50 1 (2 7)	49 4 (2 4)	-2 1 (2 7)	0.45	49 1 (3 1)	49 7 (2 8)	1 3 (2 8)	0.63
Role- physical	49 7 (2 8)	50 8 (1 4)	51 5 (2 5)	52 7 (4 7)	0 002 (2 1)	0.99	47 7 (3 4)	49 1 (1 7)	1 5 (2 2)	0.51	48 4 (3 0)	49 7 (2 3)	-0 6 (2 3)	0.81
Bodily pain	55 8 (2 1)	53 0 (1 6)	54 7 (2 5)	56 9 (1 7)	5 0 (2 3)	0.03	56 2 (2 4)	53 6 (1 5)	0 3 (2 4)	0.91	53 8 (2 2)	54 2 (2 4)	2 6 (2 5)	0.30
General health	55 0 (2 1)	50 6 (2 2)	54 1 (2 4)	54 7 (2 4)	5 0 (2 4)	0.04	57 4 (2 6)	52 8 (2 0)	1 0 (2 6)	0.69	54 4 (3 5)	51 5 (2 0)	2 7 (2 6)	0.32
Vitality	59 3 (2 0)	55 3 (1 8)	56 6 (3 1)	56 4 (2 3)	3 8 (2 4)	0.11	54 1 (3 2)	54 3 (2 8)	5 8 (2 5)	0.02	54 7 (3 2)	56 2 (1 9)	6 0 (2 5)	0.02
Social functioning	54 8 (1 4)	54 9 (2 1)	51 9 (2 4)	54 6 (1 3)	2 7 (1 9)	0.15	50 8 (2 5)	55 3 (1 1)	4 2 (1 9)	0.03	51 3 (2 6)	53 3 (1 8)	2 1 (2 0)	0.31
Role- emotional	53 8 (1 8)	52 8 (1 8)	54 1 (1 7)	54 8 (0 8)	1 8 (2 2)	0.43	51 3 (2 8)	52 7 (1 3)	2 6 (2 3)	0.28	51 0 (3 1)	51 6 (1 6)	1 9 (2 4)	0.43
Mental health	58 7 (1 2)	54 1 (2 4)	56 3 (1 6)	57 4 (1 6)	2 8 (2 2)	0.21	56 8 (2 0)	56 9 (1 9)	3 7 (2 3)	0.12	57 2 (2 1)	50 8 (2 4)	4 9 (2 4)	0.04
Physical component summary	50 8 (2 4)	51 6 (1 4)	51 1 (2 6)	53 4 (1 7)	1 6 (1 8)	0.40	51 3 (3 0)	49 3 (1 9)	-1 7 (1 9)	0.37	48 1 (3 3)	49 6 (2 1)	-0 1 (2 0)	0.97
Mental component summary	58 2 (1 2)	54 4 (2 1)	56 2 (1 8)	55 9 (1 3)	3 4 (1 9)	0.08	54 9 (2 5)	56 7 (1 8)	5 7 (2 0)	0.006	55 4 (2 1)	56 3 (2 1)	5 5 (2 1)	0.01

Data are presented as mean ± S.E.M; *P* value represents group differences in mean changes between PRT and UC at 6 weeks, 6 months and 12 months.

Supplementary Table 5. Effect of PRT on glucose tolerance and insulin indices, growth factors and adipokines: absolute values and changes over 6 weeks, 6 months and 12 months

Variables	Baseline		6 weeks		Group difference in mean change over 6 weeks PRT vs UC	P value	6 months		Group difference in mean change over 6 mo PRT vs UC	P value	12 months		Group difference in mean change over 12 mo PRT vs UC	P value
	UC (n = 12)	PRT (n = 13)	UC (n = 12)	PRT (n = 13)		P value	UC (n = 11)	PRT (n = 12)		P value	UC (n = 10)	PRT (n = 10)		P value
Glucose: baseline (mmol/L)	4 7 (0 1)	4 5 (0 1)	4 8 (0 1)	4 6 (0 1)	-0 1 (0 2)	0.73	5 2 (0 2)	5 3 (0 2)	0 2 (0 2)	0.34	5 4 (0 2)	5 3 (0 2)	0 01 (0 2)	0.94
Glucose: 30 min (mmol/L)	8 3 (0 5)	7 2 (0 4)	7 8 (0 4)	6 7 (0 3)	0 1 (0 5)	0.84	8 7 (0 4)	7 7 (0 4)	0 3 (0 5)	0.56	9 0 (0 5)	7 6 (0 4)	-0 3 (0 5)	0.53
Glucose: 60 min (mmol/L)	10 3 (0 6)	8 9 (0 5)	9 7 (0 6)	8 7 (0 5)	0 4 (0 6)	0.52	9 5 (0 5)	8 5 (0 6)	0 8 (0 6)	0.22	9 4 (0 7)	8 6 (0 5)	0 4 (0 6)	0.51
Glucose: 90 min (mmol/L)	10 4 (0 6)	9 5 (0 5)	9 6 (0 7)	9 3 (0 6)	0 6 (0 8)	0.46	9 1 (0 6)	8 2 (0 6)	0 3 (0 8)	0.73	7 4 (0 7)	8 5 (0 5)	1 9 (0 9)	0.05
Glucose: 120 min (mmol/L)	9 3 (0 5)	9 5 (0 5)	8 8 (0 4)	8 9 (0 5)	-0 1 (0 7)	0.86	7 1 (0 7)	7 5 (0 7)	0 3 (0 8)	0.74	6 7 (0 7)	7 2 (0 5)	0 02 (0 8)	0.97
Insulin (IU/L) Baseline	7 2 (1 9)	7 9 (1 3)	11 3 (5 6)	6 8 (1 4)	-5 2 (3 8)	0.18	12 2 (2 3)	11 2 (1 6)	-1 6 (3 9)	0.68	11 7 (2 3)	15 8 (3 7)	3 2 (4 1)	0.44
Insulin (IU/L) 30min	41 8 (11 2)	36 0 (4 9)	37 3 (7 0)	30 5 (6 7)	-1 0 (10 7)	0.92	62 9 (13 1)	44 7 (6 4)	-14 8 (11 1)	0.19	65 9 (17 9)	56 5 (13 7)	-3 0 (11 6)	0.80
Insulin (IU/L) 60min	58 (14 2)	47 3 (10 1)	56 (13 4)	44 7 (10 5)	-0 6 (13 9)	0.97	79 7 (19 7)	58 6 (10 9)	-9 7 (14 4)	0.50	86 2 (28 2)	66 1 (12 9)	-9 5 (15 1)	0.53
Insulin (IU/L) 90min	79 2 (15 8)	65 5 (9 4)	87 3 (25 0)	44 4 (6 9)	-29 2 (23 9)	0.23	133 1 (40 2)	63 4 (8 7)	-53 7 (24 6)	0.03	73 5 (19 8)	71 5 (14 2)	10 8 (25 8)	0.68
Insulin (IU/L) 120min	78 7 (22 7)	68 5 (14 5)	94 9 (30 8)	46 6 (10 5)	-38 2 (26 6)	0.15	115 6 (41 4)	53 3 (10 2)	-53 6 (27 4)	0.05	57 9 (10 9)	55 1 (12 5)	-2 5 (28 6)	0.93
HOMA-IR	1 5 (0 5)	1 5 (0 3)	2 5 (1 2)	1 4 (0 3)	-1 1 (0 9)	0.25	2 9 (0 6)	2 7 (0 4)	-0 2 (0 9)	0.83	2 9 (0 7)	3 9 (1 0)	0 9 (1 0)	0.34
Hepatic insulin resistance	40 7 (11 3)	32 9 (4 9)	39 2 (9 3)	26 9 (5 6)	-4 4 (13 1)	0.73	67 9 (15 4)	46 3 (6 5)	-14 8 (13 5)	0.28	74 0 (22 7)	62 5 (17 0)	-3 4 (14 2)	0.81
Muscle insulin resistance	0 9 (0 4)	0 1 (0 1)	0 6 (0 3)	0 1 (0 3)	0 3 (0 4)	0.50	1 4 (0 6)	0 6 (0 2)	0 1 (0 4)	0.77	1 3 (0 6)	0 8 (0 2)	0 4 (0 4)	0.38
Matsuda Index	6 2 (0 8)	5 9 (0 7)	5 8 (0 8)	8 1 (1 3)	2 5 (0 8)	0.004	4 3 (0 8)	4 6 (0 6)	0 4 (0 8)	0.64	4 7 (0 8)	4 1 (0 8)	-0 04 (0 9)	0.96
Disposition Index	2 6 (0 6)	3 7 (0 7)	3 0 (0 7)	5 9 (1 9)	1 7 (1 5)	0.25	2 7 (0 5)	3 4 (0 6)	-0 8 (1 50	0.61	2 9 (0 6)	3 2 (0 9)	-0 3 (1 6)	0.84
IGF-1 (ng/mL)	19 2 (1 7)	21 8 (1 2)	19 0 (1 7)	20 3 (1 1)	-1 4 (2 3)	0.55	20 9 (2 1)	20 4 (1 4)	-2 5 (2 3)	0.29	20 9 (1 4)	24 4 (2 4)	1 9 (2 4)	0.42
Leptin (pg/mL)	3788 1 (613 2)	7406 8 (2183 0)	6132 5 (1122 4)	9114 6 (2345 4)	-636 7 (3021 0)	0.84	11375 5 (3944 6)	11772 0 (2714 1)	-4083 0 (3107 8)	0.19	10492 2 (3040 5)	16367 0 (4578 0)	1499 1 (3255 9)	0.65
Adiponectin (x10^7) (pg/mL)	4 8 (0 6)	4 7 (0 6)	5 0 (0 7)	4 7 (0 6)	0 1 (1 4)	0.93	5 6 (0 8)	5 8 (0 8)	0 9 (1 5)	0.95	8 8 (2 3)	5 2 (0 7)	-3 2 (1 5)	0.04
IGFBP3 (ng/mL)	2482 9 (231 2)	3007 6 (246 7)	2932 7 (194 9)	2817 3 (261 6)	-640 1 (-193 7)	0.11	3081 (227 1)	3398 8 (240 6)	101 0 (395 0)	0.63	3318 1 (209 7)	3908 6 (246 1)	405 1 (422 7)	0.80
IGF-1:IGFBP3	0 227 (0 023)	0 215 (0 017)	0 181 (0 013)	0 163 (0 016)	0 033 (0 031)	0.06	0 188 (0 011)	0 153 (0 021)	0 032 (0 042)	0.1	0 179 (0 012)	0 184 (0 014)	0 021 (0 038)	0.07

Data are presented as mean ± S.E.M; *P* value represents group differences in mean changes between PRT and UC at 6 weeks, 6 months and 12 months. IGF-1, insulin-like growth factor; IGFBP-3, insulin-like growth factor binding protein 3

131

Chapter 6: Conclusions and future directions

6.1 Concluding summary

Testosterone is critical for the maintenance of muscle mass and function, bone mass and body composition (1). Its gradual decline with aging plays an important role in the development of sarcopenia, contributing to functional decline and physical disability which presents a substantial cost to the public health system as represented by hospitalisations, nursing home admissions and home healthcare expenditure (6). Thus, given the aging population, strategies to improve the management of sarcopenia are required.

This thesis provides an overview of the physiological actions of testosterone, particularly its role as a regulator of muscle protein balance, shifting protein balance in favour of net protein accretion leading to gains in muscle mass. In contrast, the hypogonadal state, like that during ADT, results in protein catabolism and a subsequent hydrolysis of protein into amino acids. These may then be eliminated as urea via the hepatic urea cycle, thereby representing a net loss of amino acid nitrogen. Recent evidence provided by my supervisor, Dr Vita Birzniece, and her collaborators, showed that the liver is a site of the anabolic action of testosterone (50). My work tested the hypothesis that this effect is mediated through an action of testosterone on activity of the hepatic urea cycle and used the clinical model of men with spontaneously occurring hypogonadism, or those undergoing therapeutic androgen deficiency. As examined in chapter 3, eight hypogonadal men were studied at baseline, and after two weeks of transdermal testosterone replacement. Results show, for the first time, that testosterone

replacement significantly reduces hepatic urea production in hypogonadal men. This provides the first biochemical evidence in humans that testosterone stimulates protein anabolism via inhibition of the hepatic urea cycle- to reduce irreversible nitrogen losses. These results are further substantiated in chapter 4, by the finding that induction of profound testosterone deficiency by ADT increases hepatic urea production and is associated with a whole-body catabolic effect.

Thus, this thesis contributes to current knowledge, by providing further insight into the physiological actions of testosterone in regulating protein metabolism and body composition in men. These findings are significant as it raises the possibility of using liver-targeted testosterone therapy in the prevention of protein losses through the hepatic urea cycle. For example, orally administered testosterone in a low dose could selectively expose the liver to androgen action by delivery through the portal blood flow from intestine to liver. The high first-pass metabolism of testosterone in the liver would minimize any systemic bioavailability and confine androgen action to hepatic tissue. This represents a potential intervention in the treatment of sarcopenia, enabling a whole-body anabolic action of testosterone treatment while avoiding the adverse effects of systemic administration.

Chapter 5 of the thesis presents a randomised controlled trial investigating the efficacy of a home-based PRT program in preventing the detrimental effects of ADT when instituted at the start of treatment. It is important to highlight that is the first long-term (12 month) home-based study to be conducted in prostate cancer patients. Results showed that the early implementation of this program results in beneficial effects on body composition, physical activity levels, and improvements in quality of life. This is also the first study in humans to show significant increases in IGFBP-3 (a growth factor

binding protein that not only reduces IGF-1 bioactivity, but also has independent anti-cancer effects) levels following ADT. Thus, findings from this study are clinically significant. Notably, it highlights the benefits of a home-based PRT program during prostate cancer treatment, offering clinicians a more cost-effective and viable alternative to supervised exercise programs. As there is also evidence that obesity, depression and low physical activity affects survivorship in men with prostate cancer, the benefits obtained from a home-based PRT program may translate to improved treatment outcomes and mortality rates. Furthermore, the retention of muscle mass, increased muscle contraction, and improvement in mental health during PRT may reduce systemic inflammation, stimulate development of protective myokines, and modulate epigenetic effects through reductions in miRNAs and prevention of telomere shortening which benefit men with prostate cancer as well as the aging population with sarcopenia.

In summary, this thesis has expanded our current knowledge regarding the physiology of testosterone action and benefits of exercise therapy during prostate cancer treatment specifically by:

1) Demonstrating, for the first time, biochemical evidence of a testosterone effect on the hepatic urea cycle in humans, thereby resulting in whole-body protein anabolism

2) Providing further evidence of a testosterone effect on the hepatic urea cycle by showing that ADT results in increased hepatic urea production which is associated with a whole-body catabolic effect

3) Demonstrating, for the first time in humans, an increase in serum IGFBP-3 (a cellular apoptotic protein) levels during ADT

4) Providing evidence of benefit of a 12-month home-based PRT program in preventing the adverse effect of ADT when instituted at the start of treatment

6.2 Future directions

The findings of a hepatic site of testosterone action via the urea cycle is clinically significant as it opens a novel approach of targeting the hepatic urea cycle to enhance whole-body anabolism. This liver-targeted approach has an advantage in conditions whereby the administration of systemic testosterone may be associated with adverse effects. Thus, there is the potential to develop liver-targeted testosterone as a safe and cost-effective treatment for sarcopenia. Therefore, further research is required in the use of oral testosterone in patients with sarcopenia to determine its efficacy, particularly its effects on body composition, and the magnitude of increase in LBM.

The findings of an increase in serum IGFBP-3 following ADT is significant, given the inhibitory role of IGFBP-3 in the invasion and metastasis of prostate cancer based on *in-vitro* studies (164,165). Thus, more clinical trials are needed to ascertain the clinical application of IGFBP-3 in castrate-sensitive prostate cancer, and its potential role as an adjunct to PSA for monitoring of tumour burden during ADT. The finding of a more substantial increase in serum adiponectin levels in the UC, compared to the PRT group also warrants further investigation into the pathophysiology of adiponectin in cancer, particularly in view of recent findings of a higher risk of incidental cancer in a type 2 diabetes cohort with raised adiponectin levels.

Given the benefits of a home-based PRT program on body composition, physical activity, and mental health parameters, it should be recommended following the diagnosis of prostate cancer, at the start of ADT. PRT-oriented exercise physiology clinics should ideally be offered to cancer patients, particularly those with prostate cancer, at the time of diagnosis to prevent the development of adverse effects following treatment. It is also important to note that the findings from this PhD has contributed to the establishment of this form of clinic for prostate cancer patients at Westmead Hospital (with special thanks to Dr Amy Hayden and Prof Howard Gurney). Thus, there is a need for policy change (not just in Australia but worldwide) in the provision of available services such as that of a resistance training program as part of routine prostate cancer therapy. Further research also needs to be conducted in the area of exercise medicine, particularly the impact of PRT on potential cancer signalling pathways which will result in better understanding of the mechanisms of benefit of PRT.

References

1.   Bhasin S, Woodhouse L, Casaburi R, Singh AB, Bhasin D, Berman N et al. Testosterone dose-response relationships in healthy young men. American journal of physiology endocrinology and metabolism 2001; 281:E1172-1181

2.   Bhasin S, Storer TW, Berman N, Yarasheski KE, Clevenger B, Phillips J, Lee WP, Bunnell TJ, Casaburi R. Testosterone replacement increases fat-free mass and muscle size in hypogonadal men. J Clin Endocrinol Metab 1997; 82:407-413

3.   Snyder PJ, Peachey H, Hannoush P, Berlin JA, Loh L, Lenrow DA et al. Effect of testosterone treatment on body composition and muscle strength in men over 65 years of age. J Clin Endocrinol Metab 1999; 84:2647-2653

4.   Basualto-Alarcon C, Varela D, Duran J, Maass R, Estrada M. Sarcopenia and Androgens: A Link between Pathology and Treatment. Front Endocrinol (Lausanne) 2014; 5:217

5.   Janssen I, Shepard DS, Katzmarzyk PT, Roubenoff R. The healthcare costs of sarcopenia in the United States. Journal of the American Geriatrics Society 2004; 52:80-85

6.   Beaudart C, Rizzoli R, Bruyère O, Reginster J-Y, Biver E. Sarcopenia: burden and challenges for public health. Archives of Public Health 2014; 72:45

7.   Herbst KL, Bhasin S. Testosterone action on skeletal muscle. Curr Opin Clin Nutr Metab Care 2004; 7:271-277

8.   Rossetti ML, Steiner JL, Gordon BS. Androgen-mediated regulation of skeletal muscle protein balance. Molecular and cellular endocrinology 2017; 447:35-44

9.   Saheki T, Hosoya M, Fujinami S, Katsunuma T. In: Lowenthal A, Mori A, Marescau B, eds. Urea Cycle Diseases. Advances in Experimental Medicine and Biology. Vol 153. Springer, Boston, MA: Springer; 1982.

10.   Grofte T, Wolthers T, Jensen SA, Moller N, Jorgensen JO, Tygstrup N, Orskov H, Vilstrup H. Effects of growth hormone and insulin-like growth factor-I singly and in combination on in vivo capacity of urea synthesis, gene expression of urea cycle enzymes, and organ nitrogen contents in rats. Hepatology (Baltimore, Md) 1997; 25:964-969

11.   McLean P, Gurney MW. Effect of adrenalectomy and of growth hormone on enzymes concerned with urea synthesis in rat liver. Biochem J 1963; 87:96-104

12.   Kenyon AT, Knowlton K, Sandiford I, Koch FC, Lotwin G. A Comparative study of the metabolic effects of testosterone propionate in normal men and women and in eunuchoidism. Endocrinology 1940; 26:26-45

13.   Brown GW, Jr., Cohen PP. Comparative biochemistry of urea synthesis. I. Methods for the quantitative assay of urea cycle enzymes in liver. J Biol Chem 1959; 234:1769-1774

14.   Kochakian CD. Anabolic-Androgenic Steroids. Vol 43. Heidelberg: Springer-Verlag.

15.   Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68:394-424

16. Karantanos T, Corn PG, Thompson TC. Prostate cancer progression after androgen deprivation therapy: mechanisms of castrate resistance and novel therapeutic approaches. Oncogene 2013; 32:5501-5511

17. Galvao DA, Taaffe DR, Spry N, Joseph D, Newton RU. Cardiovascular and metabolic complications during androgen deprivation: exercise as a potential countermeasure. Prostate Cancer Prostatic Dis 2009; 12:233-240

18. Chipperfield K, Fletcher J, Millar J, Brooker J, Smith R, Frydenberg M, Burney S. Predictors of depression, anxiety and quality of life in patients with prostate cancer receiving androgen deprivation therapy. Psychooncology 2013;

19. Law TD, Clark LA, Clark BC. Resistance Exercise to Prevent and Manage Sarcopenia and Dynapenia. Annu Rev Gerontol Geriatr 2016; 36:205-228

20. Hasenoehrl T, Keilani M, Sedghi Komanadj T, Mickel M, Margreiter M, Marhold M, Crevenna R. The effects of resistance exercise on physical performance and health-related quality of life in prostate cancer patients: a systematic review. Support Care Cancer 2015; 23:2479-2497

21. Galvao DA, Newton RU, Gardiner RA, Girgis A, Lepore SJ, Stiller A, Occhipinti S, Chambers SK. Compliance to exercise-oncology guidelines in prostate cancer survivors and associations with psychological distress, unmet supportive care needs, and quality of life. Psychooncology 2015; 24:1241-1249

22. Gentile MA, Nantermet PV, Vogel RL, Phillips R, Holder D, Hodor P, Cheng C, Dai H, Freedman LP, Ray WJ. Androgen-mediated improvement of body composition and muscle function involves a novel early transcriptional program including IGF1, mechano growth factor, and induction of {beta}-catenin. J Mol Endocrinol 2010; 44:55-73

23. MacLean HE, Chiu WS, Notini AJ, Axell AM, Davey RA, McManus JF, Ma C, Plant DR, Lynch GS, Zajac JD. Impaired skeletal muscle development and function in male, but not female, genomic androgen receptor knockout mice. FASEB J 2008; 22:2676-2689

24. Sinha-Hikim I, Taylor WE, Gonzalez-Cadavid NF, Zheng W, Bhasin S. Androgen receptor in human skeletal muscle and cultured muscle satellite cells: up-regulation by androgen treatment. J Clin Endocinol Metab 2004; 89:5245-5255

25. Singh R, Artaza JN, Taylor WE, Gonzalez-Cadavid NF, Bhasin S. Androgens stimulate myogenic differentiation and inhibit adipogenesis in C3H 10T1/2 pluripotent cells through an androgen receptor-mediated pathway. Endocrinology 2003; 144:5081-5088

26. Rana K, Lee NK, Zajac JD, MacLean HE. Expression of androgen receptor target genes in skeletal muscle. Asian J Androl 2014; 16:675-683

27. Thomas M, Dadgar N, Aphale A, Harrell JM, Kunkel R, Pratt WB, Lieberman AP. Androgen receptor acetylation site mutations cause trafficking defects, misfolding, and aggregation similar to expanded glutamine tracts. J Biol Chem 2004; 279:8389-8395

28. Yoo Y-E, Ko C-P. Dihydrotestosterone Ameliorates Degeneration in Muscle, Axons and Motoneurons and Improves Motor Function in Amyotrophic Lateral Sclerosis Model Mice. PloS one 2012; 7:e37258

29. Estrada M, Espinosa A, Muller M, Jaimovich E. Testosterone stimulates intracellular calcium release and mitogen-activated protein kinases via a G protein-coupled receptor in skeletal muscle cells. Endocrinology 2003; 144:3586-3597

30. Griggs RC, Kingston W, Jozefowicz RF, Herr BE, Forbes G, Halliday D. Effect of testosterone on muscle mass and muscle protein synthesis. J Appl Physiol (Bethesda, Md : 1985) 1989; 66:498-503

31. Ferrando AA, Tipton KD, Doyle D, Phillips SM, Cortiella J, Wolfe RR. Testosterone injection stimulates net protein synthesis but not tissue amino acid transport. Am J Physiol 1998; 275:E864-871

32. Sheffield-Moore M, Urban RJ, Wolf SE, Jiang J, Catlin DH, Herndon DN, Wolfe RR, Ferrando AA. Short-term oxandrolone administration stimulates net muscle protein synthesis in young men. J Clin Endocrinol Metab 1999; 84:2705-2711

33. Brodsky IG, Balagopal P, Nair KS. Effects of testosterone replacement on muscle mass and muscle protein synthesis in hypogonadal men--a clinical research center study. J Clin Endocrinol Metab 1996; 81:3469-3475

34. Urban RJ, Bodenburg YH, Gilkison C, Foxworth J, Coggan AR, Wolfe RR, Ferrando A. Testosterone administration to elderly men increases skeletal muscle strength and protein synthesis. Am J Physiol 1995; 269:E820-826

35. Church DD, Pasiakos SM, Wolfe RR, Ferrando AA. Acute testosterone administration does not affect muscle anabolism. Nutr Metab (Lond) 2019; 16:56

36. Ferrando AA, Sheffield-Moore M, Yeckel CW, Gilkison C, Jiang J, Achacosa A, Lieberman SA, Tipton K, Wolfe RR, Urban RJ. Testosterone administration to older men improves muscle function: molecular and physiological mechanisms. Am J Physiol Endocrinol Metab 2002; 282:E601-607

37. White JP, Gao S, Puppa MJ, Sato S, Welle SL, Carson JA. Testosterone regulation of Akt/mTORC1/FoxO3a signaling in skeletal muscle. Mol Cell Endocrinol 2013; 365:174-186

38. Basualto-Alarcon C, Jorquera G, Altamirano F, Jaimovich E, Estrada M. Testosterone signals through mTOR and androgen receptor to induce muscle hypertrophy.
Med Sci Sports Exerc 2013; 45:1712-1720

39. Ferrando AA, Sheffield-Moore M, Paddon-Jones D, Wolfe RR, Urban RJ. Differential anabolic effects of testosterone and amino acid feeding in older men. J Clin Endocrinol Metab 2003; 88:358-362

40. Serra C, Sandor NL, Jang H, Lee D, Toraldo G, Guarneri T, Wong S, Zhang A, Guo W, Jasuja R, Bhasin S. The effects of testosterone deprivation and supplementation on proteasomal and autophagy activity in the skeletal muscle of the male mouse: differential effects on high-androgen responder and low-androgen responder muscle groups. Endocrinology 2013; 154:4594-4606

41. Schutz Y. Protein Turnover, Ureagenesis and Gluconeogenesis. Int J Vitam Nutr Res 2011; 81:101-107

42. Consolazio CF, Nelson RA, Matoush LO, Harding RS, Canham JE. Nitrogen excretion in sweat and its relation to nitrogen balance requirements. J Nutr 1963; 79:399-406

43. Marks D, Marks A, Smith M. Basic Medical Biochemistry. Williams and Wilkins 1996.

44. Grofte T, Jensen DS, Greisen J, Tygstrup N, Vilstrup H. Growth hormone and insulin-like growth factor-I counteracts established steroid catabolism in rats by effects on hepatic amino-N degradation. J Hepatol 2001; 35:700-706

45. Grofte T, Wolthers T, Jorgensen JO, Poulsen PL, Vilstrup H, Moller N. Hepatic amino- to urea-N clearance and forearm amino-N exchange during hypoglycemic and euglycemic hyperinsulinemia in normal man. J Hepatol 1999; 30:819-825

46. Wolthers T, Hamberg O, Grofte T, Vilstrup H. Effects of budesonide and prednisolone on hepatic kinetics for urea synthesis. J Hepatol 2000; 33:549-554

47. Vilstrup H. On urea synthesis--regulation in vivo. Dan Med Bull 1989; 36:415-429

48. Okun JG, Conway S, Schmidt KV, Schumacher J, Wang X, de Guia R, Zota A, Klement J, Seibert O, Peters A et al. Molecular regulation of urea cycle function by the liver glucocorticoid receptor. Mol Metab2015; 4:732-740

49. Riggs TR, Walker LM. Diminution of arginine synthetase activity in livers of rats treated with testosterone propionate. Endocrinology 1963; 73:830-831

50. Birzniece V, Umpleby MA, Poljak A, Handelsman DJ, Ho KK. Oral low-dose testosterone administration induces whole-body protein anabolism in postmenopausal women: a novel liver-targeted therapy. Eur J Endocrinol 2013; 169:321-327

51. Birzniece V, Meinhardt UJ, Umpleby MA, Handelsman DJ, Ho KK. Interaction between testosterone and growth hormone on whole-body protein anabolism occursin the liver. J Clin Endocrinol Metab 2011; 96:1060-106

52. Birzniece V. Hepatic actions of androgens in the regulation of metabolism. Curr Opin Endocrinol Diabetes Obes 2018; 25:201-208

53. Eagon PK, Elm MS, Stafford EA, Porter LE. Androgen receptor in human liver: characterization and quantitation in normal and diseased liver. Hepatology (Baltimore, Md) 1994; 19:92-100

54. Eisenfeld AJ, Aten RF. Estrogen receptors and androgen receptors in the mammalian liver. J Steroid Biochem 1987; 27:1109-1118

55. Patel AR, Klein EA. Risk factors for prostate cancer. Nat Clin Pract Neurol 2009; 6:87

56. Roddam AW, Allen NE, Appleby P, Key TJ. Endogenous sex hormones and prostate cancer: a collaborative analysis of 18 prospective studies. J Natl Cancer Inst 2008; 100:170-183

57. Makridakis NM, Ross RK, Pike MC, Crocitto LE, Kolonel LN, Pearce CL, Henderson BE, Reichardt JK. Association of mis-sense substitution in SRD5A2 gene with prostate cancer in African-American and Hispanic men in Los Angeles, USA. Lancet (London, England) 1999; 354:975-978

58. Setiawan VW, Schumacher FR, Haiman CA, Stram DO, Albanes D, Altshuler D, Berglund G, Buring J, Calle EE, Clavel-Chapelon F et al. CYP17 genetic variation and risk of breast and prostate cancer from the National Cancer Institute Breast and Prostate Cancer Cohort Consortium (BPC3). Cancer Epidemiol Biomarkers Prev 2007; 16:2237-2246

59. Freedland SJ, Aronson WJ. Examining the relationship between obesity and prostate cancer. Rev Urol 2004; 6:73-81

60. Pelser C, Mondul AM, Hollenbeck AR, Park Y. Dietary fat, fatty acids, and risk of prostate cancer in the NIH-AARP diet and health study. Cancer Epidemiol Biomarkers Prev 2013; 22:697-707

61. Kasper JS, Giovannucci E. A meta-analysis of diabetes mellitus and the risk of prostate cancer. Cancer Epidemiol Biomarkers Prev 2006; 15:2056-2062

62. Tseng C-H. Diabetes and Risk of Prostate Cancer. Diabetes Care 2011; 34:616

63. Darbinian JA, Ferrara AM, Van Den Eeden SK, Quesenberry CP, Jr., Fireman B, Habel LA. Glycemic status and risk of prostate cancer. Cancer Epidemiol Biomarkers Prev 2008; 17:628-635

64. World Cancer Research Fund/American Institute for Cancer Research. Continuous Update Project Expert Report 2018. Diet, nutrition, physical activity and prostate cancer. Available at dietandcancerreport.org.

65. Kraemer WJ, Ratamess NA, French DN. Resistance training for health and performance. Curr Sports Med Rep2002; 1:165-171

66. Peterson MD, Sen A, Gordon PM. Influence of Resistance Exercise on Lean Body Mass in Aging Adults: A Meta-Analysis. Med Sci Sports Exerc 2011; 43:249-258

67. Cheema B, Abas H, Smith B, O'Sullivan A, Chan M, Patwardhan A, Kelly J, Gillin A, Pang G, Lloyd B, Singh MF. Progressive exercise for anabolism in kidney disease (PEAK): a randomized, controlled trial of resistance training during hemodialysis. Clin J Am Soc Nephrol 2007; 18:1594-1601

68. Grinspoon S, Corcoran C, Parlman K, Costello M, Rosenthal D, Anderson E, Stanley T, Schoenfeld D, Burrows B, Hayden D, Basgoz N, Klibanski A. Effects of testosterone and progressive resistance training in eugonadal men with AIDS wasting. A randomized, controlled trial. Ann Intern Med 2000; 133:348-355

69. Clinical Oncology Society of Australia 2017, COSA Position Statement on Exercise in Cancer Care, viewed 18th March 2020, <http://www.cosa.org.au>

70. Faris JE, Smith MR. Metabolic sequelae associated with androgen deprivation therapy for prostate cancer. Curr Opin Endocrinol Diabetes Obes 2010; 17:240-246

71. Smith JC, Bennett S, Evans LM, Kynaston HG, Parmar M, Mason MD, Cockcroft JR, Scanlon MF, Davies JS. The effects of induced hypogonadism on arterial stiffness, body composition, and metabolic parameters in males with prostate cancer. J Clin Endocrinol Metab 2001; 86:4261-4267

72. Nabid A, Carrier N, Martin A-G, Bahary J-P, Lemaire C, Vass S et al. Duration of Androgen Deprivation Therapy in High-risk Prostate Cancer: A Randomized Phase III Trial. Eur Urol 2018; 74:432-441

73. Galvao DA, Nosaka K, Taaffe DR, Spry N, Kristjanson LJ, McGuigan MR, Suzuki K, Yamaya K, Newton RU. Resistance training and reduction of treatment side effects in prostate cancer patients. Aust J Sci Med Sport 2006; 38:2045-2052

74. Hughes VA, Frontera WR, Roubenoff R, Evans WJ, Singh MA. Longitudinal changes in body composition in older men and women: role of body weight change and physical activity. Am J Clin Nutr 2002; 76:473-481

75. van Londen GJ, Levy ME, Perera S, Nelson JB, Greenspan SL. Body composition changes during androgen deprivation therapy for prostate cancer: a 2-year prospective study. Crit Rev Oncol Hematol 2008; 68:172-177

76. Hamilton EJ, Gianatti E, Strauss BJ, Wentworth J, Lim-Joon D, Bolton D, Zajac JD, Grossmann M. Increase in visceral and subcutaneous abdominal fat in men with prostate cancer treated with androgen deprivation therapy. Clinical endocrinology 2011; 74:377-383

77. Cheung AS, Gray H, Schache AG, Hoermann R, Lim Joon D, Zajac JD, Pandy MG, Grossmann M. Androgen deprivation causes selective deficits in the

biomechanical leg muscle function of men during walking: a prospective case-control study. J Cachexia Sarcopenia Muscle 2017; 8:102-112

78. Winters-Stone KM, Moe E, Graff JN, Dieckmann NF, Stoyles S, Borsch C, Alumkal JJ, Amling CL, Beer TM. Falls and Frailty in Prostate Cancer Survivors: Current, Past, and Never Users of Androgen Deprivation Therapy. J Am Geriatr Soc 2017; 65:1414-1419

79. Strom SS, Wang X, Pettaway CA, Logothetis CJ, Yamamura Y, Do KA, Babaian RJ, Troncoso P. Obesity, weight gain, and risk of biochemical failure among prostate cancer patients following prostatectomy. Clin Cancer Res 2005; 11:6889-6894

80. Efstathiou JA, Bae K, Shipley WU, Hanks GE, Pilepich MV, Sandler HM, Smith MR. Obesity and Mortality in Men With Locally Advanced Prostate Cancer: Analysis of RTOG 85-31. Cancer 2007; 110:2691-2699

81. Smith MR, Bae K, Efstathiou JA, Hanks GE, Pilepich MV, Sandler HM, Shipley WU. Diabetes and Mortality in Men With Locally Advanced Prostate Cancer: RTOG 92-02. J Clin Oncol 2008; 26:4333-4339

82. Freedland SJ, Aronson WJ, Kane CJ, Presti JC, Jr., Amling CL, Elashoff D, Terris MK. Impact of obesity on biochemical control after radical prostatectomy for clinically localized prostate cancer: a report by the Shared Equal Access Regional Cancer Hospital database study group. J Clin Oncol 2004; 22:446-453

83. Cao Y, Ma J. Body mass index, prostate cancer-specific mortality, and biochemical recurrence: a systematic review and meta-analysis. Cancer prevention research (Philadelphia, Pa) 2011; 4:486-501

84. Smith MR, Lee H, Nathan DM. Insulin sensitivity during combined androgen blockade for prostate cancer. J Clin Endocrinol Metab 2006; 91:1305-1308

85. Harrington JM, Schwenke DC, Epstein DR, Bailey DE, Jr. Androgen-deprivation therapy and metabolic syndrome in men with prostate cancer. Oncol Nurs Forum 2014; 41:21-29

86. Keating NL, O'Malley AJ, Smith MR. Diabetes and cardiovascular disease during androgen deprivation therapy for prostate cancer. J Clin Endocrinol Metab 2006; 24:4448-4456

87. Lage MJ, Barber BL, Markus RA. Association between androgen-deprivation therapy and incidence of diabetes among males with prostate cancer. Urology 2007; 70:1104-1108

88. Alibhai SM, Duong-Hua M, Sutradhar R, Fleshner NE, Warde P, Cheung AM, Paszat LF. Impact of androgen deprivation therapy on cardiovascular disease and diabetes.J Clin Oncol ; 27:3452-3458

89. Tsai HT, Keating NL, Van Den Eeden SK, Haque R, Cassidy-Bushrow AE, Ulcickas Yood M, Smith MR, Potosky AL. Risk of diabetes among patients receiving primary androgen deprivation therapy for clinically localized prostate cancer. J Urol 2015; 193:1956-1962

90. Hammarsten J, Hogstedt B. Hyperinsulinaemia: a prospective risk factor for lethal clinical prostate cancer. Eur J Cancer 2005; 41:2887-2895

91. Ma J, Li H, Giovannucci E, Mucci L, Qiu W, Nguyen PL, Gaziano JM, Pollak M, Stampfer MJ. Prediagnostic body-mass index, plasma C-peptide concentration, and prostate cancer-specific mortality in men with prostate cancer: a long-term survival analysis. The Lancet Oncology 2008; 9:1039-1047

92. Neuhouser ML, Till C, Kristal A, Goodman P, Hoque A, Platz EA, Hsing AW, Albanes D, Parnes HL, Pollak M. Finasteride modifies the relation between serum C-peptide and prostate cancer risk: results from the Prostate Cancer Prevention Trial. Cancer prevention research (Philadelphia, Pa) 2010; 3:10.1158/1940-6207.CAPR-1109-0188

93. Lee J, Giovannucci E, Jeon JY. Diabetes and mortality in patients with prostate cancer: a meta-analysis. SpringerPlus 2016; 5:1548

94. Hara N, Takizawa I, Isahaya E, Nishiyama T, Hoshii T, Ishizaki F, Takahashi K. Insulin-like growth factor-1 is associated with regulation of the luteinizing hormone production in men receiving androgen deprivation therapy with gonadotropin-releasing hormone analogues for localized prostate cancer. Urol Oncol 2012; 30:596-601

95. Dean JP, Sprenger CC, Wan J, Haugk K, Ellis WJ, Lin DW et al. Response of the insulin-like growth factor (IGF) system to IGF-IR inhibition and androgen deprivation in a neoadjuvant prostate cancer trial: effects of obesity and androgen deprivation. J Clin Endocrinol Metab 2013; 98:E820-E828

96. Rowlands MA, Holly JM, Hamdy F, Phillips J, Goodwin L, Marsden G, Gunnell D, Donovan J, Neal DE, Martin RM. Serum insulin-like growth factors and mortality in localised and advanced clinically detected prostate cancer. Cancer Causes Control 2012; 23:347-354

97. Aggarwal RR, Ryan CJ, Chan JM. Insulin-like growth factor pathway: A link between androgen deprivation therapy (ADT), insulin resistance, and disease progression in patients with prostate cancer? Urol Oncol 2013; 31:522-530

98. Baxter RC. IGF binding proteins in cancer: mechanistic and clinical insights. Nat Rev Cancer 2014; 14:329-341

99. Degraff DJ, Aguiar AA, Sikes RA. Disease evidence for IGFBP-2 as a key player in prostate cancer progression and development of osteosclerotic lesions. Am J Transl Res 2009; 1:115-130

100. Figueroa JA, De Raad S, Tadlock L, Speights VO, Rinehart JJ. Differential expression of insulin-like growth factor binding proteins in high versus low Gleason score prostate cancer. J Urol 1998; 159:1379-1383

101. Kiyama S, Morrison K, Zellweger T, Akbari M, Cox M, Yu D, Miyake H, Gleave ME. Castration-Induced Increases in Insulin-Like Growth Factor-Binding Protein 2 Promotes Proliferation of Androgen-independent Human Prostate LNCaP Tumors. Cancer Research 2003; 63:3575-3584

102. Chan JM, Stampfer MJ, Ma J, Gann P, Gaziano JM, Pollak M, Giovannucci E. Insulin-like growth factor-I (IGF-I) and IGF binding protein-3 as predictors of advanced-stage prostate cancer. Journal of the National Cancer Institute 2002; 94:1099-1106

103. Kojima S, Mulholland DJ, Ettinger S, Fazli L, Nelson CC, Gleave ME. Differential regulation of IGFBP-3 by the androgen receptor in the lineage-related androgen-dependent LNCaP and androgen-independent C4-2 prostate cancer models. The Prostate 2006; 66:971-986

104. Smith MR, Finkelstein JS, McGovern FJ, Zietman AL, Fallon MA, Schoenfeld DA, Kantoff PW. Changes in body composition during androgen deprivation therapy for prostate cancer. J Clin Endocrinol Metab 2002; 87:599-603

105. Smith MR, Lee H, McGovern F, Fallon MA, Goode M, Zietman AL, Finkelstein JS. Metabolic changes during gonadotropin-releasing hormone agonist therapy for prostate cancer: differences from the classic metabolic syndrome. Cancer 2008; 112:2188-2194

106. Stoykova GE, Schlaepfer IR. Lipid Metabolism and Endocrine Resistance in Prostate Cancer, and New Opportunities for Therapy. Int J Mol Sci 2019; 20

107. Allott EH, Howard LE, Cooperberg MR, Kane CJ, Aronson WJ, Terris MK, Amling CL, Freedland SJ. Serum lipid profile and risk of prostate cancer recurrence: Results from the SEARCH database. Cancer Epidemiol Biomarkers Prev 2014; 23:2349-2356

108. Zhang G-M, Qin X-J, Zhang H-L, Xiao W-J, Zhu Y, Gu C-Y, Dai B, Shi G-H, Ye D-W. Serum lipid profiles: novel biomarkers predicting advanced prostate cancer in patients receiving radical prostatectomy. Asian J Androl 2015; 17:239-244

109. Zhao J, Zhu S, Sun L, Meng F, Zhao L, Zhao Y, Tian H, Li P, Niu Y. Androgen deprivation therapy for prostate cancer is associated with cardiovascular morbidity and mortality: a meta-analysis of population-based observational studies. PloS one 2014; 9:e107516

110. Lu-Yao G, Stukel TA, Yao SL. Changing patterns in competing causes of death in men with prostate cancer: a population based study. J Urol 2004; 171:2285-2290

111. Zareba P, Duivenvoorden W, Leong DP, Pinthus JH. Androgen deprivation therapy and cardiovascular disease: what is the linking mechanism? Therapeutic Advances in Urology 2016; 8:118-129

112. O'Farrell S, Garmo H, Holmberg L, Adolfsson J, Stattin P, Hemelrijck MV. Risk and Timing of Cardiovascular Disease After Androgen-Deprivation Therapy in Men With Prostate Cancer. J Clin Oncol 2015; 33:1243-1251

113. US Food and Drug Administration: FDA drug safety communication: Update to ongoing safety review of GnRH agonists and notification to manufacturers of GNRH agonists to add new safety information to labelling regarding increased risk of diabetes and certain cardiovascular diseases. http://www.fda.gov/Drugs/DrugSafety/ucm229986.htm

114. Basaria S, Muller DC, Carducci MA, Egan J, Dobs AS. Hyperglycemia and insulin resistance in men with prostate carcinoma who receive androgen-deprivation therapy. Cancer 2006; 106:581-588

115. Chang S, Hursting SD, Contois JH, Strom SS, Yamamura Y, Babaian RJ et al. Leptin and prostate cancer. Prostate 2001; 46:62-67

116. Saglam K, Aydur E, Yilmaz M, Goktas S. Leptin influences cellular differentiation and progression in prostate cancer. J Urol 2003; 169:1308-1311

117. Freedland SJ, Sokoll LJ, Mangold LA, Bruzek DJ, Mohr P, Yiu SK, Epstein JI, Partin AW. Serum leptin and pathological findings at the time of radical prostatectomy. J Urol 2005; 173:773-776

118. Housa D, Vernerova Z, Heracek J, Prochazka B, Cechak P, Kuncova J, Haluzik M. Adiponectin as a potential marker of prostate cancer progression: studies in organ-confined and locally advanced prostate cancer. Physiol Res 2008; 57:451-458

119. Burton A, Martin RM, Holly J, Lane JA, Donovan JL, Hamdy FC, Neal DE, Tilling K. Associations of adiponectin and leptin with stage and grade of PSA-

detected prostate cancer: the ProtecT study. Cancer causes & control 2013; 24:323-334

120. Rubinow KB, Snyder CN, Amory JK, Hoofnagle AN, Page ST. Acute testosterone deprivation reduces insulin sensitivity in men. Clinical endocrinology 2012; 76:281-288

121. Urushima H, Inomata-Kurashiki Y, Nishimura K, Sumi R, Shimomura I, Nonomura N, Ito T, Maeda K. The effects of androgen deprivation therapy with weight management on serum aP2 and adiponectin levels in prostate cancer patients. Aging Male 2015; 18:72-76

122. Kiwata JL, Dorff TB, Schroeder ET, Gross ME, Dieli-Conwright CM. A review of clinical effects associated with metabolic syndrome and exercise in prostate cancer patients. Prostate Cancer Prostatic Dis 2016; 19:323-332

123. Norata GD, Tibolla G, Seccomandi PM, Poletti A, Catapano AL. Dihydrotestosterone decreases tumor necrosis factor-alpha and lipopolysaccharide-induced inflammatory response in human endothelial cells. J Clin Endocrinol Metab 2006; 91:546-554

124. Saylor PJ, Kozak KR, Smith MR, Ancukiewicz MA, Efstathiou JA, Zietman AL, Jain RK, Duda DG. Changes in biomarkers of inflammation and angiogenesis during androgen deprivation therapy for prostate cancer. Oncologist 2012; 17:212-219

125. Maggio M, Blackford A, Taub D, Carducci M, Ble A, Metter EJ, Braga-Basaria M, Dobs A, Basaria S. Circulating inflammatory cytokine expression in men with prostate cancer undergoing androgen deprivation therapy. J Androl 2006; 27:725-728

126. Kwon O-J, Zhang B, Zhang L, Xin L. High fat diet promotes prostatic basal-to-luminal differentiation and accelerates initiation of prostate epithelial hyperplasia originated from basal cells. Stem Cell Research 2016; 16:682-691

127. Alcover J, Filella X, Luque P, Molina R, Izquierdo L, Auge JM, Alcaraz A. Prognostic value of IL-6 in localized prostatic cancer. Anticancer Research 30:4369-4372

128. George DJ, Halabi S, Shepard TF, Sanford B, Vogelzang NJ, Small EJ, Kantoff PW. The prognostic significance of plasma interleukin-6 levels in patients with metastatic hormone-refractory prostate cancer: results from cancer and leukemia group B 9480. Clin Cancer Res 2005; 11:1815-1820

129. Akimoto S, Okumura A, Fuse H. Relationship between serum levels of interleukin-6, tumor necrosis factor-alpha and bone turnover markers in prostate cancer patients. Endocrine journal 1998; 45:183-189

130. Michalaki V, Syrigos K, Charles P, Waxman J. Serum levels of IL-6 and TNF-alpha correlate with clinicopathological features and patient survival in patients with prostate cancer. Br J Cancer 2004; 90:2312-2316

131. Nakashima J, Tachibana M, Ueno M, Miyajima A, Baba S, Murai M. Association between tumor necrosis factor in serum and cachexia in patients with prostate cancer. Clin Cancer Res 1998; 4:1743-1748

132. Kim DK, Lee JY, Kim KJ, Hong N, Kim JW, Hah YS, Koo KC, Kim JH, Cho KS. Effect of Androgen-Deprivation Therapy on Bone Mineral Density in Patients with Prostate Cancer: A Systematic Review and Meta-Analysis. J Clin Med 2019; 8:113

133. Maillefert JF, Sibilia J, Michel F, Saussine C, Javier RM, Tavernier C. Bone mineral density in men treated with synthetic gonadotropin-releasing hormone agonists for prostatic carcinoma. J Urol 1999; 161:1219-1222

134. Dalla Via J, Daly RM, Owen PJ, Mundell NL, Rantalainen T, Fraser SF. Bone mineral density, structure, distribution and strength in men with prostate cancer treated with androgen deprivation therapy. Bone 2019; 127:367-375

135. Wallander M, Axelsson KF, Lundh D, Lorentzon M. Patients with prostate cancer and androgen deprivation therapy have increased risk of fractures-a study from the fractures and fall injuries in the elderly cohort (FRAILCO). Osteoporos Int 2019; 30:115-125

136. Watts S, Leydon G, Birch B, Prescott P, Lai L, Eardley S, Lewith G. Depression and anxiety in prostate cancer: a systematic review and meta-analysis of prevalence rates. BMJ Open 2014; 4:e003901

137. Cheung AS, de Rooy C, Hoermann R, Lim Joon D, Zajac JD, Grossmann M. Quality of life decrements in men with prostate cancer undergoing androgen deprivation therapy. Clinical endocrinology 2017; 86:388-394

138. Thomas RJ, Kenfield SA, Jimenez A. Exercise-induced biochemical changes and their potential influence on cancer: a scientific review. Br J Sports Med 2017; 51:640

139. Prasad SM, Eggener SE, Lipsitz SR, Irwin MR, Ganz PA, Hu JC. Effect of depression on diagnosis, treatment, and mortality of men with clinically localized prostate cancer. J Clin Oncol 2014; 32:2471-2478

140. Aldea M, Craciun L, Tomuleasa C, Crivii C. The role of depression and neuroimmune axis in the prognosis of cancer patients. J BUON 2014; 19:5-14

141. Schoenfeld B. The Use of Specialized Training Techniques to Maximize Muscle Hypertrophy. Strength Cond J 2011; 33:60-65

142. Cheung AS, Zajac JD, Grossmann M. Muscle and bone effects of androgen deprivation therapy: current and emerging therapies. Endocrine-related cancer 2014; 21:R371-394

143. Verdijk LB, Gleeson BG, Jonkers RAM, Meijer K, Savelberg HHCM, Dendale P, van Loon LJC. Skeletal muscle hypertrophy following resistance training is accompanied by a fiber type-specific increase in satellite cell content in elderly men. J Gerontol A Biol Sci Med Sci 2009; 64:332-339

144. Tieland M, Trouwborst I, Clark BC. Skeletal muscle performance and ageing. J Cachexia Sarcopenia Muscle 2018; 9:3-19

145. Kosek DJ, Kim JS, Petrella JK, Cross JM, Bamman MM. Efficacy of 3 days/wk resistance training on myofiber hypertrophy and myogenic mechanisms in young vs. older adults. J Appl Physiol (1985) 2006; 101:531-544

146. Pesta DH, Goncalves RLS, Madiraju AK, Strasser B, Sparks LM. Resistance training to improve type 2 diabetes: working toward a prescription for the future. Nutr Metab (Lond) 2017; 14:24-24

147. Coen PM, Musci RV, Hinkley JM, Miller BF. Mitochondria as a Target for Mitigating Sarcopenia. Front Physiol 2019; 9:1883-1883

148. Segal RJ, Reid RD, Courneya KS, Malone SC, Parliament MB, Scott CG, Venner PM, Quinney HA, Jones LW, D'Angelo ME, Wells GA. Resistance exercise in men receiving androgen deprivation therapy for prostate cancer. J Clin Oncol 2003; 21:1653-1659

149. Winters-Stone KM, Dobek JC, Bennett JA, Maddalozzo GF, Ryan CW, Beer TM. Skeletal response to resistance and impact training in prostate cancer survivors. Med Sci Sports Exerc 2014; 46:1482-1488

150. Nilsen TS, Raastad T, Skovlund E, Courneya KS, Langberg CW, Lilleby W, Fossa SD, Thorsen L. Effects of strength training on body composition, physical functioning, and quality of life in prostate cancer patients during androgen deprivation therapy. Acta oncologica (Stockholm, Sweden) 2015; 54:1805-1813

151. Alberga AS, Segal RJ, Reid RD, Scott CG, Sigal RJ, Khandwala F, Jaffey J, Wells GA, Kenny GP. Age and androgen-deprivation therapy on exercise outcomes in men with prostate cancer. Support Care Cancer 2012; 20:971-981

152. Taaffe DR, Newton RU, Spry N, Joseph D, Chambers SK, Gardiner RA, Wall BA, Cormie P, Bolam KA, Galvao DA. Effects of Different Exercise Modalities on Fatigue in Prostate Cancer Patients Undergoing Androgen Deprivation Therapy: A Year-long Randomised Controlled Trial. Eur Urol 2017; 72:293-299

153. Nilsen TS, Thorsen L, Fossa SD, Wiig M, Kirkegaard C, Skovlund E, Benestad HB, Raastad T. Effects of strength training on muscle cellular outcomes in prostate cancer patients on androgen deprivation therapy. Scand J Med Sci Sports 2016; 26:1026-1035

154. Winters-Stone KM, Dieckmann N, Maddalozzo GF, Bennett JA, Ryan CW, Beer TM. Resistance Exercise Reduces Body Fat and Insulin During Androgen-Deprivation Therapy for Prostate Cancer. Oncol Nurs Forum 2015; 42:348-356

155. Bortolato B, Hyphantis TN, Valpione S, Perini G, Maes M, Morris G et al. Depression in cancer: The many biobehavioral pathways driving tumor progression. Cancer treatment reviews 2017; 52:58-70

156. Kenfield SA, Stampfer MJ, Giovannucci E, Chan JM. Physical activity and survival after prostate cancer diagnosis in the health professionals follow-up study. J Clin Oncol 2011; 29:726-732

157. Gao N, Zhang Z, Jiang BH, Shi X. Role of PI3K/AKT/mTOR signaling in the cell cycle progression of human prostate cancer. Biochem Biophys Res Commun 2003; 310:1124-1132

158. Vavvas D, Apazidis A, Saha AK, Gamble J, Patel A, Kemp BE, Witters LA, Ruderman NB. Contraction-induced changes in acetyl-CoA carboxylase and 5'-AMP-activated kinase in skeletal muscle. J Biol Chem 1997; 272:13255-13261

159. Russell RR, 3rd, Bergeron R, Shulman GI, Young LH. Translocation of myocardial GLUT-4 and increased glucose uptake through activation of AMPK by AICAR. Am J Physiol 1999; 277:H643-649

160. Wright JL, Plymate SR, Porter MP, Gore JL, Lin DW, Hu E, Zeliadt SB. Hyperglycemia and prostate cancer recurrence in men treated for localized prostate cancer. Prostate Cancer Prostatic Dis 2013; 16:204-208

161. Faubert B, Boily G, Izreig S, Griss T, Samborska B, Dong Z et al. AMPK is a negative regulator of the Warburg effect and suppresses tumor growth in vivo. Cell metabolism 2013; 17:113-124

162. Djiogue S, Kamdje AHN, Vecchio L, Kipanyula MJ, Farahna M, Aldebasi Y, Etet PFS. Insulin resistance and cancer: the role of insulin and IGFs. 2013; 20:R1

163. Santa Mina D, Connor MK, Alibhai SM, Toren P, Guglietti C, Matthew AG, Trachtenberg J, Ritvo P. Exercise effects on adipokines and the IGF axis in men

with prostate cancer treated with androgen deprivation: A randomized study. Can Urol Assoc J 2013; 7:E692-698

164. Ingermann AR, Yang YF, Han J, Mikami A, Garza AE, Mohanraj L, Fan L, Idowu M, Ware JL, Kim HS, Lee DY, Oh Y. Identification of a novel cell death receptor mediating IGFBP-3-induced anti-tumor effects in breast and prostate cancer. J Biol Chem 2010; 285:30233-30246

165. Mehta HH, Gao Q, Galet C, Paharkova V, Wan J, Said J, Sohn JJ, Lawson G, Cohen P, Cobb LJ, Lee KW. IGFBP-3 is a metastasis suppression gene in prostate cancer. Cancer Res 2011; 71:5154-5163

166. Mukherjee R, McGuinness DH, McCall P, Underwood MA, Seywright M, Orange C, Edwards J. Upregulation of MAPK pathway is associated with survival in castrate-resistant prostate cancer. Br J Cancer 2011; 104:1920-1928

167. Devin JL, Bolam KA, Jenkins DG, Skinner TL. The Influence of Exercise on the Insulin-like Growth Factor Axis in Oncology: Physiological Basis, Current, and Future Perspectives. Cancer Epidemiol Biomarkers Prev 2016; 25:239-249

168. Nishida Y, Matsubara T, Tobina T, Shindo M, Tokuyama K, Tanaka K, Tanaka H. Effect of low-intensity aerobic exercise on insulin-like growth factor-I and insulin-like growth factor-binding proteins in healthy men. Int J Endocrinol2010; 2010

169. Lemmey AB, Marcora SM, Chester K, Wilson S, Casanova F, Maddison PJ. Effects of high-intensity resistance training in patients with rheumatoid arthritis: a randomized controlled trial. Arthritis Rheum 2009; 61:1726-1734

170. Pedersen BK. Muscles and their myokines. J Exp Biol 2011; 214:337-346

171. Fatohlahi Shoorabeh F, Dabidiroshan V, Sheikh Saraf B, Nuri R. Investigating the Effects of Regular Resistance Training and Prostatic Massage on Proinflammatory Markers and Serum Prostate-Specific Antigen Levels in Males with Prostate Cancer. Middle East J Rehabil Health 2016; 3:e33651

172. Accattato F, Greco M, Pullano SA, Carè I, Fiorillo AS, Pujia A, Montalcini T, Foti DP, Brunetti A, Gulletta E. Effects of acute physical exercise on oxidative stress and inflammatory status in young, sedentary obese subjects. PloS one 2017; 12:e0178900-e0178900

173. Gupta-Elera G, Garrett AR, Robison RA, O'Neill KL. The role of oxidative stress in prostate cancer. Eur J Cancer2012; 21:155-162

174. Cakir-Atabek H, Demir S, PinarbaSili RD, Gunduz N. Effects of different resistance training intensity on indices of oxidative stress. J Strength Cond Res 2010; 24:2491-2497

175. Frohlich DA, McCabe MT, Arnold RS, Day ML. The role of Nrf2 in increased reactive oxygen species and DNA damage in prostate tumorigenesis. Oncogene 2008; 27:4353-4362

176. Iikuni N, Lam QLK, Lu L, Matarese G, La Cava A. Leptin and Inflammation. Current immunology reviews 2008; 4:70-79

177. Alshaker H, Sacco K, Alfraidi A, Muhammad A, Winkler M, Pchejetski D. Leptin signalling, obesity and prostate cancer: molecular and clinical perspective on the old dilemma. Oncotarget 2015; 6:35556-35563

178. Atashak S, Mohammad G, Faeze S. Effect of 10 week progressive resistance training on serum leptin and adiponectin concentration in obese men. British Journal of Sports Medicine 2010; 44:i29

179. Jamaluddin MS, Weakley SM, Yao Q, Chen C. Resistin: functional roles and therapeutic considerations for cardiovascular disease. British journal of pharmacology 2012; 165:622-632

180. Kim HJ, Lee YS, Won EH, Chang IH, Kim TH, Park ES, Kim MK, Kim W, Myung SC. Expression of resistin in the prostate and its stimulatory effect on prostate cancer cell proliferation. BJU Int 2011; 108:E77-83

181. Ouchi N, Walsh K. Adiponectin as an anti-inflammatory factor. Clin Chim Acta 2007; 380:24-30

182. Prestes J, Shiguemoto G, Botero JP, Frollini A, Dias R, Leite R et al. Effects of resistance training on resistin, leptin, cytokines, and muscle force in elderly post-menopausal women. Journal of sports sciences 2009; 27:1607-1615

183. Moradi F. Changes of Serum Adiponectin and Testosterone Concentrations Following Twelve Weeks Resistance Training in Obese Young Men. Asian journal of sports medicine 2015; 6:e23808-e23808

184. Chen N, Li Q, Liu J, Jia S. Irisin, an exercise-induced myokine as a metabolic regulator: an updated narrative review. Diabetes/metabolism research and reviews 2016; 32:51-59

185. Tekin S, Erden Y, Sandal S, Yilmaz B. Is Irisin an Anticarcinogenic Peptide? Vol 4.

186. Qiu S, Cai X, Sun Z, Schumann U, Zugel M, Steinacker JM. Chronic Exercise Training and Circulating Irisin in Adults: A Meta-Analysis. Sports medicine (Auckland, NZ) 2015; 45:1577-1588

187. Zhao J, Su Z, Qu C, Dong Y. Effects of 12 Weeks Resistance Training on Serum Irisin in Older Male Adults. Front Physiol 2017; 8

188. Kanzleiter T, Rath M, Gorgens SW, Jensen J, Tangen DS, Kolnes AJ et al. The myokine decorin is regulated by contraction and involved in muscle hypertrophy. Biochemical and biophysical research communications 2014; 450:1089-1094

189. Neill T, Schaefer L, Iozzo RV. Decorin as a multivalent therapeutic agent against cancer. Advanced drug delivery reviews 2016; 97:174-185

190. Hu Y, Sun H, Owens RT, Wu J, Chen YQ, Berquin IM, Perry D, O'Flaherty JT, Edwards IJ. Decorin suppresses prostate tumor growth through inhibition of epidermal growth factor and androgen receptor pathways. Neoplasia (New York, NY) 2009; 11:1042-1053

191. Xu W, Neill T, Yang Y, Hu Z, Cleveland E, Wu Y et al. The systemic delivery of an oncolytic adenovirus expressing decorin inhibits bone metastasis in a mouse model of human prostate cancer. Gene therapy 2015; 22:247-256

192. Bathina S, Das UN. Brain-derived neurotrophic factor and its clinical implications. Archives of medical science : AMS 2015; 11:1164-1178

193. Montano X, Djamgoz MB. Epidermal growth factor, neurotrophins and the metastatic cascade in prostate cancer. FEBS letters 2004; 571:1-8

194. Nuvagah Forti L, Van Roie E, Njemini R, Coudyzer W, Beyer I, Delecluse C, Bautmans I. High Versus Low Load Resistance Training: The Effect of 24 Weeks Detraining on Serum Brain Derived-Neurotrophic Factor (BDNF) in Older Adults. The Journal of frailty & aging 2017; 6:53-58

195. Magbanua MJ, Richman EL, Sosa EV, Jones LW, Simko J, Shinohara K, Haqq CM, Carroll PR, Chan JM. Physical activity and prostate gene expression in men with low-risk prostate cancer. Cancer Causes Control 2014; 25:515-523

196.   Li T, Li RS, Li YH, Zhong S, Chen YY, Zhang CM, Hu MM, Shen ZJ. miR-21 as an independent biochemical recurrence predictor and potential therapeutic target for prostate cancer. The Journal of urology 2012; 187:1466-1472

197.   Cui S, Sun B, Yin X, Guo X, Chao D, Zhang C, Zhang CY, Chen X, Ma J. Time-course responses of circulating microRNAs to three resistance training protocols in healthy young men. Scientific reports 2017; 7:2203

198.   Dimauro I, Scalabrin M, Fantini C, Grazioli E, Beltran Valls MR, Mercatelli N, Parisi A, Sabatini S, Di Luigi L, Caporossi D. Resistance training and redox homeostasis: Correlation with age-associated genomic changes. Redox Biology 2016; 10:34-44

199.   Graham MK, Meeker A. Telomeres and telomerase in prostate cancer development and therapy. Nature reviews Urology 2017; 14:607-619

200.   Ornish D, Lin J, Chan JM, Epel E, Kemp C, Weidner G et al. Effect of comprehensive lifestyle changes on telomerase activity and telomere length in men with biopsy-proven low-risk prostate cancer: 5-year follow-up of a descriptive pilot study. The Lancet Oncology 2013; 14:1112-1120

201.   Schmitz KH, Courneya KS, Matthews C, Demark-Wahnefried W, Galvao DA, Pinto BM et al. American College of Sports Medicine roundtable on exercise guidelines for cancer survivors. Medicine and science in sports and exercise 2010; 42:1409-1426

202.   Westphal T, Rinnerthaler G, Gampenrieder SP, Niebauer J, Thaler J, Pfob M, et al. Supervised versus autonomous exercise training in breast cancer patients: A multicenter randomized clinical trial. Cancer medicine 2018; 7:5962-5972

203.   Hardcastle SJ, Cohen PA. Effective Physical Activity Promotion to Survivors of Cancer Is Likely to Be Home Based and to Require Oncologist Participation. J Clin Oncol 2017; 35:3635-3637

204.   Schmidt MLK, Ostergren P, Cormie P, Ragle AM, Sonksen J, Midtgaard J. "Kicked out into the real world": prostate cancer patients' experiences with transitioning from hospital-based supervised exercise to unsupervised exercise in the community. Support Care Cancer 2019; 27:199-208

205.   Galvao DA, Newton RU, Girgis A, Lepore SJ, Stiller A, Mihalopoulos C, Gardiner RA, Taaffe DR, Occhipinti S, Chambers SK. Randomized controlled trial of a peer led multimodal intervention for men with prostate cancer to increase exercise participation. Psychooncology 2018; 27:199-207

206.   Kim SH, Seong DH, Yoon SM, Choi YD, Choi E, Song Y, Song H. The Effect on Bone Outcomes of Home-based Exercise Intervention for Prostate Cancer Survivors Receiving Androgen Deprivation Therapy: A Pilot Randomized Controlled Trial. Cancer Nurs 2018; 41:379-388

207.   Alibhai SMH, Santa Mina D, Ritvo P, Tomlinson G, Sabiston C, Krahn M et al. A phase II randomized controlled trial of three exercise delivery methods in men with prostate cancer on androgen deprivation therapy. BMC Cancer 2019; 19:2

208.   Galvao DA, Spry N, Denham J, Taaffe DR, Cormie P, Joseph D, Lamb DS, Chambers SK, Newton RU. A multicentre year-long randomised controlled trial of exercise training targeting physical functioning in men with prostate cancer previously treated with androgen suppression and radiation from TROG 03.04 RADAR. European urology 2014; 65:856-864

209. Bhasin S, Woodhouse L, Casaburi R, Singh AB, Mac RP, Lee M et al. Older men are as responsive as young men to the anabolic effects of graded doses of testosterone on the skeletal muscle. J Clin Endocrinol Metab 2005; 90:678-688

210. Snyder PJ, Peachey H, Berlin JA, Hannoush P, Haddad G, Dlewati A et al. Effects of testosterone replacement in hypogonadal men.
J Clin Endocrinol Metab 2000; 85:2670-2677

211. Kochakian CD. Comparison of protein anabolic property of various androgens in the castrated rat. Am J Physiol 1950; 160:53-61

212. Lieberman M, Marks AD, Smith CM. Marks' basic medical biochemistry. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins.

213. Matthews DE, Motil KJ, Rohrbaugh DK, Burke JF, Young VR, Bier DM. Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1-3C]leucine. Am J Physiol 1980; 238:E473-479

214. Horber FF, Horber-Feyder CM, Krayer S, Schwenk WF, Haymond MW. Plasma reciprocal pool specific activity predicts that of intracellular free leucine for protein synthesis. Am J Physiol 1989; 257:E385-399

215. Hoffman DM, Pallasser R, Duncan M, Nguyen TV, Ho KK. How is whole body protein turnover perturbed in growth hormone-deficient adults? J Clin Endocrinol Metab 1998; 83:4344-4349

216. Wolthers T, Hoffman DM, Nugent AG, Duncan MW, Umpleby M, Ho KK. Oral estrogen antagonizes the metabolic actions of growth hormone in growth hormone-deficient women. American journal of physiology Endocrinology and metabolism 2001; 281:E1191-1196

217. Nissen SL, Miles JM, Gerich JE, Haymond MW. Regulation of alpha-ketoisocaproate binding to albumin in vivo by free fatty acids. Am J Physiol 1982; 242:E67-71

218. Burt MG, Gibney J, Hoffman DM, Umpleby AM, Ho KK. Relationship between GH-induced metabolic changes and changes in body composition: a dose and time course study in GH-deficient adults. Growth Horm IGF Res 2008; 18:55-64

219. Hamberg O, Vilstrup H. A rapid method for determination of hepatic amino nitrogen to urea nitrogen conversion ('the Functional Hepatic Nitrogen Clearance'). Scand J Clin Lab Invest 1994; 54:377-383

220. Wolfe RR, Goodenough RD, Wolfe MH, Royle GT, Nadel ER. Isotopic analysis of leucine and urea metabolism in exercising humans. Appl Physiol Respir Environ Exerc Physiol 1982; 52:458-466

221. Kessler A, Siekmann L. Measurement of urea in human serum by isotope dilution mass spectrometry: a reference procedure. Clin Chem 1999; 45:1523-1529

222. Birzniece V, Khaw CH, Nelson AE, Meinhardt U, Ho KK. A critical evaluation of bioimpedance spectroscopy analysis in estimating body composition during GH treatment: comparison with bromide dilution and dual X-ray absorptiometry. European journal of endocrinology 2015; 172:21-28

223. Chambon C, Duteil D, Vignaud A, Ferry A, Messaddeq N, Malivindi R, Kato S, Chambon P, Metzger D. Myocytic androgen receptor controls the strength but not the mass of limb muscles. Proceedings of the National Academy of Sciences of the United States of America 2010; 107:14327-14332

224. Ophoff J, Van Proeyen K, Callewaert F, De Gendt K, De Bock K, Vanden Bosch A, Verhoeven G, Hespel P, Vanderschueren D. Androgen signaling in

myocytes contributes to the maintenance of muscle mass and fiber type regulation but not to muscle strength or fatigue. Endocrinology 2009; 150:3558-3566

225. Monks DA, O'Bryant EL, Jordan CL. Androgen receptor immunoreactivity in skeletal muscle: enrichment at the neuromuscular junction. J Comp Neurol 2004; 473:59-72

226. Chen Y, Zajac JD, MacLean HE. Androgen regulation of satellite cell function. The Journal of endocrinology 2005; 186:21-31

227. Foradori CD, Weiser MJ, Handa RJ. Non-genomic actions of androgens. Front Neuroendocrinol 2008; 29:169-181

228. Svensson J, Moverare-Skrtic S, Windahl S, Swanson C, Sjogren K. Stimulation of both estrogen and androgen receptors maintains skeletal muscle mass in gonadectomized male mice but mainly via different pathways. Journal of molecular endocrinology 2010; 45:45-57

229. Ackermann PG, Kheim T. The Effect of Testosterone on Plasma Amino Acid Levels in Elderly Individuals. J Gerontol 1964; 19:207-214

230. Saad F, Röhrig G, von Haehling S, Traish A. Testosterone Deficiency and Testosterone Treatment in Older Men. Gerontology 2017; 63:144-156

231. Yeap BB, Alfonso H, Chubb SAP, Handelsman DJ, Hankey GJ, Norman PE, Flicker L. Reference Ranges and Determinants of Testosterone, Dihydrotestosterone, and Estradiol Levels Measured using Liquid Chromatography-Tandem Mass Spectrometry in a Population-Based Cohort of Older Men. J Clin Endocrinol Metab 2012; 97:4030-4039

232. Yeap BB, Grossmann M, McLachlan RI, Handelsman DJ, Wittert GA, Conway AJ, Stuckey BG, Lording DW, Allan CA, Zajac JD, Burger HG. Endocrine Society of Australia position statement on male hypogonadism (part 2): treatment and therapeutic considerations. Med J Aust2016; 205:228-231

233. Roubenoff R. Sarcopenia and its implications for the elderly. European journal of clinical nutrition 2000; 54 Suppl 3:S40-47

234. Hypogonadism in the Aging Male Diagnosis, Potential Benefits, and Risks of Testosterone Replacement Therapy. International Journal of Endocrinology 2012; 2012

235. Bhasin S. Testosterone Supplementation for Aging-Associated Sarcopenia. The Journals of Gerontology: Series A 2003; 58:M1002-M1008

236. Snyder PJ, Peachey H, Hannoush P, Berlin JA, Loh L, Lenrow DA, Holmes JH, Dlewati A, Santanna J, Rosen CJ, Strom BL. Effect of Testosterone Treatment on Body Composition and Muscle Strength in Men Over 65 Years of Age1. J Clin Endocrinol Metab 1999; 84:2647-2653

237. Bhasin S, Ellenberg SS, Storer TW, Basaria S, Pahor M, Stephens-Shields AJ et al. Effect of testosterone replacement on measures of mobility in older men with mobility limitation and low testosterone concentrations: secondary analyses of the Testosterone Trials. Lancet Diabetes Endocrinol 2018; 6:879-890

238. Storer TW, Miciek R, Travison TG. Muscle function, physical performance and body composition changes in men with prostate cancer undergoing androgen deprivation therapy. Asian journal of andrology 2012; 14:204-221

239. Lam T, Poljak A, McLean M, Bahl N, Ho KK, Birzniece V. Testosterone prevents protein loss via the hepatic urea cycle in human. Eur J Endocrinol 2017; 176:489-496

240. Law TD, Clark LA, Clark BC. Resistance Exercise to Prevent and Manage Sarcopenia and Dynapenia. Annual review of gerontology & geriatrics 2016; 36:205-228

241. Cormie P, Galvao DA, Spry N, Joseph D, Chee R, Taaffe DR, Chambers SK, Newton RU. Can supervised exercise prevent treatment toxicity in patients with prostate cancer initiating androgen-deprivation therapy: a randomised controlled trial. BJU Int 2015; 115:256-266

242. Hayes SC, Spence RR, Galvao DA, Newton RU. Australian Association for Exercise and Sport Science position stand: optimising cancer outcomes through exercise. J Sci Med Sport 2009; 12:428-434

243. R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

244. Lin R, Snodgrass P, Rabier D. Induction of urea cycle enzymes by glucagon and dexamethasone in monolayer culture of adult rat hepatocytes. The Journal of Biological Chemistry 1982; 257:5061-5067

245. Lexell J, Henriksson-Larsen K, Winblad B, Sjostrom M. Distribution of different fiber types in human skeletal muscles: effects of aging studied in whole muscle cross sections. Muscle & nerve 1983; 6:588-595

246. Bhasin S. Testosterone supplementation for aging-associated sarcopenia. The journals of gerontology Series A, Biological sciences and medical sciences 2003; 58:1002-1008

247. de Rooy C, Grossmann M, Zajac JD, Cheung AS. Targeting muscle signaling pathways to minimize adverse effects of androgen deprivation. Endocrine-related cancer 2016; 23:R15-26

248. Basaria S, Bhasin S. Targeting the skeletal muscle-metabolism axis in prostate-cancer therapy. The New England journal of medicine 2012; 367:965-967

249. Leuprolide- LiverTox Clinical and Research Information on Drug-Induced Liver Injury- National Institutes of Health. http://livertox.nih.gov/Leuprolide.htm (accessed 3rd May 2018).

250. Shen M, Shi H. Sex Hormones and Their Receptors Regulate Liver Energy Homeostasis. International journal of endocrinology 2015; 2015:294278

251. Schmitz KH, Courneya KS, Matthews C, Demark-Wahnefried W, Galvao DA, Pinto BM et al. American College of Sports M. American College of Sports Medicine roundtable on exercise guidelines for cancer survivors. Med Sci Sports Exerc 2010; 42:1409-1426

252. Galvao DA, Newton RU, Girgis A, Lepore SJ, Stiller A, Mihalopoulos C, Gardiner RA, Taaffe DR, Occhipinti S, Chambers SK. Randomized controlled trial of a peer led multimodal intervention for men with prostate cancer to increase exercise participation. Psychooncology 2018; 27:199-207

253. Fenicchia LM, Kanaley JA, Azevedo JL, Jr., Miller CS, Weinstock RS, Carhart RL, Ploutz-Snyder LL. Influence of resistance exercise training on glucose control in women with type 2 diabetes. Metabolism: clinical and experimental 2004; 53:284-289

254. Hanson ED, Nelson AR, West DW, Violet JA, O'Keefe L, Phillips SM, Hayes A. Attenuation of Resting but Not Load-Mediated Protein Synthesis in Prostate Cancer Patients on Androgen Deprivation. J Clin Endocrinol Metab 2017; 102:1076-1083

255. Yarasheski KE, Pak-Loduca J, Hasten DL, Obert KA, Brown MB, Sinacore DR. Resistance exercise training increases mixed muscle protein synthesis rate in frail women and men >/=76 yr old. Am J Physiol 1999; 277:E118-125

256. Carraro F, Kimbrough TD, Wolfe RR. Urea kinetics in humans at two levels of exercise intensity. J Appl Physiol (1985)1993; 75:1180-1185

257. Fielding RA. Effects of exercise training in the elderly: impact of progressive-resistance training on skeletal muscle and whole-body protein metabolism. The Proceedings of the Nutrition Society 1995; 54:665-675

258. Cheung AS, Tinson AJ, Milevski SV, Hoermann R, Zajac JD, Grossmann M. Persisting adverse body composition changes 2 years after cessation of androgen deprivation therapy for localised prostate cancer. Eur J Endocrinol 2018; 179:21-29

259. Smith MR, Saad F, Egerdie B, Sieber PR, Tammela TL, Ke C, Leder BZ, Goessl C. Sarcopenia during androgen-deprivation therapy for prostate cancer. J Clin Oncol 2012; 30:3271-3276

260. Lam T, McLean M, Hayden A, Poljak A, Cheema B, Gurney H, Stone G, Bahl N, Reddy N, Shahidipour H, Birzniece V. A potent liver mediated mechanism for loss of muscle mass during androgen deprivation therapy. Endocr Connect 2019;

261. Bohannon RW, Bubela DJ, Magasi SR, Wang Y-C, Gershon RC. Sit-to-stand test: Performance and determinants across the age-span. Isokinet Exerc Sci 2010; 18:235-240

262. Herman T, Giladi N, Hausdorff JM. Properties of the 'timed up and go' test: more than meets the eye. Gerontology 2011; 57:203-210

263. Sturnieks DL, Arnold R, Lord SR. Validity and reliability of the Swaymeter device for measuring postural sway. BMC geriatrics 2011; 11:63

264. Nr S, Nmdb R. Reliability Evaluation of the Physiological Profile Assessment to Assess Fall Risk in Older People. Journal of Gerontology & Geriatric Research 2014; 03

265. Abdul-Ghani MA, Matsuda M, Balas B, DeFronzo RA. Muscle and liver insulin resistance indexes derived from the oral glucose tolerance test. Diabetes Care 2007; 30:89-94

266. Utzschneider KM, Prigeon RL, Faulenbach MV, Tong J, Carr DB, Boyko EJ, Leonetti DL, McNeely MJ, Fujimoto WY, Kahn SE. Oral disposition index predicts the development of future diabetes above and beyond fasting and 2-h glucose levels. Diabetes Care 2009; 32:335-341

267. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999; 22:1462-1470

268. Kiwata JL, Dorff TB, Schroeder ET, Dieli-Conwright CM. Effect of a supervised exercise intervention on sarcopenic obesity and metabolic syndrome in prostate cancer patients: A randomized pilot study. Cancer Research Conference: American Association for Cancer Research Annual Meeting 2017; 77

269. Dickerman BA, Torfadottir JE, Valdimarsdottir UA, Giovannucci E, Wilson KM, Aspelund T et al. Body fat distribution on computed tomography imaging and prostate cancer risk and mortality in the AGES-Reykjavik study. Cancer 2019; 125:2877-2885

270. Dunstan DW, Daly RM, Owen N, Jolley D, Vulikh E, Shaw J, Zimmet P. Home-based resistance training is not sufficient to maintain improved glycemic control following supervised training in older individuals with type 2 diabetes. Diabetes Care 2005; 28:3-9

271. Phillips SM, Stampfer MJ, Chan JM, Giovannucci EL, Kenfield SA. Physical activity, sedentary behavior, and health-related quality of life in prostate cancer survivors in the health professionals follow-up study. J Cancer Surviv = 2015; 9:500-511

272. Bourke L, Smith D, Steed L, Hooper R, Carter A, Catto J, Albertsen PC, Tombal B, Payne HA, Rosario DJ. Exercise for Men with Prostate Cancer: A Systematic Review and Meta-analysis. European urology 2016; 69:693-703

273. Silva TD, Boing L, Dias M, Pazin J, Guimarães ACdA. Prostate cancer: quality of life and physical activity level of patients Journal of Physical Education 2018; 29

274. Wang A, Karunasinghe N, Plank L, Zhu S, Osborne S, Bishop K et al. Effect of Androgen Deprivation Therapy on Bone Mineral Density in a Prostate Cancer Cohort in New Zealand: A Pilot Study. Clinical Medicine Insights Oncology 2017; 11:1179554917733449

275. Lau YK, Lee E, Prior HJ, Lix LM, Metge CJ, Leslie WD. Fracture risk in androgen deprivation therapy: a Canadian population based analysis. Can J Urol 2009; 16:4908-4914

276. Chambers SK, Dunn J, Lazenby SC, Newton RU, Cormie P, Lowe A, Sandoe D, Gardiner RA. Proscare: A psychological care model for men with prostate cancer. 2013. Prostate Cancer Foundation of Australia and Griffith University, Australia. .

277. Kollberg KS, Wilderäng U, Thorsteinsdottir T, Hugosson J, Wiklund P, Bjartell A, Carlsson S, Stranne J, Haglind E, Steineck G. Psychological Well-being and Private and Professional Psychosocial Support After Prostate Cancer Surgery: A Follow-up at 3, 12, and 24 Months After Surgery. Eur Urol Focus 2016; 2:418-425

278. Frederiksen L, Hojlund K, Hougaard DM, Mosbech TH, Larsen R, Flyvbjerg A, Frystyk J, Brixen K, Andersen M. Testosterone therapy decreases subcutaneous fat and adiponectin in aging men. European journal of endocrinology 2012; 166:469-476

279. Lee CH, Lui DTW, Cheung CYY, Fong CHY, Yuen MMA, Chow WS, Woo YC, Xu A, Lam KSL. Higher Circulating Adiponectin Concentrations Predict Incident Cancer in Type 2 Diabetes – The Adiponectin Paradox. The Journal of Clinical Endocrinology & Metabolism 2020;

280. Prostate Cancer Foundation of Australia 2014, Prostate cancer and exercise, viewed 18 February 2020, <http://prostate.org.au>.

Appendix 1

Resistance Training Logbook

(Phase 1)

Name: _____

Date of next visit: _____

- All exercises below should be performed for 8-12 reps per set.

- Take 1-2 min of rest between sets and exercises.

- If you are able to perform 12 reps easily, the weight should be increased, or you

  should go deeper with the movement (in the case of leg exercises)

Incline push-up

Target muscle group: pectorals

Breathing: inhale when lowering, exhale when raising

Basic instructions: (1) stabilize chair/bench against wall, (2) head, trunk and body should be in a straight line, hands wider than shoulder width apart, (3) lower yourself to a 90 degree (or slightly less) arm bend, then raise yourself until arms fully extended (complete 8-12 repetitions).

More difficult option: use a lower bench or the floor (standard push up) if the incline is too easy

Easier option: use a more vertical position (e.g. standing up against wall) OR perform a modified push-up (i.e. with knees on floor)

Bent over row

Target muscle group: latissimus dorsi, posterior deltoid

Breathing: inhale when lowering, exhale when raising

Basic instructions: (1) stabilize yourself in 'tripod' position (place your opposing arm on chair/bench), keep your back parallel to floor or chest slightly raised, (2) raise dumbbell up to the side of your rib cage, then lower (complete 8-12 repetitions on each side)

Biscuits curl

Target muscle group: biceps

Breathing: inhale when lowering, exhale when raising

Basic instructions: (1) elbow is kept next to trunk for the full movement, only the forearm moves, (2) as you raise the dumbbell, twist of the wrist externally 90 degrees or slightly more - this activates more of the biceps muscle, (3) when lowering, return the wrist to its original position (4) alternate left and right arm, (5) do not swing the weight; keep movement controlled (complete 8-12 repetitions on each side)

Triceps extension

Target muscle group: triceps

Breathing: inhale when lowering, exhale when raising

Basic instructions: raise arm with weight above head; this arm should be kept as close to perpendicular to the floor as you can, only the forearm is meant to move during this exercise. Complete 8-12 repetitions on each side.

Easier option: perform with no weight or with arm slightly less than perpendicular to the floor. As a goal, improve your technique (flexibility) over time.
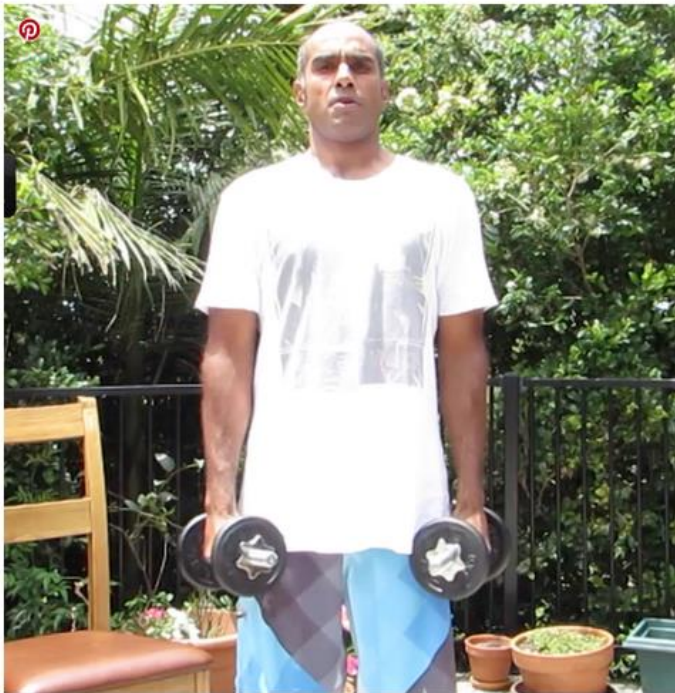

160

Side shoulder raise

Target muscle group: deltoids

Breathing: inhale when lowering, exhale when raising

Basic instructions: arms should be kept straight for the entire movement, raise arms to parallel to the floor of slightly more elevated (complete 8-12 repetitions)

Dumbbell squat

Target muscle group: quadriceps, glutes

Breathing: inhale when lowering, exhale when raising

Basic instructions: (1) feet wider than hip width apart and toes slightly pointed outward, (2) dumbbells held in front of the shoulders, chest out, strong through the torso, (3) as you squat the centre of gravity should be kept over the feet (the dumbbells should not move forward or backward but only straight up and down during the movement), (4) squat down aiming to get your thighs parallel to the floor, return to upright position (complete 8-12 repetitions).

Safety: if needed, position a chair behind you for safety

Easier option: do this exercise without dumbbells and/or do not go as deep with the movement
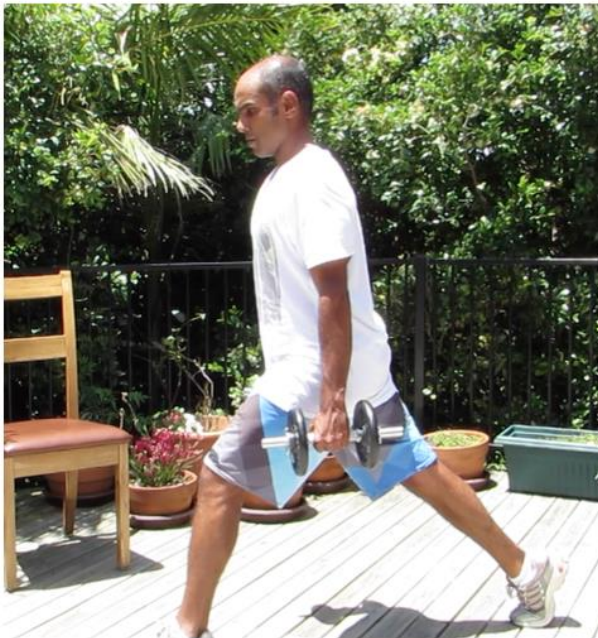
Split squat

Target muscle group: quadriceps, glutes, calf muscles

Breathing: inhale when lowering, exhale when raising

Basic instructions: (1) feet shoulder width apart to slightly wider; front foot 1 m or more in front of the other, (2) trunk remains perpendicular to the floor, (3) flex front knee until thigh is parallel to the floor, the return to starting position (repeat), (4) your front knee should not extend beyond your toes (places too much strain on the knee joint), (5) back leg should bend naturally, you may feel a stretch through the quadriceps muscle. Complete 8-12 repetitions on each side.

Safety and Easier Option: if needed, perform this exercise without weight and/or do not go as deep with the movement, and position a chair beside you for safety

More difficult option: Once you have mastered this version of the split squat, try the Romanian version of the split squat (place back foot on a bench or chair)

Straight leg deadlift

Target muscle group: hamstrings, lower back

Breathing: inhale when lowering, exhale when raising

Basic instructions: (1) feet shoulder width apart, legs relatively straight (knees slightly flexed, not locked), (2) lower trunk until you feel a stretch on the hamstrings, (3) keep trunk strong and stable, back neutral (no rounding the back at the bottom of the movement), (4), return to starting position by placing emphasis on contracting the hamstrings and back extensors. Complete 8-12 repetitions.

Easier option: do this exercise without weights

164
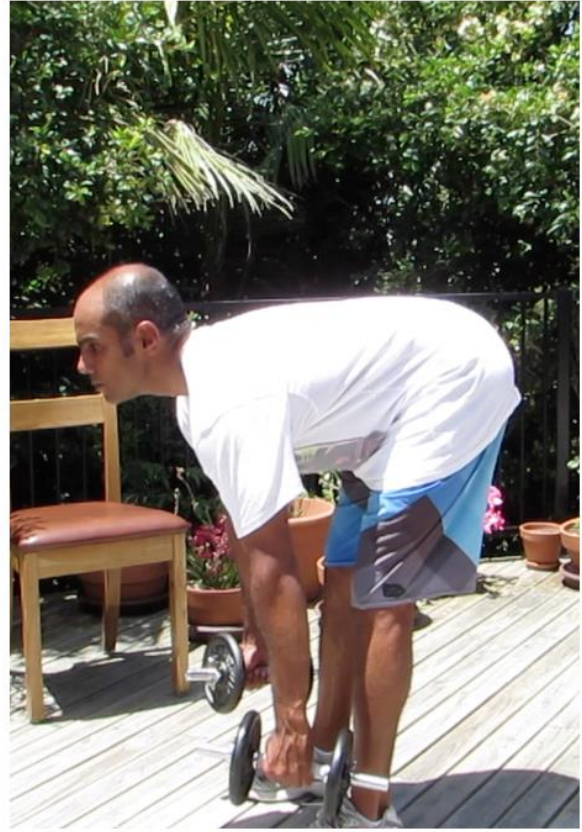
Resistance Training Logbook:

Phase 2

Name: _____

Date of next visit: _____

- All exercises below should be performed for 8-12 reps per set.

- Take 1-2 min of rest between sets and exercises.

- If you are able to perform 12 reps easily, the weight should be increased, or you should go deeper with the movement (in the case of leg exercises)

- Exercise sessions should be completed on non-consecutive days to allow for optimal recovery

Shoulder Press

This exercise requires a fixed seat (with no wheels!)

Target muscle group: deltoids (shoulders)

Breathing: exhale when lifting, inhale when lowering

Basic instructions: Raise dumbbells to approximately 90-degree bend at the elbows (can be slightly less than 90; this is the starting position), raise dumbbells together until arms fully extended (repeat)

Starting position: elbows at less than 90-deg   Full range of motion: arms fully extended

Resistance Training Logbook:

Phase 3

Name: _____

Date of next visit: _____

- All exercises below should be performed for 8-12 reps per set.

- Take 1-2 min of rest between sets and exercises.

- If you are able to perform 12 reps easily, the weight should be increased, or you should go deeper with the movement (in the case of leg exercises)

- Exercise sessions should be completed on non-consecutive days to allow for optimal recovery

Standard Push-up

Target muscle group: pectorals (chest)

Breathing: inhale when lowering, exhale when lifting,

Basic instructions: Start with arms extended fully, then lower to approximately 90-degree bend at the elbows (repeat). Keep rest of body rigid (all muscles activated).

Decline Push-up

Target muscle group: pectorals (upper chest)

Breathing: inhale when lowering, exhale when lifting,

Basic instructions: Feet on chair (tip toes). Start with arms extended fully, then lower to approximately 90-degree bend at the elbows (repeat). Keep rest of body rigid (all muscles activated).
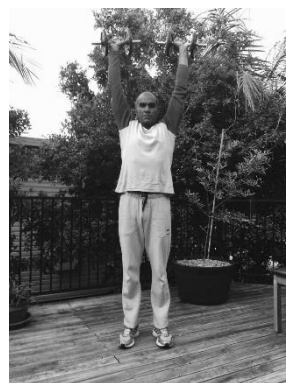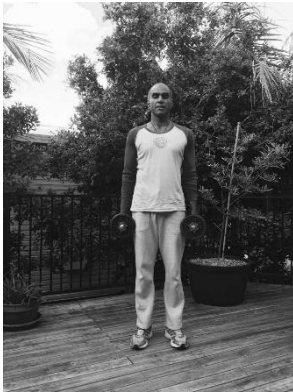
Curl to Press

Target muscle group: biceps and deltoids (shoulders)

Breathing: exhale when lifting, inhale when lowering

Basic instructions: This exercise combines the biceps curl and shoulder press. Perform in standing position.
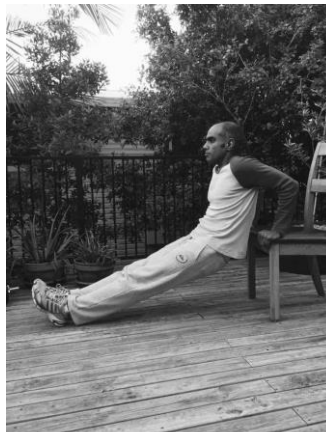
Chair Dips

Target muscle group: triceps

Breathing: inhale when lowering, exhale when lifting,

Basic instructions: Start with arms extended fully, feet positioned as far forward as possible. All weight should be placed in the heels. Lower to approximately 90-degree bend at the elbows (repeat).
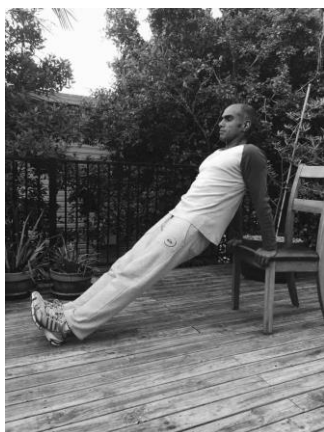
Lunge

Target muscle group: quadriceps and glutes

Breathing: inhale when lowering, exhale when lifting,

Basic instructions: This exercise is an advanced version of the split squat. In confined space step outward and back. In open space, lunge repeatedly across the surface/floor in a forward direction to achieve 8-12 repetitions for each leg. On lowering, both knees should be at approximately 90-degree bend; the back knee should nearly touch the ground.

Appendix 2: Exercise logbooks

Exercises (✔ when complete)	Phase 1								Week #: 1
	Date:			Date:			Date:		
	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3
Incline push-up									
Bent over row									
Biceps curl									
Triceps extension									
Side shoulder raise									
Dumbbell Squat									
Split squat									
Straight leg deadlift									

Exercises (✔ when complete)	Phase 2						Week #: 13		
	Date:			Date:			Date:		
	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3
Incline (or standard*) push-up									
Bent over row									
Biceps curl									
Triceps extension									
Side shoulder raise									
Shoulder Press**									
Dumbbell Squat									
Split squat									
Straight leg deadlift									

*Advance to standard push-up <u>only</u> if the incline push-up has been mastered (i.e. 12 reps can completed with ease); the standard push-up is to be performed on the floor
**The shoulder press is a new exercise – see instructions

Exercises (✔ when complete)	Date:			Date:			Date:		
	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3
Standard Push-up* (new) *(or incline push-up)*									
Decline Push-up* (new)									
Curl to Press* *(or biceps curl and shoulder press)*									
Chair Dips* *(or triceps extension)*									
Side shoulder raise (old)									
Dumbbell Squat (old)									
Lunge* *(or split squat)*									
Straight leg deadlift (old)									

Phase 3   **Week #: 37**

*New exercise – see instructions; advance to standard and decline push-up <u>only</u> if the incline push-up has been mastered (i.e. 12 reps can completed with ease); the standard push-up is to be performed on the floor, the decline push-up with feet up on a chair

173

Appendix 3: Physical activity diary

PHYSICAL ACTIVITY DIARY

Name: _____

Date of next visit: _____

Date to wear pedometer: _____

Please keep a record of all <u>light, moderate and high intensity</u> activities that you perform one week before your study visit. You will receive a telephone reminder a week prior to your study visit. Please ensure all times are recorded.

Examples of types of activities and intensity

<u>Light</u>

- Light walking (eg taking a walk around the neighbourhood)

<u>Moderate</u>

- Brisk walking faster than 5 km/hr
- Bicycling slower than 14 km/hr
- General gardening
- Golf, wheeling or carrying clubs
- Recreational swimming

<u>High</u>

- Race walking or jogging (faster than 8 km/hr)
- Swimming laps
- Tennis (singles)
- Bicycling faster than 16 km/hr
- Heavy gardening (continuous digging or hoeing)
- Hiking uphill or with a heavy backpack

Physical Activity Diary (One week prior to study visit)

Date	Type of exercise	Duration	Intensity		
			Light	Moderate	High