Molecular regulation of skeletal muscle metabolism by testosterone and exercise in younger and older age

Nima Gharahdaghi

Thesis submitted to the University of Nottingham

for the degree of Doctor of Philosophy

October 2019

Acknowledgement

I would like to begin by expressing my gratitude towards my PhD supervisors Professors Philip Atherton, Ken Smith and Nate Szewczyk for giving me the opportunity to study and work in one of the most well-equipped and advanced laboratory in the world. Their invaluable guidance, insightful suggestions and constant encouragement were instrumental in ensuring that my research reached its full potential. In particular, Phil's passion and hard-work is infectious, and I hope that I still approach science with his same enthusiasm when I reach a similar stage in my career. He has taught me how to stay focused and fully develop thoughts in order to stay productive.

I would also like to thank United Kingdom for accepting me as a student and University of Nottingham for giving me the full tuition fee scholarship (Vice-Chancellor's Scholarship for Research Excellence) that allowed me to attend my PhD program.

Thank you to Mrs Amanda Gates and Dr Supreeth Rudrappa for their help in clinical works; and also thanks to everybody within the laboratory for their support, encouragement and companionship throughout.

Finally, I want to thank my amazing family for all their love and support through this process. Words cannot describe how much you mean to me. And I promise that this is my last degree!

Molecular regulation of skeletal muscle metabolism by testosterone and exercise in younger and older age

Chapter 1 Introduction: Skeletal Muscle Mass in Health, Disease and Ageing 1

1.1 Skeletal muscle- structure and function 2

1.2 Skeletal muscle development 4

1.3 Pathways regulating skeletal muscle protein turnover 7

1.4 Key hormones regulating postnatal muscle growth 14

1.4.1 Mechanism of testosterone action on skeletal muscle growth 20

1.5 Muscle growth regulation by skeletal muscle loading 26

1.5.1 Muscle growth regulation by muscle loading and nutrient interactions 28

1.5.2 Skeletal muscle mechanotransduction 31

1.6 Skeletal muscle glucose uptake and environmental stimuli 39

1.6.1 Mechanism of insulin action on skeletal muscle glucose uptake 40

1.6.2 Androgens and skeletal muscle glucose uptake 43

1.6.3 Exercise and skeletal muscle glucose uptake 46

1.7 Factors associated with muscle loss 48

1.7.1 Aging and skeletal muscle mass loss 51

1.7.2 Ageing and muscle loading 54

1.7.3 Aging, muscle loss and androgens 56

1.8 Aims of the study 64

Chapter 2 General Methods 66

2.1 Study ethics and participants 67

2.2 Study conduct 68

2.3 Physiological and functional measurements 70

2.3.1 Resistance training procedures and strength assessments 70

2.3.2 Muscle architecture by ultrasound and DXA-derived muscle mass 71

2.4 Metabolic and biochemical measurements 72

2.4.1 Oral Glucose Tolerance Test (OGTT) 72

2.4.1.1 Calculation of insulin resistance/sensitivity 73

2.4.2 Testosterone ELISA assay 74

2.4.3 Muscle immunohistochemistry 74

2.4.4 Body water and protein-bound alanine myofibrillar protein synthesis (MPS) 75

2.4.4.1 Calculation of synthetic fractional rate 77

2.4.5 Muscle protein breakdown (MPB) measures using D_3-3MH 78

2.4.6 Muscle RNA, DNA and protein content 78

2.4.7 Immunoblotting for anabolic/catabolic signalling 79

2.4.8 Gene expression analysis of myogenesis, IGF-1 related and testosterone processing 80

2.4.9 Mitochondrial citrate synthase activity and DNA copy number 82

2.5 Statistical analyses 84

Chapter 3 Pharmaceutical depletion of endogenous testosterone blunts resistance exercise-induced hypertrophy in younger men via impairing muscle growth programming 85

3.1 Abstract 86

3.2 Introduction 88

3.3 Methods 90

3.4 Results 91

3.4.1 Body composition 92

3.4.2 Muscle architecture 93

3.4.3 Fibre type satellite cell, myonuclei number and CSA 95

3.4.4 Muscular performance 97

3.4.5 Muscle protein synthesis and [extrapolated] breakdown 98

3.4.6 Muscle ASP, DNA and RNA content 100

3.4.7 Intramuscular signalling pathways 102

3.4.8 Muscle gene expression 104

3.4.9 Mitochondrial oxidative phosphorylation capacity 106

3.5 Discussion 108

Chapter 4 Testosterone therapy induces molecular programming augmenting physiological adaptations to resistance exercise in older men 118

4.1 Abstract 119

4.2 Introduction 121

4.3 Methods 124

4.4 Results 124

4.4.1 Body composition 126

4.4.2 Muscle architecture 128

4.4.3 Fibre type CSA 130

4.4.4 Muscular performance 131

4.4.5 Muscle protein synthesis and [extrapolated] breakdown 133

4.4.6 Muscle ASP, DNA and RNA content 135

4.4.7 Intramuscular signalling pathways 137

4.4.8 Muscle gene expression 139

4.4.9 Mitochondrial oxidative phosphorylation capacity 142

4.5 Discussion 144

Chapter 5A The role of endogenous testosterone (T) in regulating resistance exercise-induced modulation of glycaemic control in young men 155

5.1 Abstract 156

5.2 Introduction 158

5.3 Methods 160

5.4 Results 160

5.4.1 Body composition 161

5.4.2 Glucose, insulin and C-peptide levels 161

5.4.3 Correlations and ratios 164

5.5 Discussion 167

Chapter 5B The role of exogenous testosterone (T) therapy in regulating resistance exercise-induced modulation of glycaemic control in older men 171

5.1 Abstract 172

5.2 Introduction 174

5.3 Methods 177

5.4 Results 177

5.4.1 Body composition 177

5.4.2 Glucose, insulin and C-peptide levels 178

5.4.3 Insulin resistance and sensitivity 180

5.4.4 Correlations and ratios 181

5.5 Discussion 184

Chapter 6 Overview 188

6.1 Overview of the study results and general conclusion 189

6.2 Future research directions 193

References **196**

Appendices **234**

Appendix 1 235

Appendix 2 236

Appendix 3 238

Appendix 4 246

Appendix 5 255

Appendix 6 257

Publications, Presentations, Awards and funding 259

List of Figures

Figure 1-1 Cellular events leading to the preservation or loss of muscle functional reserve

Figure 1-2 Hierarchical structure of muscle

Figure 1-3 Satellite cell (SC) location and function

Figure 1-4 Akt/TSC2/mTOR pathway

Figure 1-5 RNA composition of the transcriptome

Figure 1-6 Mechanisms of testosterone action on skeletal muscle

Figure 1-7 Endocrine and local regulation of Leydig cell steroidogenesis by LH and locally produced factors

Figure 1-8 Pathway of testosterone biosynthesis

Figure 1-9 Potential mechanisms of the anabolic effects of testosterone on the skeletal muscle

Figure 1-10 Resistance exercise stimulates MPS that can remain elevated for ≥ 24 h

Figure 1-11 Schematic representation of cellular mechanotransduction layers

Figure 1-12 Skeletal muscle that can lead to training adaptation

Figure 1-13 Regulation of ribosome biogenesis during skeletal muscle hypertrophy

Figure 1-14 Molecular mechanisms of skeletal muscle glucose uptake

Figure 1-15 Scheme of the different etiological sarcopenia mechanism and their consequences

Figure 1-16 Normal (A) and aged (B) myogenic program in response to an anabolic stimulus

Figure 1-17 Changes in muscular function and architecture over 10-wks of T administration

Figure 2-1 Study protocol

Figure 3-1 Plasma testosterone concentrations during the study

Figure 3-2 Muscle mass and body composition changes

Figure 3-3 Fold changes in muscle architecture (A-D)

Figure 3-4 Fibre type number (A-C), satellite cell number (D-E), myonuclei number (F-G) and CSA (H-I) and correlation between fibre type and quadriceps CSA (J-K)

Figure 3-5 Time course of changes in Total strength (A) and muscle strength (B-D), and correlation between FFM and total strength (E)

Figure 3-6. Muscle protein synthesis, fractional growth rate, absolute synthetic rate, absolute break-down rate and protein turnover

Figure 3-7 Intramuscular anabolic signalling pathways

Figure 3-8 Muscle gene expression Changes in T and P in T metabolism gene expression (A-C), anabolism (D-F), and myogenic (G-L) and myogenesis inhibitor (M) from baseline to wk 6

Figure 3-9 Mitochondrial oxidative phosphorylation capacity markers

Figure 4-1 Schematic diagram of the study protocol

Figure 4-2 Muscle mass and body composition changes. (A–F)

Figure 4-3 Fold change in muscle architecture

Figure 4-4 Time course of changes in fibre-type cross-section area (CSA)

Figure 4-5 Time course of changes in (A) sum strength and (B–D) muscle strength and (E) correlation between fat-free mass (FFM) and sum strength.

Figure 4-6 Muscle protein synthesis, fractional growth rate (FGR), absolute synthetic rate (ASR), absolute breakdown rate (ABR), and protein turnover

Figure 4-7 Intramuscular signalling pathways

Figure 4-8 Muscle gene expression. Changes in testosterone and placebo in (A–D) testosterone metabolism gene expression, (E, F) anabolism, (G, H) fibre type, and (I–N) myogenic from baseline to Week 6

Figure 4-9 Mitochondrial oxidative phosphorylation capacity markers

Figure 5A-1 changes in insulin, glucose and C-peptide (A-F)

Figure 5A-2 Correlations (A-E) and ratios (F-I)

Figure 5B-1 changes in insulin, glucose and C-peptide (A-F)

Figure 5B-2 Correlations (A-E) and ratios (F-I)

List of Tables

Table 2-1 Primer sequences used in PCR

Table 3-1 Participants Characteristics

Table 3-2 Muscle protein, RNA, DNA and related ratios

Table 4-1 Participants Characteristics

Table 4-2 Muscle protein, RNA, DNA and related ratios

Table 5A-1 Glycaemic control-related calculations.

Table 5B-1 Glycaemic control-related calculations.

Abbreviations

1-RM: One repetition maximum

3MH: 3-Methylhistidine

ABR: Absolute breakdown rate

Akt: Protein Kinase B

AMPK: Adenosine monophosphate-activated protein kinase

AS160: Akt substrate of 160 kDa

ASP: alkaline soluble protein

ASR: Absolute synthetic rate

AUC; Area under curve

CaMK: Ca2+/calmodulin-dependent protein kinase.

CS: Citrate synthase

CSA: Cross sectional area

DXA: Dual-energy X-ray absorptiometry

ERK ½: extracellular signal-regulated kinase ½

FBR: Fractional breakdown rate

FFM: Fat free mass

FGR: Fractional growth rate

FSR: fractional synthetic rate

GIR: Glucose infusion rate

GLUT: Glucose Transporter

HOMA: Homeostatic model assessment

IR: Insulin Receptor

LM: Legs fat free mass

MPB: Muscle protein breakdown

MPS: Muscle protein synthesis

MRF: Myogenic regulatory factor

MT: Muscle thickness

mtDNA: mitochondrial DNA

mTOR: Mammalian target of rapamycin

MVC: Maximal voluntary contraction

OGTT: Oral glucose tolerance rate

P: Placebo

p70S6K: Ribosomal protein S6 kinase

PA: Pennation angle

PDK: phosphoinositide-dependent kinase

PI3K: Phosphatidylinositol-3-kinase

RET: Resistance exercise training

T: Testosterone

Tfam: Transcription factor A

TFM: Total fat mass

TFP: Total fat percentage

TSC2: Tuberous sclerosis complex 2

VL: Vastus lateralis

Chapter 1

Introduction: Skeletal Muscle Mass in Health, Disease and Ageing

1.1 Skeletal muscle- structure and function

Skeletal muscle plays a principal role in producing body movement, force, storage of protein (Fanzani et al. 2012), maintenance of posture and locomotion and heat production (Goldspink 2005). This range of roles are performed through many complex factors; e.g. architectural component, contractile fiber types, metabolic composition (Frontera and Ochala 2015). Further to this, skeletal muscle has an essential role as an endocrine organ by regulating energy metabolism, and secreting cytokines and myokines (Zurlo et al. 1990). However, muscle mass, along with strength and function, is lost with age; and understanding the mechanisms underlying muscle loss is essential in developing therapies that potentially restore muscle mass and strength; therefore, improving daily activities.

Skeletal muscle is the largest organ (~40% of body weight) in the body and loss in muscle mass, muscle strength; so called sarcopenia, has significant effects on whole body metabolism (Mitchell, Atherton, et al. 2012). It is reported that (Mitchell, Atherton, et al. 2012) the median annual muscle loss in adults over 75 years old was 0.8-0.9% in men and 0.6-0.7% in women, but a disproportionate amount of strength was observed corresponding to 3.0-4.0% per year in men and 2.5-5.0% per year in women (Guralnik et al. 1995). Further, hormones, i.e. growth hormone (GH), insulin-like growth factor (IGF-1) and androgens, which regulate muscle growth and result in muscle hypertrophy, also decrease in old age (Burton and Sumukadas 2010a). These age-associated attenuation have

serious consequences on quality of life, daily activities, and mortality (Guralnik et al. 1995; Burton and Sumukadas 2010b). In addition, risk factors for sarcopenia include sedentary lifestyle, low physical activity, bedrest, chronic disease, certain drug treatments, intermuscular adipose tissue infiltration, and decreased neuromuscular activation which result in anabolic resistance (Cruz-Jentoft et al. 2010; Sayer et al. 2008). But physical activity, specifically resistance exercise training (RET) (Rolland et al. 2011), and hormonal therapy (Burton and Sumukadas 2010a) seem to have an important role in both prevention and treatment of sarcopenia. This highlights the importance of understanding factors that determine protein balance and turnover in skeletal muscle, and the need to adopt the best strategy to promote muscle quantity and quality. The balance between the rates of protein synthesis and breakdown is crucial in determining whether muscles grow (i.e. accumulate protein) or undergo atrophy (i.e. a net loss of protein). Both chemical signals (e.g. hormones, growth factors and branched chain amino acids) and mechanical signals (e.g. stretch or different patterns of activity) associated with exercise can influence protein turnover at all stages of life, potentially improving the functional reserve of the skeletal muscles. This complicated relationship is partially depicted in Figure 1-1 (Goldspink 2005).

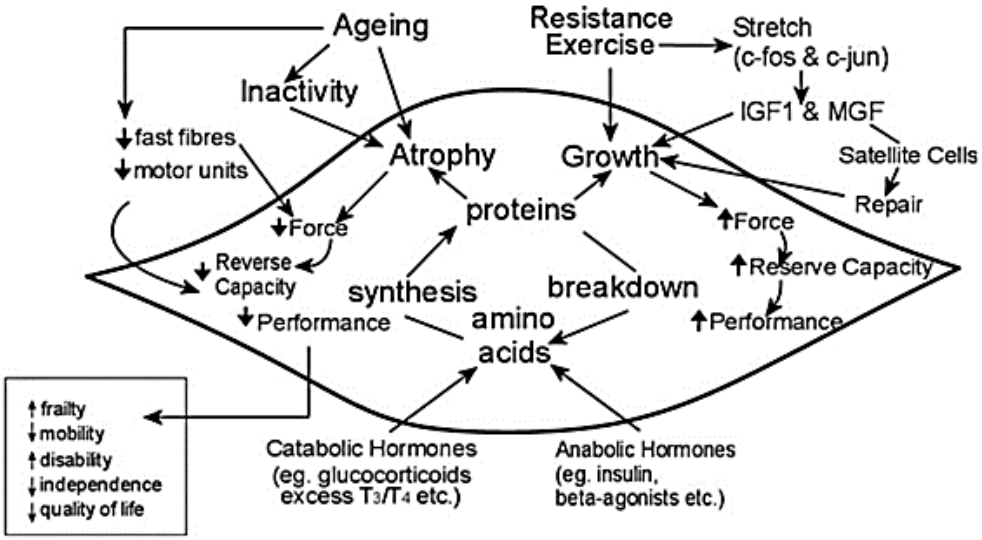


Figure 1-1 Cellular events leading to the preservation or loss of muscle functional reserve (Goldspink 2005).

Thus, in this chapter, the mechanisms of muscle development and then muscle growth will be reviewed; and afterward, the anabolic stimuli and the factors affect muscle mass/metabolism will be explained and finally, factors and metabolic syndrome which are associated with muscle loss, ageing and the strategies to combat them will be reviewed.

1.2 **Skeletal muscle development**

Skeletal muscles composed of myofibers; in which, when they are arranged in series provide length and in parallel provide cross sectional area (CSA) of the muscle (Figure 1-2) (Haun et al. 2019). Each myofiber is surrounded by endomysium, a specialized basal lamina, which protects cells sarcolemma (Sanes 2003). The myofibers contain a highly organized

cytoskeleton composed of myofibrils; which are formed by fusion of myoblasts to produce myotubes which then mature into myofibers (Abmayr and Pavlath 2012). Myofibrils are composed of sarcomeres which are contractile modules and express different isoforms of myosin heavy chains (MyHC), which are associated with different electrophysiological properties and metabolism; e.g. oxidative slow twitch fibers express slow MyHC (type I), and glycolytic fast twitch ones express fast MyHC (types IIa, IIb, IIx). However, the processes of myoblast-myoblast fusion and myoblast-myotube fusion are still poorly understood but are highly regulated during development (Chal and Pourquié 2017).

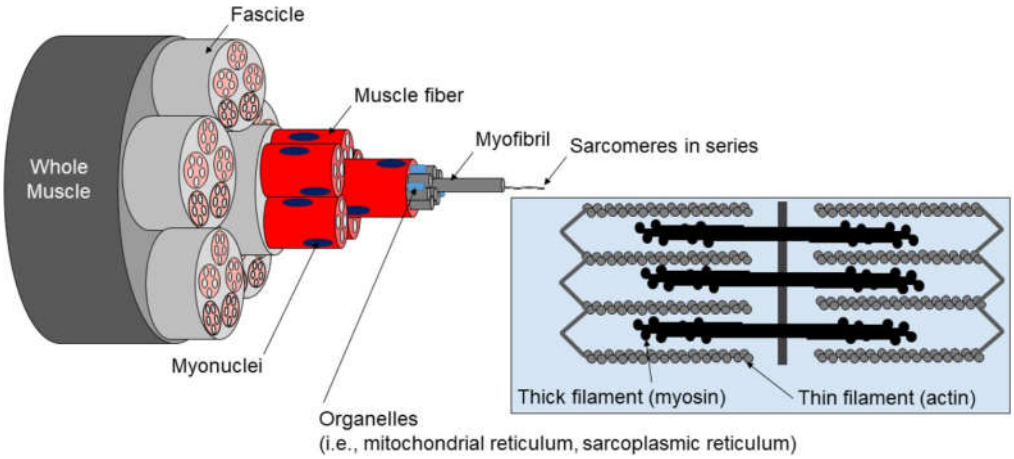


Figure 1-2 Hierarchical structure of muscle (Haun et al. 2019).

During prenatal development, myoblasts are developed from myogenic precursor cells; which then could be differentiated into myotubes and finally modulated to muscle fibers (Dayanidhi and Lieber 2014). This is the mechanism of developing/regulating muscle mass during prenatal development, but during the postnatal period, hypertrophy of an individual

fiber is shown to be the main reason of whole muscle hypertrophy rather than increase in fiber numbers (hyperplasia) (Rehfeldt et al. 2000; Swatland 1976). This postnatal development is associated with proliferation of muscle stem cells (i.e. satellite cells); which are responsible for composing new nuclei in the muscle fibers. Satellite cells are basically located between muscle sarcolemma and basal lamina and the process of transforming satellite cells to myofibers consists of quiescence, to activation, proliferation, differentiation and finally, cell fusion (Figure 1-3) (Dayanidhi and Lieber 2014). Once the satellite cells are activated, they commence the myogenesis cycle during which the myogenic regulatory factors (MRFs) will be expressed (Pownall, Gustafsson, and Emerson Jr 2002); which followed by proliferation (i.e. amplifying cell numbers), in which myoblasts will be formed. Formed myoblasts will then be differentiated to create myotubes which express myosin heavy chain which is the major muscle contractile protein (Wang and Rudnicki 2012). However, satellite cells are not only responsible of cell growth and repair, but also they need to perform self-renewal; without which the pool of satellite cells would be exhausted over time (Dayanidhi and Lieber 2014).

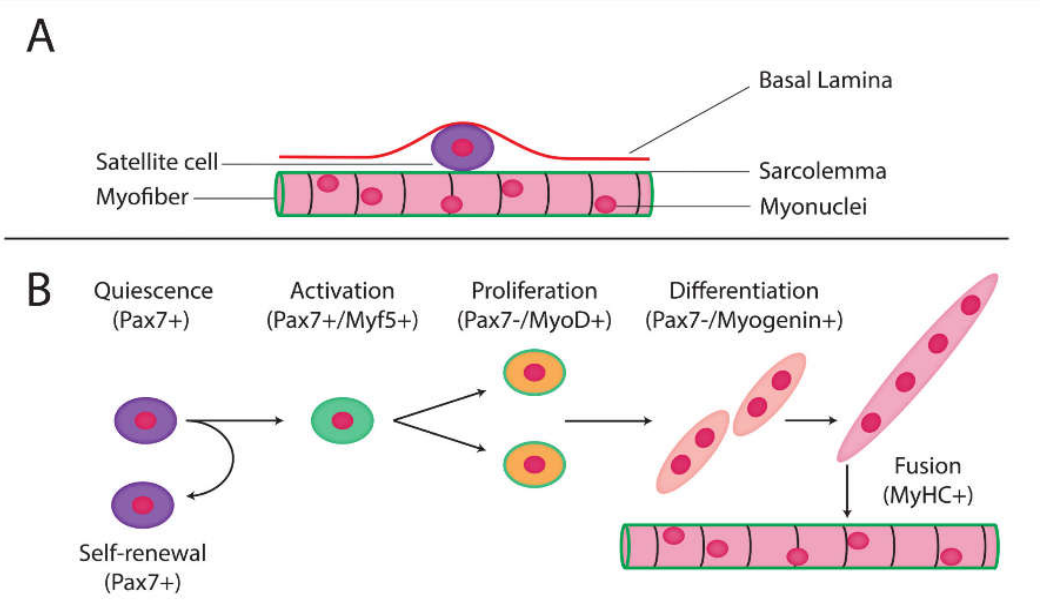


Figure 1-3 Satellite cell (SC) location and function. A) The SC is anatomically located between the myofiber basal lamina and sarcolemma. B) The relationship between gene expression and SC activation, self-renewal, proliferation, differentiation and fusion with existing myofiber (Dayanidhi and Lieber 2014).

Finally, after forming a single muscle fiber, which with other fibers, form whole skeletal muscle, each fiber starts to grow and increase the size during the life. The main pathways are responsible to regulate muscle mass growth will be explained in the next section.

1.3 **Pathways regulating skeletal muscle protein turnover**

As it has been mentioned above, postnatal muscle growth, specifically, relies on individual muscle fiber growth rather than increases in number of muscle fibers. In this section, the main pathway regulating muscle fiber

growth will be reviewed and then the interventions and corresponding mechanisms which boost muscle growth will be explained.

The understanding of skeletal muscle growth is critical in human medicine. Postnatal skeletal muscle growth substantially depends upon increase in individual fiber cross section area (i.e. size) which relies on the ratio between muscle protein synthesis (MPS) and muscle protein breakdown (MPB). Indeed, the enhancement of muscle fiber size occurs when the rate of protein synthesis is higher than the rate of breakdown of the proteins (MPS>MPB) (Sandri 2008). Accordingly, protein turnover is a constant and crucial process for survival, tissue renewal and repair (Sartorelli and Fulco 2004). Protein breakdown also represents a quality control mechanism, eliminating damaged or abnormally folded proteins (Chondrogianni and Gonos 2007). During postnatal muscle growth, satellite cells, the stem cells of skeletal muscle, undergo proliferation and fuse to constitute new muscle fibers with increasing protein synthesis (Moss and Leblond 1971); nevertheless, muscle fiber number seems to remain almost constant during the life (i.e. postnatal growth) since multinucleated myofibers form from myoblasts prenatally (Rehfeldt et al. 1999); therefore, factors affecting fiber morphology; via MPS>MPB, are fundamental in controlling muscle growth during postnatal period. Indeed, muscle protein synthesis (MPS) is highly dependent upon a number of key pathways resulting in myofibrillar protein synthesis and subsequent muscle hypertrophy; involving the translational ‘efficiency’ of ribosomes (Van der Meer, Jaspers, and Degens 2011) corresponding to the

translational rate per ribosome (Chaillou 2017), translational capacity, corresponding to the translational rate per ribosome ribosomal biogenesis, and transcriptional capacity (available mRNA) (Van der Meer, Jaspers, and Degens 2011). In accordance, anabolic stimuli and growth factors are primary regulators of net protein balance and myofiber hypertrophy (Moss and Leblond 1971), albeit through ill-defined mechanism.

The mammalian target of rapamycin (mTOR) pathway is a regulator of translational efficiency which leads to an increase in muscle mass which is resulted from mechanical loading (i.e. RET) (Bodine et al. 2001). Regulation of translation initiation is a key in muscle protein synthesis (Baar and Esser 1999; Dennis, Jefferson, and Kimball 2012) and mTOR complex 1 (mTORC1) regulates translation initiation and elongation by coordinating the activity of eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1) and p70S6 kinase (p70S6k) (Kimball and Jefferson 2010). It has been reported that the timing and location of the growth are dependent upon this particular pathway (Wullschleger, Loewith, and Hall 2006). In addition, phosphorylation of 4E-BP1 by mTORC1 modifies the formation of the eIF4E-4EBP1 complex and allows the eIF4E-eIF4G complex formation promoting translation initiation (Kimball, Horetsky, and Jefferson 1998). The p70S6k (another downstream protein) has numerous effectors including ribosomal protein S6 (rpS6) (up-regulated) and eukaryotic elongation factor 2 kinase (eEF2K) (down-regulated) (Ruvinsky and Meyuhas 2006). The rpS6

induces the up-regulation of specific mRNA with a 5'-oligopyrimidine tract, which encode for proteins of the translational apparatus (Meyuhas 2000) while eEF2K phosphorylates and down-regulates the eukaryotic elongation factor 2 (eEF2) mediated translation elongation (Pigott et al. 2012). On the other hand, upstream of mTOR is Akt (protein kinase B) (Figure 1-4). Phosphorylation of Akt through interaction with phosphatidylinositol 3 kinase (PI3K) results in downstream activation of mTOR pathway (Krakstad and Chekenya 2010). The best-known function of Akt is the promotion of cell survival (Datta et al. 1997). Akt phosphorylation also activates cell cycle progression (Graff et al. 2000). Akt can phosphorylate and inactivate glycogen synthase kinase 3 beta (GSK3β) which, other than playing an important role in glucose metabolism, also phosphorylates the cell cycle regulator cyclin D1 (Diehl et al. 1998). Phosphorylated cyclin D1 is targeted for degradation, but Akt phosphorylation of GSK3β prevents this effect and causes cyclin D1 to accumulate and induce cell cycle progression (Graff et al. 2000). However, one of the best-known targets of Akt is the mTOR pathway. mTOR and its effectors are essential for a hypertrophic response as a result of increased muscle loading (Reynolds, Bodine, and Lawrence 2002). In general, activation of the PI3K/Akt/mTOR pathway happens in immediate (i.e. 1-2 hours) and later fashions after RE. The later activation can happen via a number of factors (i.e. autocrine or paracrine) including various nutrients such as amino acids or muscle growth factors (MGFs) such as IGF-1 (Rennie and Wackerhage 2003; Hornberger and Chien 2006). Conversely, blocking the mTOR pathway prevents muscle growth

during development and under overload conditions (Bodine 2006). However, the immediate response is believed to originate from the mechanical stimuli (Reynolds, Bodine, and Lawrence 2002) and activation of mTOR increases protein synthesis by subsequent phosphorylation of the serine/threonine kinase p70S6 (S6k) which phosphorylates the S6 ribosomal protein (S6) (Baar and Esser 1999).

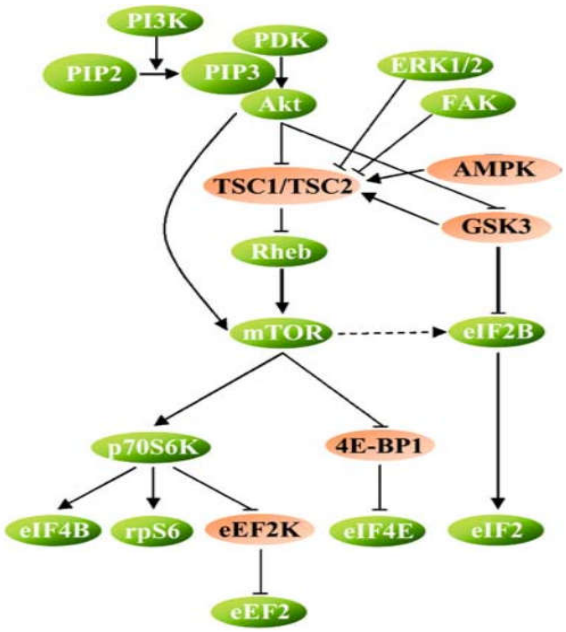


Figure 1-4 Akt/TSC2/mTOR pathway. Akt is phosphorylated by PDK and phosphorylates the tuberous sclerosis complex (TSC) 2, leading to the release of the inhibition on Ras homologue enriched in brain (Rheb), which activates the mammalian target of rapamycin (mTOR) and then ribosomal protein S6 kinase (p70S6K) and prevents binding of 4E-BP1 to eurakyotic initiation factor (eIF) 4E. Phosphatidylinositol-3-kinase (PI3K), phosphatidylinositol-biphosphate (PIP2), phosphatidylinositol-triphosphate (PIP3), phosphoinositide-dependent protein kinase (PDK), glycogen synthase kinase (GSK) 3, AMP kinase (AMPK), extracellular signal-regulated kinase (ERK) 1/2, focal adhesion kinase (FAK) (Favier, Benoit, and Freyssenet 2008).

In addition, 5' adenosine monophosphate-activated protein kinase (AMPK) is a highly conserved metabolic sensor expressed in a wide range of eukaryotes (Steinberg and Kemp 2009). AMPKs inhibition of anabolic

processes extends to the inhibition of the mTOR pathway in skeletal muscle (Bolster et al. 2002; Thomson, Fick, and Gordon 2008). This inhibition attenuates protein synthesis and ultimately blunt muscle growth (Mounier et al. 2011). AMPK can block the mTOR pathway in a few ways such as: phosphorylation of tuberous sclerosis complex 2 (TSC2) an upstream regulator of mTOR (Inoki, Zhu, and Guan 2003) or phosphorylation of raptor, a member of the mTOR complex that is essential for mTOR activity (Gwinn et al. 2008).

Apart from translation efficiency, as mentioned above, translational capacity is important in muscle protein synthesis which is determined by the total ribosomal content and often is expressed as the total RNA content per unit tissue (Figueiredo and McCarthy 2018). The ribosome is responsible for the translation of mRNA into protein, i.e., protein synthesis; and in the muscle cell, the ribosomal RNAs compose the vast majority of total cellular RNA (~70-90%), and therefore any change in total cellular RNA content, as the result of ribosome biogenesis, is generally attributed to a change in ribosomal RNA and indicating an increase in the translational capacity of the cell (O'Neil, Glowatz, and Schlumpberger 2013) (Figure 1-5).

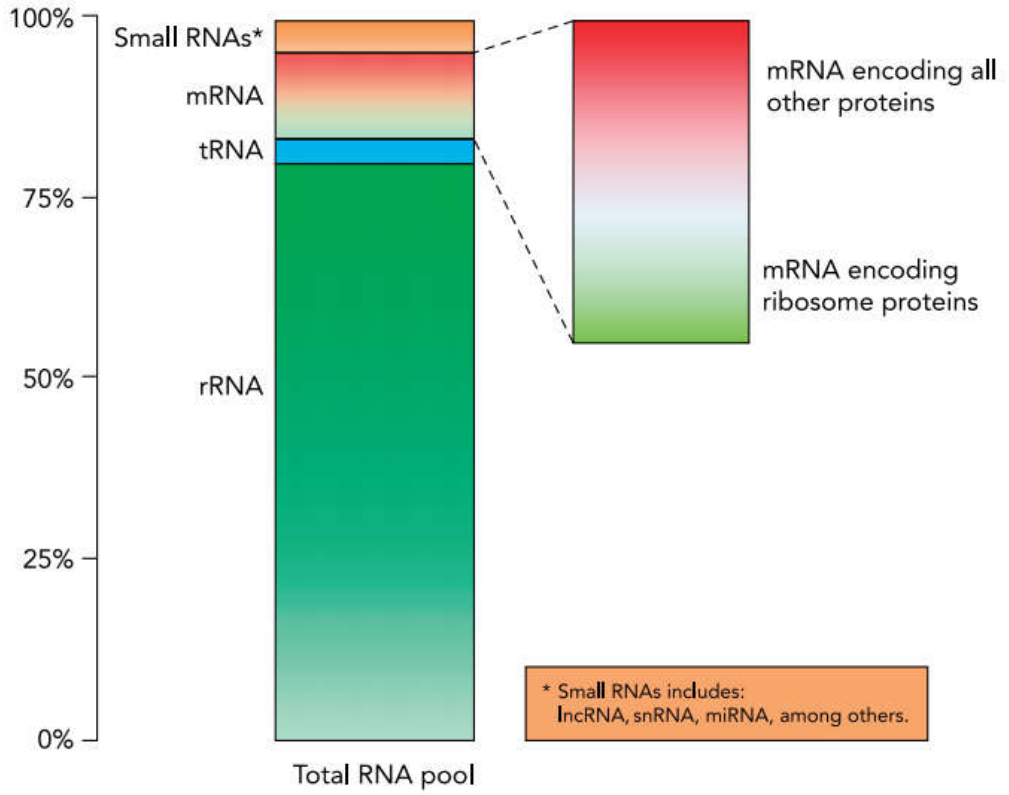


Figure 1-5 RNA composition of the transcriptome. The total RNA content within the cell is composed of ~80% ribosomal RNA. The remainder of the RNA content of the cell is composed of small noncoding RNAs such as transfer RNA, long noncoding RNAs, and microRNAs (Figueiredo and McCarthy 2018).

Synthesis of anabolic protein via gene expression includes transcription of sections of genetic code from DNA to messenger (m)RNA which is subsequently translated into a new functional protein. Generally, the transient increase in rates of gene transcription and subsequent increase in mRNA content leads to an increased protein content of that specific protein (Flück and Hoppeler 2003). It is a highly coordinated process where various environmental cues and signaling pathways integrate to regulate the formation of muscle mass (Lassar and Münsterberg 1994; Naya and Olson 1999).

As it has been mentioned already, the basic helix-loop-helix myogenic regulatory factors (MRFs) have critical roles in muscle development and hypertrophy (Berkes and Tapscott 2005). The primary MRFs, MyoD and Myf5, are required at the determination step for commitment of the proliferating somatic cells to the myogenic lineage. The committed cells (myoblasts) can proliferate and further differentiate into myocytes and mature into myofibers under the action of the secondary MRFs, myogenin and MRF4 (Berkes and Tapscott 2005). The myocyte enhancer factor-2 (MEF2) family of transcription factors is also involved in the activation of muscle specific gene expression. Together with the MRFs, these transcription factors are responsible for coordinating muscle-specific gene expression in the developing embryo (Berkes and Tapscott 2005).

1.4 **Key hormones regulating postnatal muscle growth**

As it was explained in the previous section, the net protein accretion occurs only when protein synthesis exceeds protein breakdown (MPS>MPB) which leads to the muscle growth. To do so, the endocrine system has the possible role; albeit through the complex and poorly defined interactions. However, it is reported that testosterone, growth hormone (GH), IGF-I and glucocorticoids are important regulators of muscle protein turnover and remodeling which result in postnatal muscle growth and hypertrophy (Breier and Gluckman 1991). Although many issues of hormone action remain unresolved with regard to skeletal muscle, we discuss here our current understanding of the actions of

testosterone, GH – IGF-I, estrogen, insulin, myostatin and GCs on skeletal muscle anabolism and catabolism (White et al. 2010). GH seems to have uncertain/minimal effect on muscle growth but it stimulates IGF-1 and androgen receptor synthesis in the muscle which result in muscle hypertrophy (Hayes et al. 2001). The expression of IGF-1 is primarily responsible for stimulating muscle growth likely via stimulation of satellite cells replication and then accelerating the progression of cell division (Fiorotto, Schwartz, and Delaughter 2003). In addition, beside regulation of glucose uptake, insulin is also an important regulator of muscle growth (Sheffield-Moore and Urban 2004). However, the mechanism of insulin action on muscle growth has been widely debated and further studies are needed.

In addition to all other anabolic hormones, the idea that androgens have anabolic effects on the muscle is not novel; nevertheless, Information on the association of androgens and muscle mass is scarce. Initially, testosterone (T) production is initiated through neuroendocrine regulation in the brain. Luteinizing hormone releasing hormone (LHRH), also called gonadotropin releasing hormone, is released in pulses from the hypothalamus (Molina and Education 2006). LHRH travels to the anterior pituitary gland and stimulates the synthesis and release of two gonadotropins: leutinizing hormone (LH) and follicle stimulating hormone (FSH) (Molina and Education 2006). In response to LH stimulation, testosterone is produced in the leydig cells of the testes, which are embedded in the interstitial tissues (Behre and Nieschlag 2012).

Testosterone levels are then maintained through a negative feedback loop. The inhibitory effect of testosterone on LH release is mediated predominantly by a locally produced metabolite of testosterone aromatization namely 17β-estradiol (Behre and Nieschlag 2012). Indeed, estradiol can inhibit LH release indirectly at the level of the hypothalamus through inhibition of LHRH release or directly on the pituitary gland (Behre and Nieschlag 2012).

Testosterone is an anabolic steroid hormone produced by both men and women, though to a much larger level in men (15-70 ng/dl in women vs. ~320 ng/dl in men). However, testosterone during postnatal period may not increase muscle fiber number (Galavazi and Szirmai 1971), but in later postnatal periods, it augments muscle growth via stimulating satellite cell proliferation and MPS (Urban et al. 1995b; Joubert and Tobin 1995); partially explain muscle mass differences between men and women. However, muscles in men are generally faster and have a higher maximum power output than women muscles. On the other hand, muscles in women are generally more fatigue resistant and recover faster and show less mechanical damage after each exercise training (Nygaard 1981; Haizlip, Harrison, and Leinwand 2015). In addition, given this fact that androgens during postnatal augments muscle fiber size further in men compared to women (i.e. type-I fibers are 19% larger, type-IIA fibers are 59% larger, and type-IIX fibers are 66% larger in men than in women) (Galavazi and Szirmai 1971; Haizlip, Harrison, and Leinwand 2015), therefore, sex differences in fiber numbers maybe rely on prenatal

testosterone level differences (Rehfeldt et al. 1999). Further, sex differences in fiber number may be due to different physical activity levels between men and women (Rehfeldt et al. 1999). It is reported that exercise training (especially resistance exercise training (RET)) influences minimally postnatal muscle fiber number and mainly fiber size, whereas inactivity for long term may be followed by decrease in muscle fiber number and muscle fiber size which both lead to muscle atrophy (Rehfeldt et al. 1999) .

On the other hand, healthy, hypogonadal men have lower muscle mass and higher fat mass than eugonadal men (Katznelson et al. 1998; Katznelson et al. 1996). To support, it is shown endogenous testosterone levels have been correlated with appendicular muscle mass and the strength in men (Baumgartner 2000); and low bioavailable testosterone levels were associated with lower levels of muscle function and increased risk of falls (Orwoll et al. 2006). Epidemiological studies have reported that there is a negative correlation between testosterone levels and visceral fat mass and obesity (Khaw and Barrett-Connor 1992); as well as glucose, insulin and C-peptide concentrations (Seidell et al. 1990). Like GH (Sattler 2013), testosterone production begins to decline in the late 20's and then continues to decline ~1% per year after age 40 in men (Gray et al. 1991); and the decline in testosterone levels, in both older males and females, is ~ 60% and 30%, respectively (van den Beld et al. 2000). This anabolic hormone is inactive when bound to the sex hormone-binding globulin (SHBG), which increases more than 2-fold after age 50 in men (van den

Beld et al. 2000). It is reported that provision of testosterone (or precursors) in aging men helps to combat sarcopenia effects. Further, it is reported that testosterone therapy (exogenous testosterone), 200 mg injection biweekly for 12 weeks has a benefit effect on increasing strength and muscle mass in older men (Ottenbacher et al. 2006). Anabolic effects of testosterone on muscle mass are dependent upon testosterone doses and resulting circulating concentrations, and often requires supra-physiological doses in healthy subjects (Bhasin et al. 2001b). Accordingly, dose-dependent increases in type I and type II fibre areas were found in relation to testosterone injection titrations (25-600 $mg \cdot wk^{-1}$) in men >65 y (Sinha-Hikim et al. 2006a). The testosterone-induced hypertrophy is characterized by muscle fiber hypertrophy of both type I and type II (Sinha-Hikim et al. 2002b); which is resulted from an increase in muscle protein synthesis (Ferrando et al. 1998a) and from a stimulation of satellite cell proliferation and differentiation (Sinha-Hikim et al. 2006b) (Figure 1-6). Moreover, testosterone therapy for 12 days in mice promoted myogenic differentiation of pluripotent mesenchymental stem cells (Singh et al. 2003b), and downregulates myostatin expression (Mendler et al. 2007) and activity (Singh et al. 2008). Nevertheless, it is reported that there is no association between testosterone and muscle mass in older men (van den Beld et al. 2000; Vermeulen, Verdonck, and Kaufman 1999). Similarly, Roy et al. (2002) found no association of muscle mass with testosterone, independent of age, in 20-90 years old men (Roy et al. 2002). One major confounder is that neither exercise nor diet was prescribed or controlled for in most of the studies. This would affect the

potential for therapeutic benefit to be gained by improving the anabolic environment (with anabolic hormonal supplementation) but not providing a potent anabolic stimulus (RET or protein ingestion). Therefore, perhaps combining RET with testosterone provision could be a better strategy. In addition, most of these studies involve restoring hypogonadic males to age normal levels of testosterone, restoring to high-normal levels of young men or to slightly supraphysiological levels would have a greater impact (Deschenes et al. 1994).

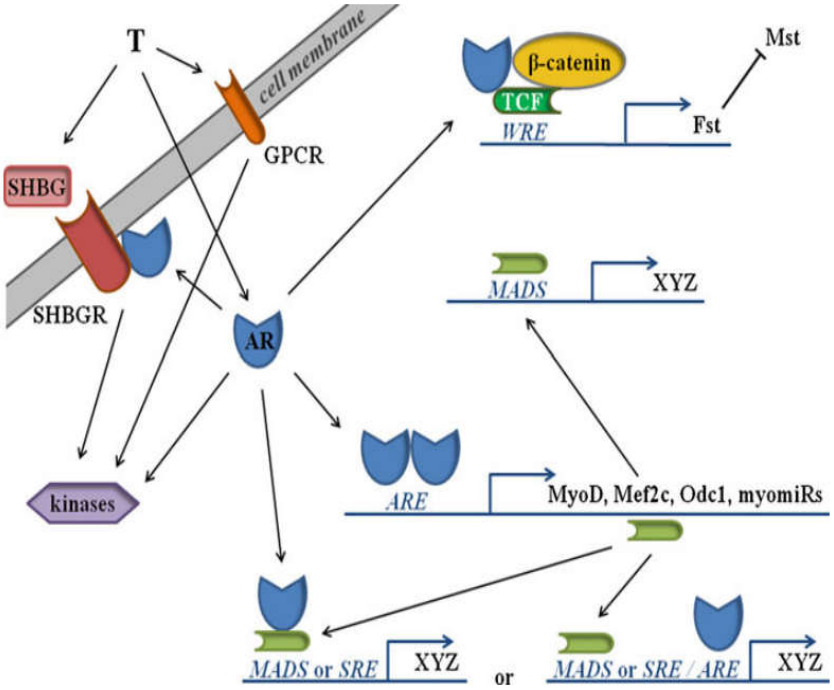


Figure 1-6 Mechanisms of testosterone action on skeletal muscle (Dubois et al. 2012)

However, the interventions increase muscle protein accretion and muscle mass are still under review and the corresponding mechanisms are not well-defined. In the following sections, factors and the corresponding

molecular mechanisms controlling muscle mass via MPS (i.e. androgens and RET) and MPB (i.e. metabolic syndrome and diseases) will be discussed in details.

1.4.1 Mechanism of testosterone action on skeletal muscle growth

Androgens' physiological roles rely on different factors; e.g. androgen molecule numbers, distribution of androgens and related metabolites into the muscle cells, interaction with their receptors (i.e. androgen receptors) and receptor activation (Palazzolo et al. 2008). To achieve enough exposure to androgens in muscle cells the mechanisms of transportation must be effectively operated (Palazzolo et al. 2008).

About 95% of circulating testosterone (6-6.5 mg daily) in men is produced by Leydig cells in testicles (Figure 1-7). Testosterone synthesis requires the conversion of cholesterol to T. This transformation is achieved via five different enzymatic steps, at the end of which the side chain of cholesterol is shortened through oxidation from 27C to 19C (Jarow et al. 2001). As far as almost all peripheral testosterone is derived from testicular Leydig cells, then it is expected that testosterone concentration exceed their binding proteins (i.e. sex hormone-binding globulin (SHBG)/androgen binding protein (ABP)) by ~200 fold (Jarow et al. 2001). The Leydig cells are the only cells expressing all required enzymes for testosterone metabolism (i.e. converting cholesterol to testosterone). Thereafter, testosterone is metabolized further into dihydrotestosterone (DHT) or

estradiol (Hammes et al. 2005). Basically, pituitary LH and local factors regulate and control synthesis and secretion of testosterone from Leydig cells (Lei et al. 2001; Sriraman, Anbalagan, and Rao 2005). Beside Leydig cells, adrenal steroidogenesis is responsible for the remaining physiological testosterone production (Rommerts 1998).

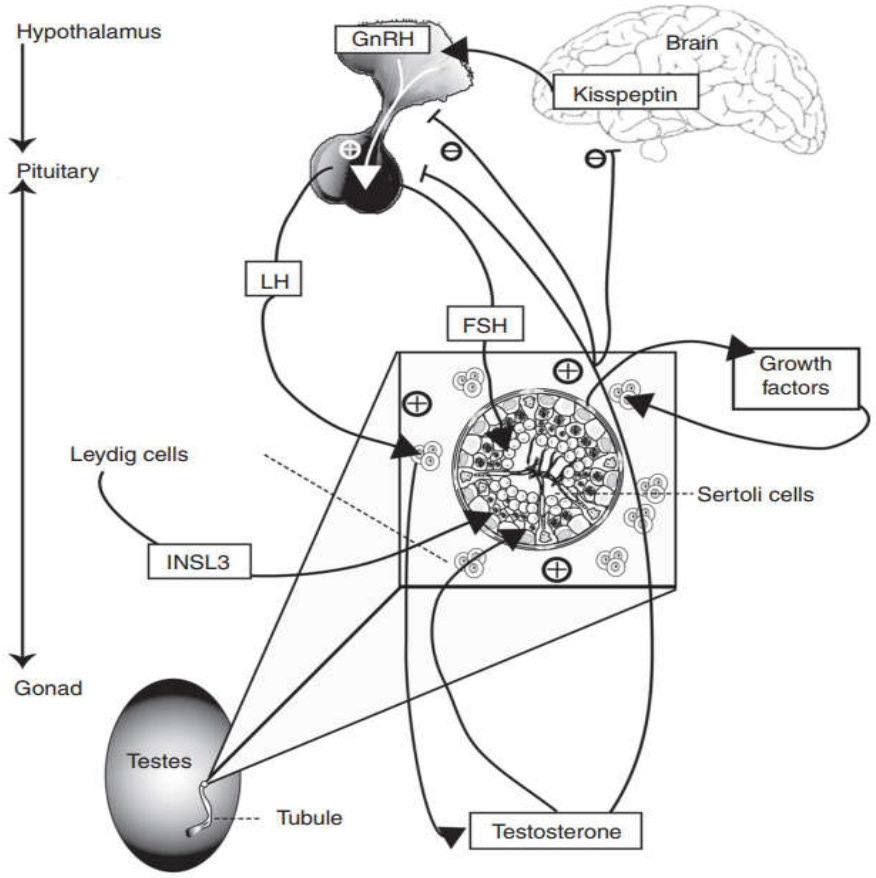


Figure 1-7 Endocrine and local regulation of Leydig cell steroidogenesis by LH and locally produced factors. Luteinizing hormone is the key regulator of Leydig cell function, and testosterone is the key product of the Leydig cell. A sensitive and rapid negative-feedback loop exists between the Leydig cells and the brain that is mediated via testosterone (and its metabolites DHT and estradiol) (Luetjens and Weinbauer 2012).

Testosterone biosynthesis by the Leydig cells of the testis proceeds via a sequence of enzymatic steps under the control of LH secretion. The

pathways of testicular steroid biosynthesis are shown in Figure 1-8. There is a negative feedback of testosterone on LH secretion both directly at the pituitary and indirectly at the hypothalamic level. The feedback relationship is developed in early infancy.

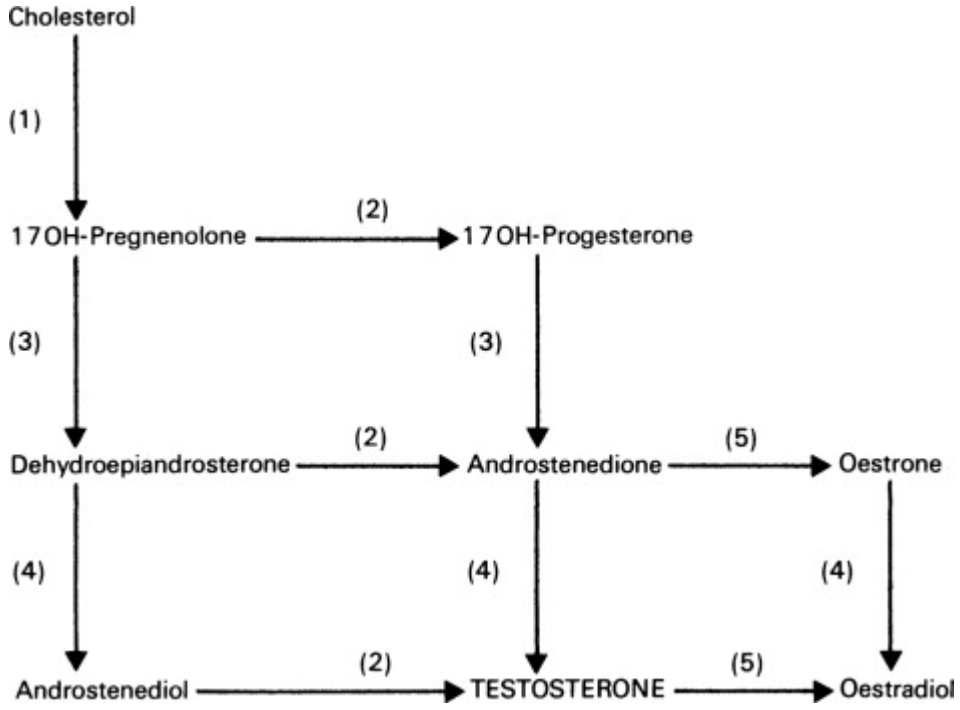


Figure 1-8 Pathway of testosterone biosynthesis. Enzymes: (1) desmolase; (2) 3 β-hydroxysteroid dehydrogenase; (3) 17, 20-desmolase; (4) 17 β-hydroxysteroid dehydrogenase; (5) aromatase (Hughes 1986).

Testosterone is mainly bound to albumin or SHBG in plasma to transport to target cells. Albumin-binding protein which has similar T-binding

characteristics is produced by Sertoli cells in testicles (Luetjens and Weinbauer 2012). However, only ~2% of total testosterone is circulating freely in blood, while 44% and 54% of total testosterone are bound to SHBG and albumin, respectively. It seems dissociation of testosterone from SHBG or albumin takes place in capillaries. The interaction of SHBG and albumin with endothelial glycocalyx results in a physical modification of binding site and therefore change in the affinity; and then testosterone would be free to enter into the target cell (e.g. muscle cells) or bind together with SHBG to megalin (an importer protein) (Hammes et al. 2005). The first step of testosterone action is binding to its receptor (androgen receptor (AR)). AR which is known as the transcriptional factors binds to its cytosolic ligand; inducing conformational changes, loss of chaperones, dimerization and nuclear translocation. In the nucleus, RNA synthesis is stimulated via binding ligands and ARs to specific sequences of genomic DNA (Wu and Zhang 2009). Aside from delayed genomic action, androgens are also inducing the rapid non-genomic effects, contribution to physiological effects (Losel et al. 2003). This rapid effects of testosterone modulate transcriptional activity of ARs and/or other transcription-independent pathways (e.g. calcium fluxes or MAP-kinase pathway) (Rahman and Christian 2007; Castoria et al. 2003).

While it is well-explained that AR mediates anabolic effects of testosterone on skeletal muscle (Figure 1-9), the non-genomic mechanism of testosterone in muscle growth is ill-defined and the results of previous studies are controversial (i.e. effects do not depend on gene

transcription or protein synthesis and involve steroid-induced modulation of cytoplasmic or cell membrane-bound regulatory proteins). However, it seems testosterone-induced augmentation in muscle mass is associated with both muscle fibers type I and II hypertrophy, albeit without any effect on number of muscle fibers (Sinha-Hikim et al. 2002c). Further, it is shown that 300 mg testosterone administration for 20 weeks is associated with increase in satellite cell and myonuclei numbers, without any effect on myonuclear domain size (the ratio of muscle fiber CSA to myonuclear number) in older men (Sinha-Hikim et al. 2003).

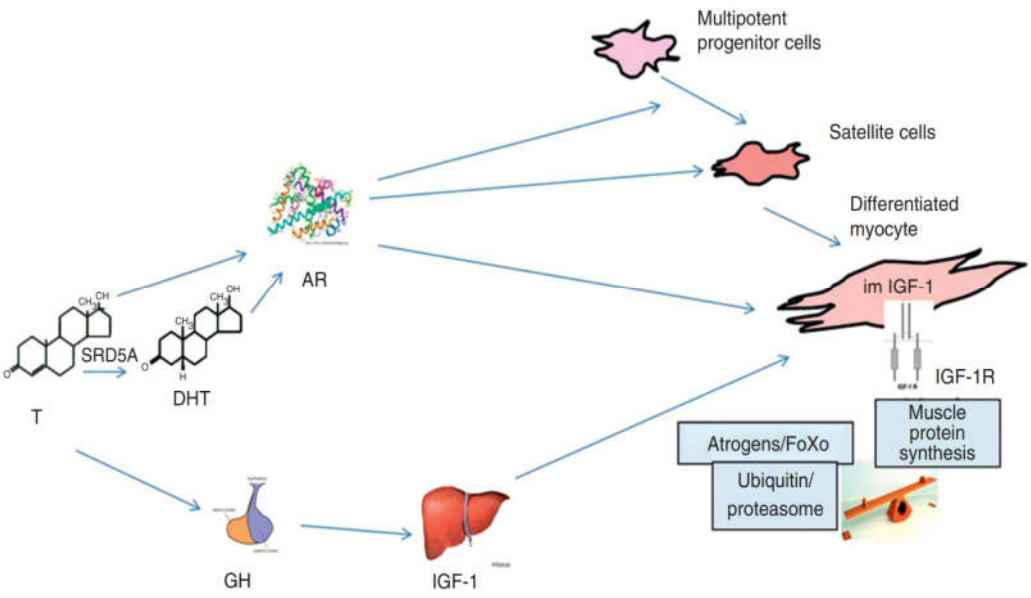


Figure 1-9 Potential mechanisms of the anabolic effects of testosterone on the skeletal muscle. Testosterone (T) could potentially increase muscle mass through multiple mechanisms. The vast majority of evidence suggests that testosterone's effects are mediated through the AR (Sinha-Hikim et al. 2002c).

Another observation is that testosterone increases MPS via increasing the nitrogen retention in hypogonadal men (Urban et al. 1995b; Rossetti,

Steiner, and Gordon 2017). In addition, studies using stable isotopes showed that testosterone therapy increases myofibrillar fractional protein synthesis and reutilization of amino acids (Sheffield-Moore et al. 1999). Moreover, testosterone might regulate the differentiation of mesenchymal multipotent cells, promoting their differentiation into the myogenic lineage and inhibiting adipogenic differentiation (Sinha-Hikim et al. 2004). Further, testosterone upregulates AR mRNA and expression of MyoD, and MHC (Gupta et al. 2008).

Noteworthy, testosterone effects on muscle mass are mediated by the androgen receptor (AR) and by the PI3K (phosphoinositide-3 kinase)/Akt pathway (Basualto-Alarcón et al. 2013). Interestingly, testosterone stimulates the expression of muscle IGF-I (Lewis et al. 2002) and the activation of S6K (ribosomal S6 kinase) (Xu et al. 2004). However, IGF-IR signaling is not mandatory for the anabolic effect of testosterone on muscle (Serra et al. 2011); but IGF-1 is key in regulating apoptosis and denervation, as well as in satellite cell activation and differentiation (Messi and Delbono 2003). Testosterone depletion in mice induces down regulation of Akt/mTORC1 signaling (White et al. 2013a), decrease in muscle IGF-1 mRNA expression (Mateescu and Thonney 2005) while testosterone administration upregulated/increased mentioned hypertrophy pathways in rats (Öner et al. 2008). Nevertheless, 3 months castration and testosterone administration afterwards had no effect on activation of Akt or related downstream targets (Hourde et al. 2009). However, given that the effects of testosterone on muscle protein

degradation are even less clear, further studies are needed to determine the mechanism of testosterone action. Additionally, further studies are needed to determine the clinically significant reduction in endogenous testosterone that could impact muscle Akt/mTOR signaling. This will also allow for a better understanding of the physiological implication of testosterone on the control of skeletal muscle protein turnover/growth.

1.5 **Muscle growth regulation by skeletal muscle loading**

Over the last ten years a number of important discoveries have shed light on how loading determines muscle mass, especially in fed state (Baar, Nader, and Bodine 2006). A type of muscle loading which accompanies with progressive muscle contraction against the resistance; so called resistance exercise training (RET), consists of mechanical stimuli which increase muscle mass and strength. This increase in muscle mass is characterized by an increase in muscle fiber size which is resulted from a stimulation of muscle protein synthesis (Phillips, Hartman, and Wilkinson 2005). Many studies have shown that RET increases muscle mass and strength in the younger adults as well as elderly individuals (Melov et al. 2007b; Phillips 2007). Thus, RET combats much of age-related muscle dysfunction; e.g. RET Improves fatty acid oxidation, body composition, insulin response, increased capillary density (Thompson 1994). While exercise response is blunted in the elderly in comparison to younger adults (Leiter, Peeler, and Anderson 2011), it still can cause increases in anabolic hormone, growth factor and paracrine/autocrine production,

cause muscle cell remodeling and hypertrophy (clearing out old damaged proteins and adding new, fresh proteins and contractile elements), increase strength and power production thereby improving functional ability, an increased satellite cell pool with improved proliferative capacity and many other positive changes to both the muscle fiber, and muscle programming (Leiter, Peeler, and Anderson 2011). However, the accretion of muscle mass (i.e. muscle hypertrophy) is the primary adaptation in skeletal muscle during long-term RET (Folland and Williams 2007). Augmentation in muscle CSA is induced from increase in individual fiber's CSA (i.e., muscle fibre hypertrophy) (Folland and Williams 2007). Changes in muscle fiber CSA are resulted from accretion of protein turnover in skeletal muscle which leads to fiber hypertrophy if MPS>MPB (Rasmussen and Phillips 2003). However, there are no measurable increases in MPS after a single bout of exercise (Atherton and Smith 2012), even MPS is suppressed for more than 3 h after high intensity eccentric contractions (Cuthbertson et al. 2006), and less than 1 h after low intensity resistance exercise (Kumar et al. 2009). After the initial silent period, MPS increases between 45 and 150 min and could last for ~4 h after resistance exercise in the fasted state (Kumar et al. 2009), while it can be sustained over two days after one single RE in the fed state (Churchward-Venne, Burd, and Phillips 2012). Given that whole muscle protein turnover increases after RE, it also increases (albeit smaller) the rates of MPB which lasts as long as MPS lasts (i.e. 24 h) (Phillips et al. 1997).

Aside from increases in contractile protein content, muscle fiber hypertrophy is also associated with incorporation of myonuclei which originating from satellite cells (Pallafacchina, Blaauw, and Schiaffino 2013). Satellite cells, located dominantly in basal lamina surrounding each myofiber, are activated during resistance exercise; and then active satellite cells initially proliferate as myoblasts before undergoing myogenic differentiation and finally contribute to the production of new contractile proteins (Ten Broek, Grefte, and Von den Hoff 2010). As the contentious result, augmentation of muscle mass has been reported in spite of significant satellite cell depletion (>90%)(McCarthy et al. 2011). However, the obligatory role of satellite cells and contribution of myogenesis during muscle loading which result in increase in MPS and then muscle fiber hypertrophy and finally whole muscle hypertrophy are still not very well defined (especially when RET is coupled with anabolic therapy). Nevertheless, as it has been mentioned above, the levels of increases in MPS vs. MPB after RE depends upon the feeding state; therefore, the interaction of RET with nutrient will be explained in the next section.

1.5.1 Muscle growth regulation by muscle loading and nutrient interactions

Regardless of age or gender, resistance training or provision of adequate amounts of dietary protein or essential amino acids can increase MPS in healthy adults. Combined protein ingestion proximal to resistance training, however, can augment the post-exercise MPS response and has been

shown to elicit a greater anabolic effect than exercise plus carbohydrate (Hulmi, Lockwood, and Stout 2010). Normally, muscle protein turnover rates are ~1.2% per day, which in the fed state MPS exceeds MPB (i.e. MPS>MPB or anabolic phase); and in the fasted state MPB exceeds MPS (i.e. MPB>MPS or catabolic phase) (Phillips et al. 1997). The acute augmentation in MPS after RE in the absence of EAA; on the other hand, provide a more prolonged increase in MPB such that the net effect is negative muscle protein turnover (Biolo et al. 1995). Therefore, the impacts of availability of the nutrient on adaptation to RET is considerable (Hawley et al. 2010) (figure 1-10). The extent to which substrate levels are altered before and/or during exercise and in the subsequent post-exercise recovery period is an important factor in modifying subsequent training adaptations; e.g. muscle glycogen replenishment during recovery via ingestion of carbohydrate to enhance subsequent performance has been established (Hawley, Tipton, and Millard-Stafford 2006). Similarly, it is reported that ingestion of protein and amino acid stimulate MPS, reduce MPB which both result in promoted net muscle protein synthesis (Rennie et al. 1982; Phillips, Hartman, and Wilkinson 2005)

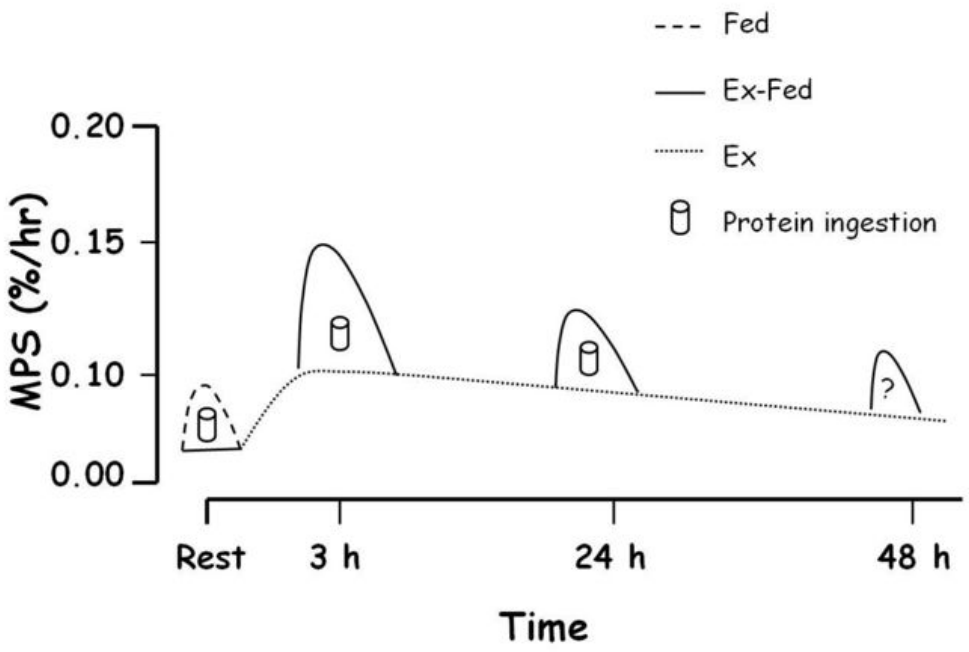


Figure 1-10 Resistance exercise stimulates MPS that can remain elevated for ≥ 24 h (Churchward-Venne, Burd, and Phillips 2012).

However, it seems the time of feeding (i.e. pre, during or after RE) is not critical in increase in MPS. This is in part due to a delay in muscle full response to RE which lasts at least 24h (Burd et al. 2011). Thus, sufficient nutrient is more important than timing of feeding to result in augmented hypertrophy adaptations (Ferguson-Stegall et al. 2011). Moreover, there are still limits to protein loading. It is reported that saturable responses will happen after loading ~20 g while doses higher than 20 g increases amino acid oxidation and then the excess protein will be catabolized (Moore et al. 2008). Given that RET-induced muscle hypertrophy is primarily resulted from post exercise MPS accretion (Glynn et al. 2010) (especially in the fed state), the following section will discuss the corresponding molecular regulation of MPS.

1.5.2 Skeletal muscle mechanotransduction

Cells are constantly exposed to mechanical stimuli arising from the surrounding extracellular matrix (ECM) or from other cells. The process of transferring a mechanical stimuli (i.e. muscle contraction) to an internal cellular response (i.e. initiating signal pathways) is termed mechanotransduction (Wu et al. 2011). Mechanotransduction is fundamentally important to help the cell to be adapted to the continuous dynamic modifications of the microenvironment. Local changes in ECM composition and mechanics are driven by a feed forward interplay between the cell and the matrix itself, with the first depositing ECM proteins that in turn will impact on the surrounding cells. Indeed, these changes occur regularly during tissue development and process of aging. In this section, the molecular basis of cellular mechanotransduction, by analyzing how mechanical stimuli get transformed into a given biological response through the activation of a peculiar genetic program, will be reviewed.

Cells perceive mechanical stimuli through diverse mechanosensitive molecules at the cell membrane including integrins, stretch-activated ion channels, G protein coupled-receptors, growth factor receptors, activating different mechanotransduction pathways. Muscle cells in contact with a stimuli typically develop discrete multiprotein complexes under the membrane named focal adhesions, which are the main hub of cell-ECM interaction. The mechanical information arising from modifications of the ECM, perceived by the FAs and propagated at the cytoskeleton level,

impacts on proteins residing at the membrane or in the cytoplasm and induces their structural modification and their subsequent shuttling to the nucleus. The interpretation of mechanical cues by the cell is completed by the activation of a given genetic program which induces the cell to adapt to the new conditions (Figure 1-11) (Martino et al. 2018).

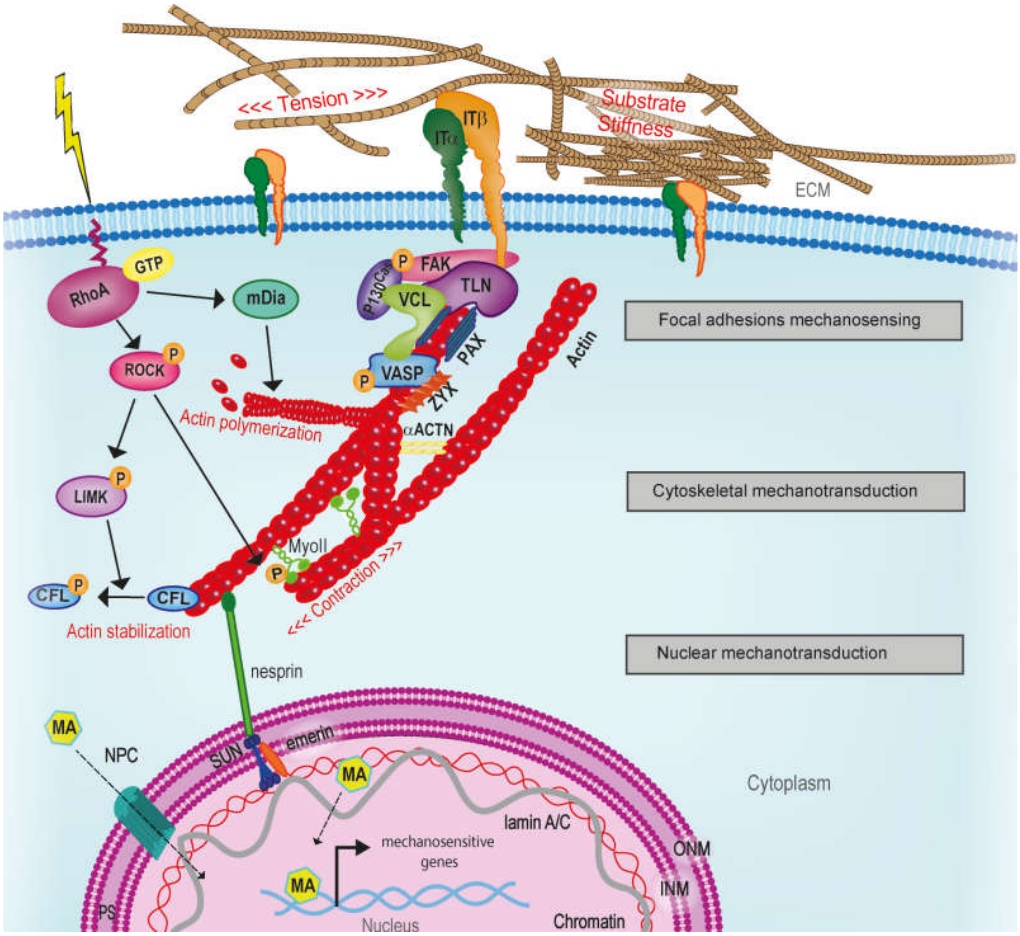


Figure 1-11 Schematic representation of cellular mechanotransduction layers. Extracellular physical stimuli are perceived by FAs at the cell-ECM interface; the signals are propagate by the cytoskeleton and transferred to the nucleus where mechanosensitive genes are activated by mechanoactuators (MA). MA can be shuttling mechanotransducers or mechanosensitive transcription factors. ACTN, actinin; CFL, cofilin; FAK, focal adhesion kinase; INM, inner nuclear membrane; IT, integrin; LIMK, LIM kinase; mDia, Diaphanous-related formin-1; MyoII, myosin II; NPC, nuclear pore complex; ONM, outer nuclear membrane; PAX, paxillin; PS, perinuclear space; ROCK, Rho-associated protein kinase; TLN, talin; VASP, vasodilator-stimulated phosphoprotein; ZYX, zyxin (Martino et al. 2018).

However, it is reported that mechanical stimuli (e.g. RE) acutely increases the levels of catabolism (e.g. cortisol) and anabolism (e.g. testosterone). Testosterone is known as the potent stimulator of MPS and muscle growth and strength in men, which increases immediately after high intensity RE

(Hakkinen and Pakarinen 1993). Nevertheless, it is reported that the levels of testosterone has not changed after RE (Koziris et al. 2000). Generally, testosterone levels increase immediately after high intensity (>80% 1-RM) RE and return to the baseline levels within 30 mins (Kvorning et al. 2007a; Kraemer et al. 1998); and this acute RET-related response is related to ensuing muscle hypertrophy in younger men (Brook, Wilkinson, Mitchell, et al. 2016), suggesting a link between testosterone and muscle mass gains with RET. To regulate muscle mass growth, it seems MPS is more important factor regulating protein turnover and augmenting muscle growth during RET rather than MPB (Phillips et al. 1997). To demonstrate this, a moderate attenuation (~20 - 30%) of MPB with RET is generally reported only when exogenous essential amino acids are ingested during recovery (Rasmussen et al. 2000; Wilkinson et al. 2007b). As it explained above, the important factors regulating MPS and then muscle growth to an anabolic stimuli (testosterone or RET) are transcription and translation, and subsequent synthesis of proteins (Coffey and Hawley 2007). Regarding RET-induced muscle growth, the chronic structural and metabolic adaptations are resulted from the cumulative effects of each repeated exercise bouts (Perry et al. 2010; Wilkinson et al. 2008b). Therefore, molecular adaptations to RET range from immediately post-exercise to weeks (Figure 1.12). To support, it is reported that numerous genes are activated in the muscle cells immediately after a single bout of RE which are responsible of anabolic and catabolic responses (Coffey, Shield, et al. 2006; Harber et al. 2009). Predominantly, RE-induced acute gene

expression mainly involve in transcriptional cell regulation (Hoppeler and Flück 2002; Booth and Neufer 2005). This response is then followed by an augmentation in many other genes expression approximately 3-12 h after RE; before returning to the resting levels after 24 h (Yang et al. 2005). Accordingly, repeated bouts of similar contractile activity induce early gene responses and protein synthesis in skeletal muscle that ultimately result in an altered phenotype (Coffey and Hawley 2007).

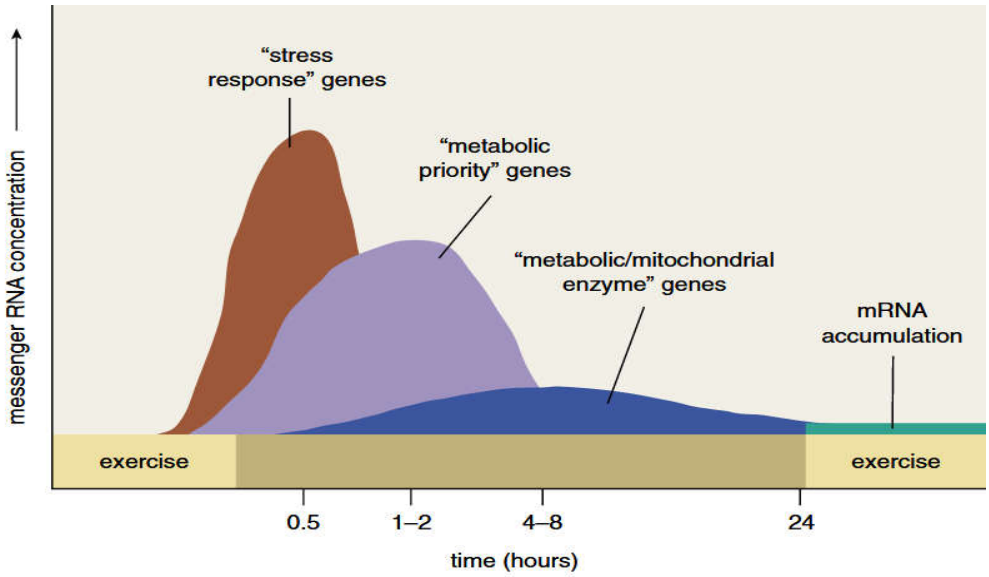


Figure 1-12 Skeletal muscle that can lead to training adaptation (Booth and Neufer 2005).

It is well-defined that RET increases the phosphorylation of proteins of the hypertrophy signaling pathway (Burd et al. 2010; Terzis et al. 2010); i.e. mTOR pathway, a key regulator of the mRNA translation into proteins and more globally of the cell size (Drummond et al. 2009). Interestingly, RET increases the muscle expression of IGF-I, a stimulus of mTOR and a positive regulator of muscle mass (Yang et al. 1996). Consistently, it is

reported that an increase in the phosphorylation of the main proteins involved in mTOR-mediated signalling pathway is observed within 4 h after a resistant exercise (RE) bout. In particular, the phosphorylation of p70S6K is increased during 1-2 h post RE. The phosphorylation of p70S6k appears to be the best available surrogate marker of skeletal muscle anabolism outside of direct measures of fractional synthetic rate, and has been shown to have a modest correlation with acute increases in muscle protein synthesis (Burd, Wall, and van Loon 2012) and chronic changes in whole body muscle mass and type IIA fibre cross sectional area (CSA) after 16 weeks of RET (Burd et al. 2010; Terzis et al. 2010). The 4E-BP1 phosphorylation following RE has also been reported as the marker of the myofibrillar fractional synthesis rate (FSR) 24 h after a bout of RE (Burton and Sumukadas 2010b). Further, acute activation of hypertrophic proteins regulating translation initiation and elongation for MPS including Akt (~ 2 fold), mTOR (~ 2 fold), ERK (~ 3 fold), p70S6K (~ 4 fold), rpS6 (~ 4 fold), 4E-BP1 (~ 5 fold) and eEF2 (~ 2 fold) which all are induced after RE (Terzis et al. 2010; Dreyer et al. 2006; Coffey, Zhong, et al. 2006; Eliasson et al. 2006). These acute responses are even augmented further (~3 - 20 fold) with ingestion of protein following RE (Dreyer et al. 2008; Koopman et al. 2007). Nevertheless, chronic adaptation of activated signaling proteins after each RE during RET are not fully defined. However, it is reported that phosphorylation of $mTOR^{Ser2448}$ (~ 200%) and $p70S6K^{Thr389}$ (~ 400%) were increased vs. resting levels after 10-wks of RET (Vissing, McGee, Farup, Kjølhede, et al. 2013). Moreover, activation of signaling proteins as well as rate of

mitochondrial biogenesis at rest and acutely after RE increased by 10-wks RET (Wilkinson et al. 2008b) (Figure 1-13). Higher resting phosphorylation of anabolic proteins might be associated with higher resting MPS after RET (Wilkinson et al. 2008b; Phillips et al. 2002). Thus, it seems progressive RET increases protein turnover (i.e. MPS>MPB) immediately after each RE and during recovery period in the myofibrillar state which may lead to muscle growth (Kim, Staron, and Phillips 2005; Wilkinson et al. 2008b; Phillips et al. 2002).

Aside from anabolic pathways, AMPK is involved in repressing the mTORC1 (Gwinn et al. 2008; Zoncu, Efeyan, and Sabatini 2011). It is reported that not only AMPK attenuates mTOR signalling by phosphorylation of TSC2 (Inoki, Zhu, and Guan 2003); but it also can directly phosphorylate and inhibit mTORC1 at the Thr2446 residue (Zoncu, Efeyan, and Sabatini 2011). However, AMPK activity increases following RE (Dreyer et al. 2006) while MPS increases meanwhile (Moore et al. 2005). It is suggested that concomitant increase in AMPK activity and MPS during post-RE may reflect AMPK's roles in other metabolic processes such as glucose uptake (Richter et al. 1989) and/or glycogen synthesis (Richter et al. 1982). However, AMPK-induced inhibition of hypertrophic pathways after RE in skeletal muscle remains to be established. Moreover, investigating the molecular mechanisms regulate MPS and protein turnover induced by RET are therefore essential to understanding the regulation of chronic adaptations to RET in skeletal muscle. Such information may improve understanding of how these

pathways become deregulated in disease states characterized by significant muscle wasting (e.g., sarcopenia, diabtes, and cancer cachexia)

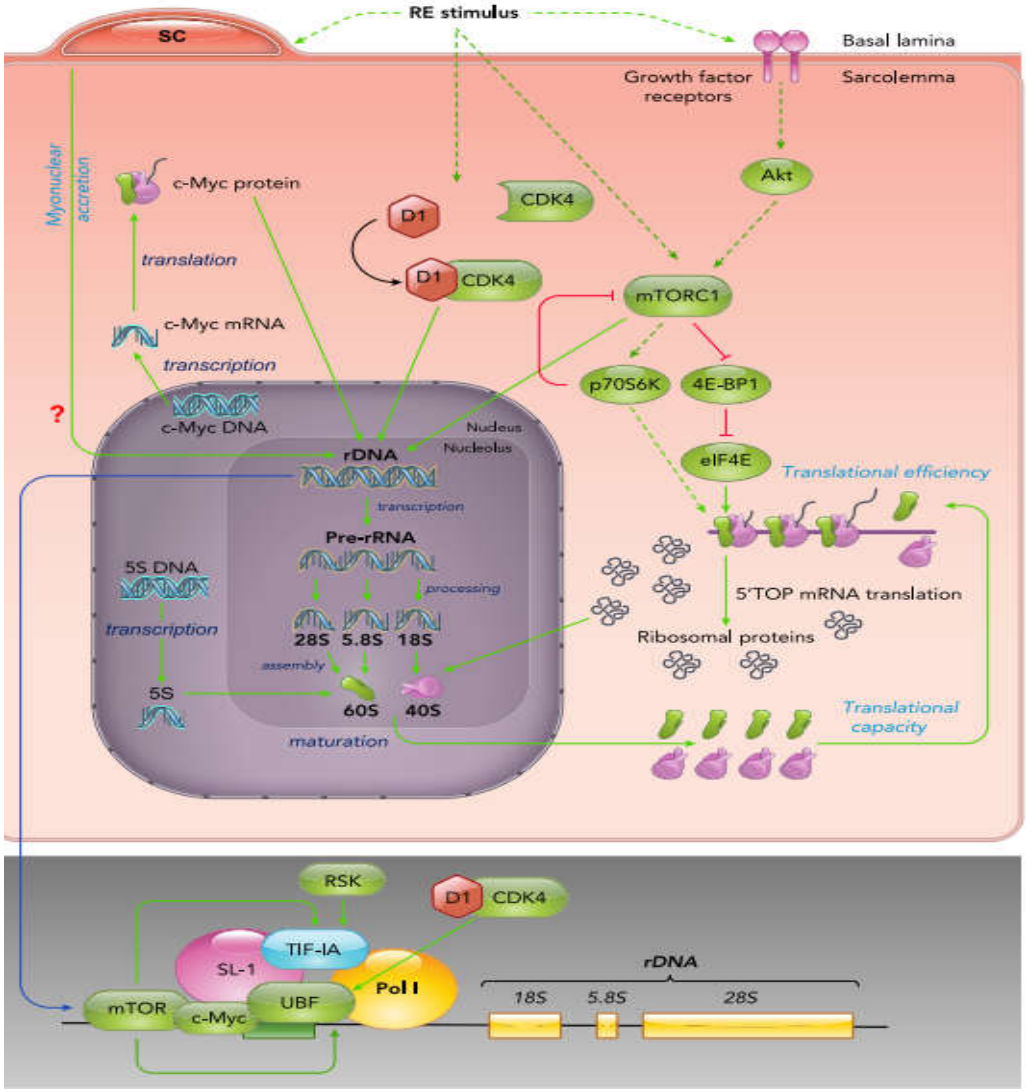


Figure 1-13 Regulation of ribosome biogenesis during skeletal muscle hypertrophy. Relevant signaling transduction pathways to ribosome biogenesis are shown where active rRNA genes are being transcribed leading to processing, maturation, and assembly of ribosomes. This will result in increased number of ribosomes and increased translational capacity. Efficiency also contributes with translational capacity by promoting the specific translation of key proteins involved in ribosome biogenesis, such as the ribosomal proteins. The role of satellite cell by donating rDNA template is shown with a question mark to denote the need for further investigation (Kim, Guo, and Nader 2019).

However, one important energy source which affects available demanded energy during RE is the levels of blood glucose. Glucose, an essential energy source for all cells, is initially transported from blood; which comes from digested foods. Like many other nutrients in the body, nutrients in the blood will be transported into various tissues to provide the nutrient requirements for the cells over a period of physiological actions (Yang 2014). However, in addition to RET-induced muscle mass growth which have positive effects on movement, quality of life and general health, it is reported that increase in muscle mass regulates fat and glucose metabolism which results in a remarkable improvement of insulin sensitivity (Atlantis et al. 2009). Therefore, it is important to understand that maintaining/improving muscle mass at any age would have positive effects on metabolic syndrome (e.g. insulin resistance) through improving glucose homeostasis (Atlantis et al. 2009). Thus, in the next section, the importance of muscle mass over glycaemia control and the environmental interventions affecting it will be explained.

1.6 Skeletal muscle glucose uptake and environmental stimuli

Glycemic control refers to the control of blood sugar glucose. This control is critical as long-term hyperglycemia, which is associated with insulin resistance, might lead to microvascular complications and diabetes (Tarnow et al. 2007). This control has long been the golden standard and fundamental for diabetes management (Chiang et al. 2014). Indeed, good

control of glycaemic is critical during therapeutic interventions in diabetes mellitus (Adams 2008). In accordance, perfect glycemic control is corresponding with normal glucose levels (70–130 mg/dl, or 3.9–7.2 mmol/L). Poor glycemic control, on the other hand, refers to high blood glucose (200–500 mg/dl, or 11–28 mmol/L). Thus, given the poor-defined insulin action mechanism in muscle glucose uptake, there remains a need for studies to investigate the mechanisms of insulin action and beneficial effects of therapeutic implications to counteract the poor glycaemic control (e.g. insulin resistant) and associated metabolic syndrome (i.e. diabetes). Therefore, in the following sections, the possible mechanism of insulin action in glucose uptake will be explained; and then, the effects and limitations of two favorable therapies (exercise and hormone therapy) will be reviewed.

1.6.1 Mechanism of insulin action on skeletal muscle glucose uptake

In general, glycaemic control is set through insulin-dependent and insulin-independent cell signaling pathways. The independent manner involves AMPK and calcium/calmodulin-dependent protein kinase (CaMKK) (Witczak et al. 2007). Glucose enters into the muscle cells through binding to its receptor (i.e. insulin receptor or IR) (Leney and Tavare 2009). The receptor has intrinsic tyrosine kinase activity which is phosphorylated and activated once bound to the glucose. Once activated, IR phosphorylates the different substrates; i.e. insulin receptor substrate 1 and 2 (IRS-1 and

IRS-2), which then activate phosphatidylinositol-3 kinase enzyme (PI3K). PI3K activates phosphoinositide-dependent kinase-1 (PDK-1) and then Akt which is key regulator of insulin signaling pathway (Leney and Tavare 2009). Akt is responsible of activation of AS160 (Leney and Tavare 2009) which has two main roles in GLUT-4 translocation: 1) Reducing the tethering of the vesicle (via TUG) and releasing the GLUT-4 towards membrane of the cells; 2) Activating the Rab proteins which translocate the vesicles contain GLUT-4 to the periphery. Thus, the levels of GLUT-4 and the capacity of the cells to translocate them to the sarcoplasm are essential for glycaemic control of muscle cells (Leney and Tavare 2009). It is not surprising that this pathway is downregulated in the metabolic diseases such as diabetes (Hotamisligil 2006). However, there are some other alternative pathways that regulate glucose uptake; e.g. IGF-1 pathway which is not affected when insulin signaling has been downregulated, suggesting an alternative pathway may regulate glucose metabolism (He et al. 2006). Indeed, in the absence of insulin, IGF-1R receives insulin signal and stimulates the same pathway (He et al. 2006).

It is reported that phosphorylation of AMPK in muscle cells increases lipid oxidation and glycogen synthesis during and after exercise in response of muscle contraction and increase of calcium release (Jeon 2016). During exercise, ATP levels drop which results in an increase in ADP:ATP and AMP:ATP ratios. This increases the phosphorylation of AMPK and then AS160 (Stanford and Goodyear 2014; Treebak et al. 2006) (Figure 1-14); which stimulates the translocation of GLUT-4 to translocate the glucose

into the cells (Cartee 2015). Additionally, AMPK phosphorylates and activates of MEF2 and GEF; which are transcription factors in the GLUT4 expression in skeletal muscle (McGee et al. 2006).

Furthermore, it is reported that caffeine-induced calcium release from sarcoplasmic reticulum was enough to uptake the glucose without any membrane depolarization (i.e. muscle contraction) (Holloszy and Narahara 1967a). It is suggested that caffeine stimulates calcium ion pumps from sarcoplasmic reticulum which increases cellular energy demand and ATP consumption (Holloszy and Narahara 1967b); and as it is explained above, this increases AMP:ATP ratio and activates AMPK and finally results in translocating GLUT-4 from intracellular vesicles to the plasma membrane (Witczak et al. 2007). Thus, Ca^{2+} indirectly increases glucose uptake. In addition to the indirect action of Ca^{2+}, it is reported that calcium kinase-dependent protein/calmodulin (CaMKKs) has the role in phosphorylation and activation of AMPK and then increase glucose uptake directly (Hawley et al. 2005); independent from cellular energy consumption.

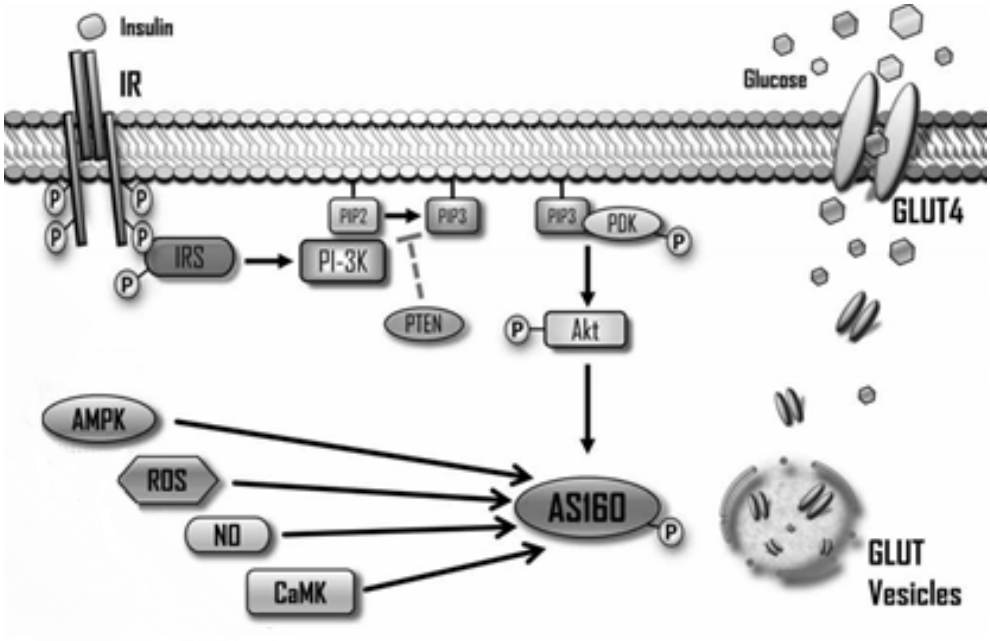


Figure 1-14 Molecular mechanisms of skeletal muscle glucose uptake (Mul et al. 2015).

1.6.2 Androgens and skeletal muscle glucose uptake

Diabetes is a progressive condition characterized by insulin resistance and eventual inability to compensate with increased insulin secretion, resulting in hyperglycemia (Alberti and Zimmet 1998). It is demonstrated that sustained improvements in glycaemia and metabolic parameters could be achieved in individuals with diabetes who lost approximately 10% of their body weight after diagnosis (Feldstein et al. 2008). In general, the prevalence of obesity (a major risk factor for the development of diabetes and related death) has dramatically increased worldwide over

the last 30 years and the number of people living with diabetes worldwide is projected to double by 2030 (Wild et al. 2004).

Endogenous testosterone levels are inversely associated with diabetes (Corona et al. 2011) and low testosterone levels in men with androgen deficiency or due to androgen deprivation therapy for prostate cancer is accompanied by unfavorable body composition changes, increased insulin resistance and elevated risk of diabetes (Groth et al. 2013; Taylor, Canfield, and Du 2009). Testosterone levels correlate positively with functional and genetic markers of mitochondrial function (e.g. PGC-1α or citrate synthase) associated with increased insulin sensitivity in skeletal muscle from men with and without diabetes (Pitteloud, Mootha, et al. 2005a). Hence, testosterone provision was associated with decreased total fat mass in young hypogonadal men, older men with low/normal testosterone levels, and men with diabetes (Isidori et al. 2005). However, testosterone injection for 30-wks did not improve insulin resistance and muscle mass in type 2 diabetes (Hackett et al. 2014). Conversely, transdermal testosterone gel for 6 months improved insulin resistance, without changing the muscle mass and fat mass (Jones et al. 2011). Further, 30-wks testosterone injection improved insulin sensitivity with considerable weight loss (Kalinchenko et al. 2010) and muscle mass gains (Aversa et al. 2010). Pitteloud et al. reported that men with hypogonadal testosterone levels were twice as insulin resistant as their eugonadal counterparts, confirming a positive correlation between testosterone levels and insulin sensitivity across full spectrum of glucose

tolerance (Pitteloud, Mootha et al. 2005); indicating this is an effective strategy in men with persistent insulin resistant state and hypogonadism to combat attenuated muscle mass and insulin sensitivity, and increase in total fat mass (TFM) (Dhindsa, Ghanim et al. 2016). However, the role of testosterone in prevalence of diabetes is ill-defined; i.e. only <19% of men suffered from type 2 diabetes had total testosterone <231 ng.dl-1 (Grossmann 2014). Nevertheless, provision of exogenous testosterone (200 mg injection every 2-wks for 3-monts (Kapoor et al. 2006) or oral 160 mg/day for 6-months) (Emmelot-Vonk et al. 2008) has been shown to reduce insulin resistance and improve glycaemic control in hypogonadal men with type 2 diabetes and healthy older men, respectively. Controversially, it is reported testosterone therapy for 40-wks (1000 mg injection, every ~2 month) did not improve insulin resistance and glycaemic control in hypogonadal men with type 2 diabetes (Gianatti, Dupuis et al. 2014). Therefore, given the positive association between glycaemic control and testosterone levels (Haffner, Karhapää et al. 1994) and men with diabetes have lower testosterone levels (Barrett-Connor 1992), it is reported that testosterone levels might not have correlation with insulin sensitivity (Birkeland, Hanssen et al. 1993, Abate, Haffner et al. 2002). However, moderately short-term (i.e. 12-wks) (Smith, Lee, and Nathan 2006a) and long term (i.e. 24-months) (Haidar et al. 2007) androgen-deprivation therapy in men decreased Insulin sensitivity and increased TFM which were associated with an increased risk for diabetes (Kintzel et al. 2008). Thus, besides administering long-term exogenous testosterone, interventions that boost endogenous sex hormones i.e.

exercise, might be of greater benefit (Hawkins, Foster-Schubert et al. 2008). Therefore, the interventions that increase testosterone levels and muscle mass-i.e. RET- may also affect muscle glycaemic control.

1.6.3 Exercise and skeletal muscle glucose uptake

Hyperglycemia is associated with various metabolic syndromes and diseases. Further, diabetes is a well-established risk factor for sarcopenia (Wong et al. 2013; Kalyani, Corriere, and Ferrucci 2014), and increase the rate of muscle mass loss over ageing (Park et al. 2007). However, related mechanism is still ill-defined; nevertheless, it seems given that insulin is a strong stimulator of MPS and muscle growth, abnormalities in insulin action is likely associated with muscle mass decline (O'Neill et al. 2016). It is suggested that hyperglycemia increases Krüppel-like factor 15 (KLF15) levels via downregulation of the E3 ubiquitin protein ligase 1 (WWP1); which result in increase in MPB and muscle atrophy (Hirata et al. 2019). Therefore, the strategies stimulate MPS and muscle growth might be considerable as an effective strategy to combat muscle metabolic disorders such as diabetes.

It is reported that exercise training increases the effects of insulin action on muscle cells which results in higher glucose uptake and internalization through muscle cells, which is critical in diabetes (Lawrence 1926). On the other hand, insulin injection prior to exercise session decreases glucose levels in the blood during the exercise training (Hagenfeldt and Wahren 1971). However, type, during and intensity of the exercise are

important in the magnitude of the effects of it on glucose metabolism (Coyle et al. 1983). In addition, transportation of the glucose is increased by insulin or muscle membrane transporter (i.e. GLUT-4) (Host et al. 1998). The effects of exercise on GLUT-4 may remain for up to 48 h post-exercise which showing the need of chronic adaptation to obtain continuous benefits. In this regard, it is reported that exercise training increases the expression of GLUT-4 which leads to promoting glucose uptake in skeletal muscle (Christ-Roberts et al. 2004).

In addition to muscle hypertrophy, RET is emerging as an exercise modality associated with improvements in age-related muscle function decline, oxidative capacity and mitochondrial function and insulin sensitivity (Hall et al. 2013; Kim et al. 2015). RET acutely increases insulin sensitivity and increases insulin-mediated suppression of endogenous glucose production (Kim et al. 2015). RET promotes muscle mass and anabolism (even in older men (Ribeiro et al. 2017; Phillips et al. 2017)) and stimulates muscle glycaemic control (Lee, Kim, and Kim 2017); these effects are also associated with enhanced endogenous testosterone in response to acute bouts of RE (Rønnestad, Nygaard, and Raastad 2011). Nonetheless, hypertrophic and glycaemic responses to RET are blunted in older age (vs. younger cohorts), so called “anabolic resistance” (Zachwieja et al. 1996a), suggesting the need for strategies beyond, or adjuvant to RET (Rønnestad, Nygaard, and Raastad 2011), in order to maximize efficacy on muscle mass and glycaemic control. The effects of RET on insulin sensitivity appear to occur preferentially in muscle over the

liver, and may be mediated by increases in key enzymes and proteins in glucose metabolism including hexokinase, GLUT4, Akt, insulin receptor, PKB, and glycogen synthase (Croymans et al. 2013). RET increases IGF-1, which binds its receptor and activates tyrosine kinase, stimulating/phosphorylating insulin receptor substrate. Phosphorylated IRS recruits and activates hypertrophic pathways; e.g. PI3K and mTOR pathways (Vega et al. 2010). Nevertheless, despite positive effects of RET on muscle mass and subsequent glycaemic control, 2-weeks withdrawal of testosterone led to significant decrements in insulin sensitivity without changing FFM and TFM, suggesting testosterone may exert direct effects on metabolic regulators or signalling, rather than by effecting changes in body composition (Yialamas et al. 2007). In contrast, it is reported testosterone deprivation for 4-wks did not change glycaemic control in younger men (Rabiee et al. 2010). Thus, glycaemic control improvement during RET cannot be ascribed to a mere increase in FFM and likely via factors independent of changes in FFM; perhaps via endogenous androgen hormones.

1.7 **Factors associated with muscle loss**

Given the two dimensions affecting muscle protein turnover (i.e. MPS and MPB), the effects of MPB will be explained in this section and the resulted medical conditions will be reviewed.

Pathological disorders (i.e. muscular dystrophies) (Emery 2002), chronic diseases (i.e. cachexia) (Evans et al. 2008), starvation (Pasiakos et al. 2010), andropause, and immobilization (Urso 2009) as well as ageing (i.e. sarcopenia) (Baumgartner et al. 1999) are associated with MPB which likely result in muscle loss (i.e. dystrophy). One of the main reason leads to most of the above health disorders is low levels of activity or severe inactivity (e.g. long-term bed rest) (Glass 2003). Almost all of the muscle disuse-induced muscle protein breakdown is caused by reduced neuromuscular activity which results in extensive loss of skeletal muscle mass (up to 30%) and function (Narici and De Boer 2011).

During muscle protein breakdown, removing the dysfunctional proteins is critical to generate free amino acids for protein synthesis and/or metabolism (Mitch and Goldberg 1996a). Thus, it is not surprising that muscle contractions during RE increase proteolysis which is required for muscle protein remodeling and adaptation (Zhang, Chinkes, and Wolfe 2002). The regulation and activity of MPB pathways in skeletal muscle in response to RE is not well-defined. However, it is suggested that three major proteolytic systems work independently or together to promote protein breakdown during protein turnover in skeletal muscle: the calcium-dependent calpain (Murphy 2010), the lysosomal protease/cathepsins (Bird et al. 1980) and the ubiquitin-proteasome systems (Fagan, Waxman, and Goldberg 1987); but the time course of activation and potential role of each in the adaptation responses to RE or during catabolism in human skeletal muscle is not clear. It is reported that RE increases MuRF1 mRNA

abundance and total protein in human skeletal muscle during the post-exercise recovery period (Glynn et al. 2010; Fry et al. 2012). Conversely, atrogin-1 expression can remain unchanged (Coffey, Shield, et al. 2006) or is reduced (Kostek et al. 2007) following acute RE. Indeed, changes in the transcriptional activity of atrogenes (genes related to muscle atrophy) are induced in response to heavy RE showing increase in muscle protein turnover. It is reported that atrogenes decrease at rest and following RE when branched chain amino acids are ingested (Borgenvik, Apró, and Blomstrand 2011), indicating a nutrient-mediated effect on atrogene expression that may modulate remodeling/ hypertrophy in skeletal muscle. Importantly, a clear association between changes in atrogene transcriptional activity/total protein and measures of proteasome activity or muscle protein breakdown in skeletal muscle has yet to be established. MPB increases up to 35% after RE and returns to resting levels 24 h post-exercise (Phillips et al. 1999). Interestingly, this response is only reported in untrained individuals, indicating RE-induced MPB is attenuated with RET (Phillips et al. 1999). However, further studies measuring the fractional breakdown rates of skeletal muscle following both RE and RET are required to clarify the extent of breakdown and its effects on muscle mass. However, as it was explained in previous sections, even if MPB increased following RE, normally, it does not exceed MPS (MPB<MPS) which results in muscle hypertrophy after RE sessions are repeated. But in some cases, MPB exceeds MPS which leads in muscle loss and atrophy; e.g. ageing or metabolic syndrome. In the following section, the

association of ageing and related metabolic syndrome with muscle mass and related therapy strategies will be reviewed.

1.7.1 Aging and skeletal muscle mass loss

The global population of older adults continues to grow at an exponential rate; i.e. there were 205 million over the age of 60 in 1950, and this population was estimated to be almost 810 million in 2010, and by 2050 it is estimated that over 2 billion people will be >60 years old (Desa 2014). During aging, humans undergo a physiological process (i.e. skeletal muscle wasting), which characterized by a reduction in muscle mass, due to a loss of net protein content in myofibres. Several factors seem to be involved, including a reduction in protein synthesis and an increase in intracellular proteolysis, due to activation of different pathways (Sandri 2013; Murton, Constantin, and Greenhaff 2008). Given that skeletal muscle is the major protein reservoir in the body, muscle wasting has beneficial effect on its initial stage; indeed, it provides the organism with an ample pool of free amino acids that can be reused for energy production or protein synthesis in vital organs (Mitch and Goldberg 1996b). However, in a variety of pathologies like cancer, sepsis and metabolic syndrome, this catabolic state is maintained over a long period of time and might be a life-threatening factor (Mitch and Goldberg 1996b). Metabolic disorders-induced sarcopenia is due to a decrease in both fiber size and fiber number. The primary muscle loss occurs in the fast-twitch

muscle fibers, with increased fibrosis and adipose tissue (Evans and Lexell 1995). It has been reported that muscle loss is about 1-2% per year after the age of 50 years; and 10% of older people<70 years old suffer from sarcopenia, whereas the prevalence increases to 23% in people above 80 years old; and by the age of 80, peak muscle mass is reduced by 40% (Deschenes 2004b).

The etiology and pathology of sarcopenia are multi-factorial. Aging is associated with multiple catabolic conditions, such as decreased activity, denervation, increased oxidative stress, chronic inflammation, age-related hormone changes, and satellite cells (Walston 2012). The preferential loss of fast-twitch muscle fibers is likely due to a decline in the number of neuromuscular junctions (Williams, Higgins, and Lewek 2002; Chai et al. 2011). In contrast to the conditions that mainly contribute to the catabolism of muscle with aging, the age-related reduction of growth hormone, IGF-1 or testosterone (T) (i.e. andropause) is primarily associated with attenuated protein synthesis (Narici and Maffulli 2010). Given the decrease in satellite cell numbers and impaired muscle regeneration with aging, satellite cells, the stem cells of skeletal muscle, likely play a role in the etiology of sarcopenia. For instance, the number of satellite cell in the fast-twitch fibers dramatically decreased by ~50% in vastus lateralis muscle after 75-year old (Verdijk et al. 2007). An etiological model of sarcopenia can be seen in Figure 1-15.

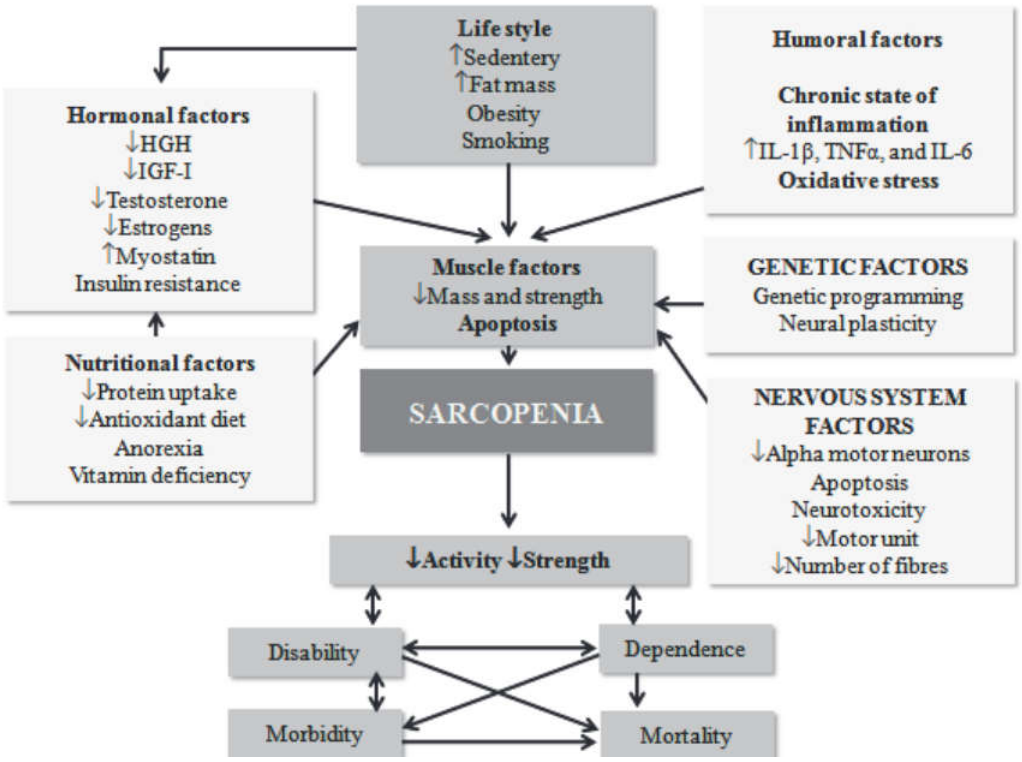


Figure 1-15 Scheme of the different etiological sarcopenia mechanism and their consequences (Zembroń-Łacny et al. 2014).

However, the balance between the rates of protein synthesis and breakdown is crucial in determining whether muscles grow (i.e. accumulate protein) or undergo atrophy (i.e. sarcopenia). Both chemical signals (e.g. hormones, growth factors and branched-chain amino acids) and mechanical signals (e.g. exercise training) can influence protein turnover at all stages of life (Goldspink 1991). Age-related lowering of myosin heavy chain (MHC) type IIa and IIx has been reported (Balagopal et al. ay2001); in addition to associated reduced insulin-like growth factor-I (IGF-1) signaling and elevated amounts of circulating cytokines which result in cell oxidative stress, and loss of spinal motor neurons due to apoptosis, which is paralleled by a reduction in muscle fiber number and

size that impairs performance and reduces functional capacity of daily activities and eventually increase the rate of mortality (Aagaard et al. 2010). Thus, as the ageing populace grows, there remains a need for therapies to counteract the associated muscle wasting and dysfunction.

1.7.2 Ageing and muscle loading

Despite of considerable investment in pharmacological treatment/interventions, resistance exercise (RE) is likely the most effective and also safe strategy to combat ageing-induced muscle loss in older population, especially with appropriate supportive nutrition (Yarasheski, Zachwieja, and Bier 1993; Kumar et al. 2012). Nevertheless, while RE *per se* is supposed to be a potent anabolic stimulus, ageing is associated with attenuated MPS responses to RE (i.e. anabolic resistance) across a wide range of intensities (30-90 % 1-RM) (Fry et al. 2011). These blunted responses in older individuals are accompanied by decreases in mTORC1 activation and other related downstream signaling proteins (i.e. P70S6K1 and 4EBP1) acutely after each RE (Kumar et al. 2009; Fry et al. 2011). Conversely, the mentioned blunted anabolic signaling have not been confirmed by all studies (Drummond et al. 2008; Mayhew et al. 2009). However, it has shown constantly that anabolic resistance in older individuals needs greater amount of exercises to stimulate a maximal anabolic responses, even with appropriate amino acid feeding (Moore et al. 2014). It is suggested that despite blunted responses to exercise stimuli with ageing, it may be possible to overcome

some of this blunting by introducing low-load high-volume exercises (Kumar et al. 2012).

However, It is reported that repeated RE (i.e. RET) may lead to an augmented protein turnover (MPS>MPB), which results in muscle hypertrophy (Wilkinson et al. 2013). Nevertheless, some studies have reported that whole-body progressive RET augments muscle mass (Lemmer et al. 2000; Phillips et al. 2011) and CSA (Welle, Totterman, and Thornton 1996) in younger rather than older individuals. Conversely, others reported no differences between younger and older adults (Martel et al. 2006; Mayhew et al. 2009). These discrepancies are likely resulted from different RET programs and/or nutritional supports (Brook, Wilkinson, Phillips, et al. 2016). However, RET surely improves muscle strength/function in older men (Peterson, Sen, and Gordon 2011). The mechanisms of blunted responses of MPS to RE and hypertrophic adaptations to RET in older men are poorly defined. However, in terms of potential role, it is suggested that MRFs role in satellite cell proliferation and differentiation have altered the regulation of muscle growth during RET in older men; which possibly is associated with anabolic resistance in older individuals (Snijders et al. 2014; Petrella et al. 2006). Further, there have been blunted myogenic responses, showing impaired satellite cells activation responses to RE in older age (Snijders et al. 2014) (Figure 1-16). Given that anabolic resistance may be mitigated via muscle loading and feeding in older men (Brook, Wilkinson, Phillips, et al. 2016), it is perhaps more beneficial if other anabolic supplementation (e.g. androgen

provision) were administrated adjuvant to RET. Thus, next section is to explain details of effects of androgens (i.e. testosterone) on anabolic resistance during RET in older men.

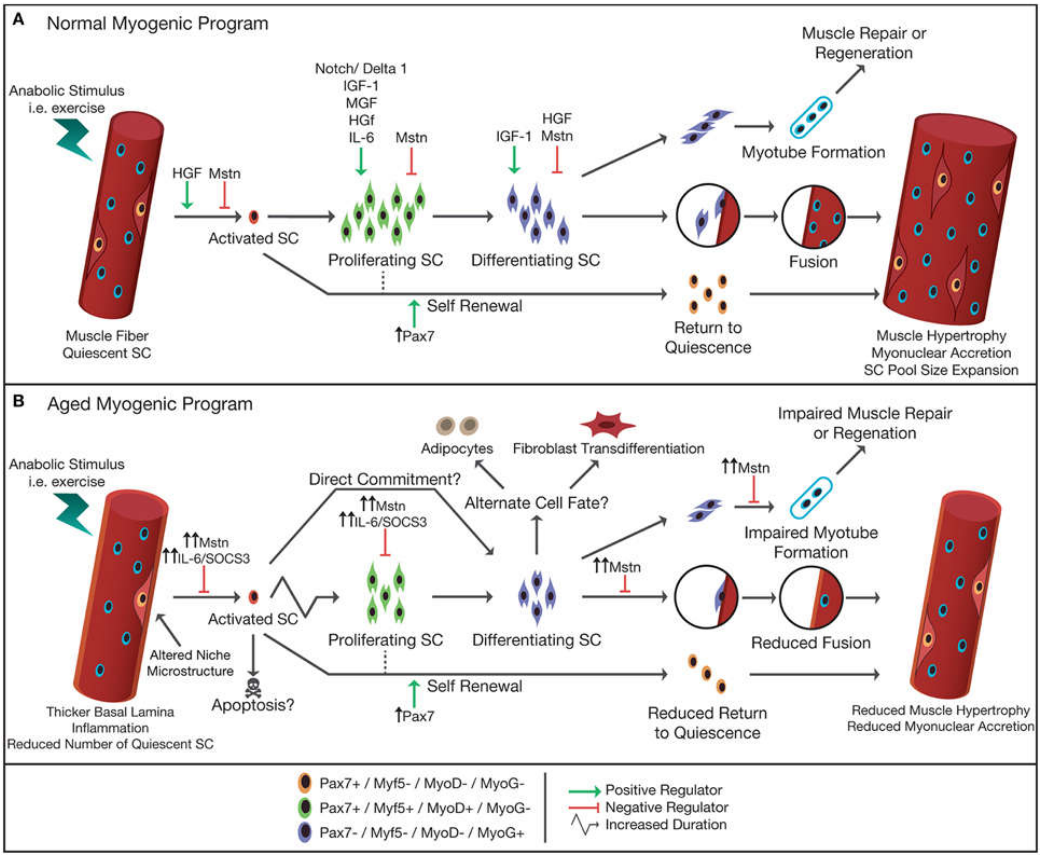


Figure 1-16 normal (A) and aged (B) myogenic program in response to an anabolic stimulus (Snijders et al. 2015)

1.7.3 Aging, muscle loss and androgens

Muscle loss and weakness are important components of frailty in older age, contributing to functional limitation in daily activities and related problems such as an increased risk for falls (Rubenstein, Josephson, and Robbins 1994; Guralnik et al. 1995). Anabolic hormones decline with age and may be one of the causes of loss of muscle mass (sarcopenia) out

of many (Sipilä et al. 2013). Fractional synthetic rate of mixed muscle protein, myofibrillar protein (actin and myosin) (Balagopal et al. 1997), and mitochondrial proteins also decrease with age (Thomas 2007). This loss of muscle mass/quality (sarcopenia) is not only one of the most serious problems associated with ageing, but also it is becoming one of the major health care problems with the change in the age profile of society (Goldspink and Harridge 2004). Regardless of the exact mechanism, muscle atrophy occurs when protein breakdown exceeds synthesis. This decrease in basal muscle synthetic rate is associated with a reduction in mRNA responsible for gene expression of myofibrillar protein (Thomas 2007). This decrease in muscle mass and cross-sectional area associated with age appears to be due to a decrease in size of type II muscle fibres compared to Type I muscle fibres, and also loss of muscle fibre number (Lexell et al. 1988). Accordingly, it is shown that age-associated loss of motoneurons may be an important contributing factor to reduce muscle fibre number and muscle mass (Thomas 2007).

Indeed, testosterone administration in myotubes of rats significantly increased Akt and mTOR phosphorylation, while decreased levels of phosphorylated Akt accompanied by an upregulation of MuRF-1 and MAFbx were observed after orchidectomy in rats (Jones et al. 2010). Expectedly, both consequences were reversed during testosterone therapy (Jones et al. 2010). Activation of Akt by testosterone is mediated by a direct interaction of the AR with the p85 which is regulatory subunit of PI3K, leading to its activation and following upregulation of Akt

phosphorylation (Dubois et al. 2012). Generally, it is shown that muscle hypertrophy under influence of testosterone in mice is achieved by inhibiting c-Jun NH2-terminal kinase (JNK) and activating p38 mitogen-activated protein kinase (MAPK) (Brown et al. 2009). In addition, a study exploring testosterone effects on aged muscle showed that testosterone administration was able to reverse the age-related increase in p21 and limit satellite cell regenerative capacity (Dubois et al. 2012). Furthermore, it is demonstrated that while protein breakdown remained unchanged, testosterone administration increases net protein synthesis (Urban et al. 1995), by which stimulates skeletal muscle for growth (Ferrando et al. 2002c; Sheffield-Moore 2000) and may be an efficient re-utilization of intracellular amino acids (derived from protein breakdown) for protein synthesis (Ferrando et al. 2002c; Sheffield-Moore 2000). However, ample amino acid precursors are always ready to support the initial rate of protein synthesis even if breakdown decreases. Not only the appearance of amino acids from protein breakdown is reduced, but those that are derived from protein breakdown are also efficiently utilized to maintain protein synthesis (Ferrando et al. 1998b). Thus, testosterone administration may ameliorate the loss of nitrogen in skeletal muscle in older population through preventing the loss of intracellular amino acids. Further, testosterone administration leads to some noteworthy effects on AR and IGF-I expression in skeletal muscles, as well. Sheffield-Moore (2000) reported that IGF-I mRNA increased in skeletal muscle of older men treated with 4-week of testosterone therapy. On the other hand, marked decline in IGF-I mRNA concentrations occur following induction

of major hormone deficiency in normal young men (Mauras et al. 1998). Moreover, given that ribosomal RNA accounts for approximately 80-85% of the total RNA pool, changes in the total RNA concentration are generally regarded to reflect a change in the translational capacity and protein synthesis (Van der Meer, Jaspers, and Degens 2011). It is reported increasing RNA content concomitant with upregulation in gene expression-related to myogenesis and hypertrophy may result in muscle hypertrophy (Wilborn et al. 2009). Further, increases in total RNA content per “cell unit” (RNA:DNA ratio), an indicator of ribosomal abundance (Kemp et al. 2013), is critical for anabolic potential and further hypertrophy (Wilborn et al. 2009). Total RNA:ASP ratio, as an index of synthetic capacity (Brook, Wilkinson, Mitchell, et al. 2016), it was already reported that testosterone administration could increase RNA:DNA ratio and RNA content in young castrated rats (Saunders, Steciw, and Kline 1962) although, conversely, it is reported that administrating testosterone (15d; 9 mg/d) could not change MPS and RNA-related ratios (Lobley et al. 1990).

In addition, testosterone is an important determinant of regional fat distribution and metabolism in men. Accordingly, testosterone levels correlate negatively with visceral fat and positively with plasma high-density lipoprotein (HDL) concentrations (Bhasin, Woodhouse, and Storer 2001). Moreover, testosterone administration in middle-aged men with visceral obesity, improves insulin sensitivity and decreases blood glucose and blood pressure (Bhasin, Woodhouse, and Storer 2001). Thus,

testosterone administration not only positively affects body fat tissue and insulin sensitivity, but it also increases muscle mass (Ferrando et al. 2002a), and strength (Urban et al. 1995); especially adjuvant to RET (Bhasin, Woodhouse, and Storer 2001); e.g. 12-week testosterone provision augmented isometric strength, maximal voluntary contraction, and isokinetic strength (peak torque) (Caminiti et al. 2009).

However, RET alone for 10 (Kraemer et al. 1999), 12 (Bellew 2002) or even 24 (Hakkinen et al. 2000) weeks did not increase chronic total testosterone level or hypertrophic adaptation. Nevertheless, it is reported that 12 months RET as a long term intervention could increase muscle mass without altering testosterone level and metabolism in older men (Ahtiainen et al. 2015). They concluded that changes in endogenous testosterone level did not seem to have an effect on RET-induced adaptations of muscular characteristics (Ahtiainen et al. 2015). However, suppression of endogenous testosterone production blunted the adaptation to 8-wk RET in young men (Kvorning et al. 2006b). It is shown that testosterone could influence neurotransmitter synthesis (Hakkinen et al. 2000) and reduce fluctuations during MVCs (Tracy, Byrnes, and Enoka 2004) due to summation of motor unit forces and/or the timing of the action potential discharged by the motor neurons (Tracy, Byrnes, and Enoka 2004), accompanied by recruitment of the larger and faster motor units (fast twitch fibres) (Bellew 2002). More importantly, the adaptations in these mechanisms are independent of those responsible for dynamic strength gains (Tracy, Byrnes, and Enoka 2004). Nonetheless, Bellew

(Bellew 2002) reported an increase in MVC in older males without neither receiving testosterone nor muscle mass gain, but after 12-wk RET (Caserotti et al. 2008). It was demonstrated that decreased muscle mass was associated with castration-induced androgen withdrawal and subsequent suppressed myofibrillar protein synthesis, and down-regulation of Akt/mTORC1 signalling pathway (White et al. 2013b). In addition, it has been consistently reported that testosterone administration improves MPS (Brodsky, Balagopal, and Nair 1996; Urban et al. 1995a), but thorough ill-defined mechanism.

Similar to young men (Bhasin et al. 2005), exposure of older men to different testosterone doses varying from hypogonadal to supra-physiological serum levels resulted in dose-dependent increases of muscle mass and strength (Bhasin et al. 2005; Sattler et al. 2009). It is reported, long term testosterone administration (i.e. > 3 months) in older men with low or normal testosterone levels increased muscle mass (Ferrando et al. 2002b; Basaria et al. 2010) (Figure 1-17).

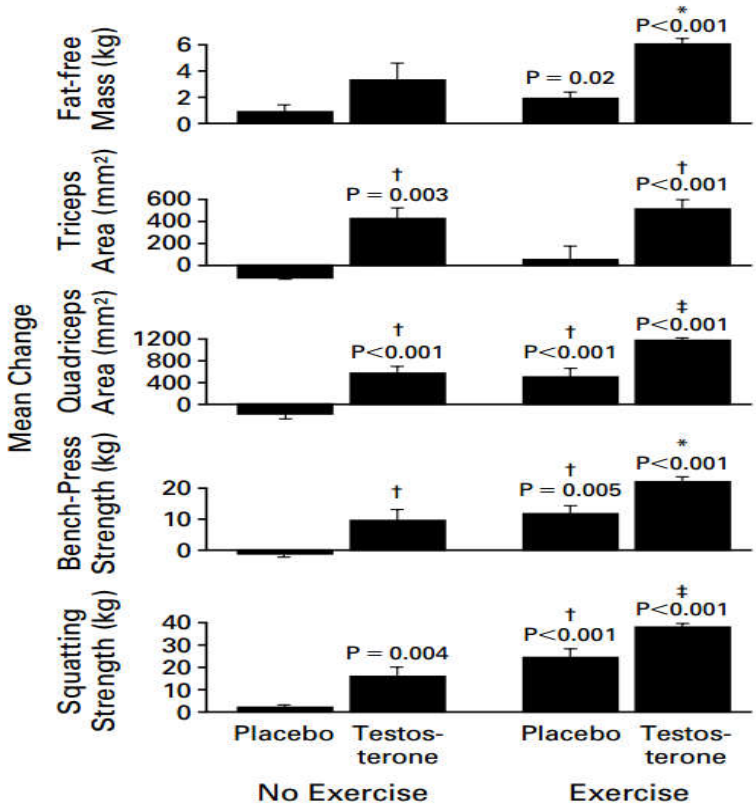


Figure 1-17 Changes in muscular function and architecture over 10-wks of T administration. The asterisks indicate P<0.05 for the comparison between the change indicated and that in either no-exercise group; the daggers, P<0.05 for the comparison between the change indicated and that in the group assigned to placebo with no exercise; and the double daggers, P<0.05 for the comparison between the change indicated and the changes in all three other groups (Bhasin et al. 1996).

This section indicated that an age-associated muscle loss might play a contributory role in the frailty of older men, which is multifactorially determined, and that the older men can respond to testosterone provision, in particular if the testosterone level was initially low. However, whether testosterone therapy plays a role in the management of sarcopenia and frailty in older men is not clear, as several issues need to be clarified, such as optimal treatment modalities, safety in frail elderly, and related cellular mechanisms.

Therefore, there is a significant clinical need to identify contributing factors to this muscle loss so that they can be specifically targeted for intervention (i.e., pharmacological hormonal therapies). However, the mechanisms by which testosterone regulates muscle metabolism and glycaemic control are ill-defined and the results about these concepts are contentious.

1.8 **Aims of the study**

As discussed in the introduction section, aging is a complex phenomenon that affects muscle mass at a different rate. In accordance, with advancing age, the skeletal muscle undergoes a progressive loss of mass and strength, a process known as sarcopenia that eventually leads to a decreased mobility and increased risk of falls and metabolic issues (e.g. diabetes) and invalidity and death; which all are in line with decrease in testosterone levels. In addition, the molecular mechanisms involved in these peculiar aging patterns and the effects of endogenous testosterone are still largely unknown. Thus:

1) Firstly, we aim to investigate the impact of systemic testosterone levels on control of muscle mass in healthy young men undertaking a RET program. We hypothesize that reduction of testosterone levels in systemically normal young men will impair muscle protein programming and muscle mass gains in response to RET.

2) Secondly, we aim to investigate the impact of enhancing testosterone levels in older men on responsiveness to RET and the contribution of systemic testosterone levels to muscle mass regulation in ageing. We hypothesize that increasing testosterone levels in older males will improve responsiveness to anabolic stimuli (RET).

3) Further, we aim to investigate the impact of testosterone suppression on muscle glycaemic control during RET in younger men. We hypothesize that testosterone suppression will attenuate glycaemic control in response to RET in young men.

4) Finally, we aim to investigate the impact of testosterone provision on muscle glycaemic control during RET in older men. We hypothesize that testosterone therapy will improve glycaemic control in response to RET in older men.

Chapter 2

General Methods

2.1 **Study ethics and participants**

This study was approved by The University of Nottingham Faculty of Medicine and Health Sciences Research Ethics Committee (G11082015 SoMS MSGEM), was conducted according to the declaration of Helsinki, and was pre-registered at clinicaltrials.gov (NCT02152839). Before entry into the study, participants provided written informed consent to participate after all procedures and risks were explained to them. All participants performed activities of daily living and were recreationally active, but had not partaken in RET within the previous 12 months. Participants were screened by medical questionnaire, physical examination, routine blood chemistry and a resting electrocardiogram. Participants who presented with metabolic, respiratory or cardiovascular disorders or who were prescribed medication (e.g. beta-adrenergic blocking agents, statins, anti-inflammatory drugs) and any other medications which could influence testosterone metabolism were excluded. Based on the primary endpoint of this study, muscle protein synthesis, a power calculation suggested that we need minimum 8 participants per group to detect with $\alpha=0.05$ and $\beta=0.85$ a difference between and within groups of 25%. Therefore, of the pre-screened volunteers, eighteen older and sixteen younger non-hypogonadal healthy normotensive (<140/90) volunteers males with morning normal serum testosterone concentrations were assigned in a random double-blinded fashion to receive bi-weekly either: placebo (P, saline n=9) or testosterone (Sustanon 250-mg, n=9) injections in older groups and one

off either: placebo (n=8) or GnRH analogous: Goserelin, so called Zoladex (Z) (3.6-mg, n=8) injections in younger groups over 6-wks of whole-body supervised RET. All participants involved in the study were monitored throughout the study for any negative side effects of testosterone injections. No adverse events were reported during or after completion of the study.

2.2 **Study conduct**

Following baseline measurements of maximal voluntary contraction (MVC) and 1-repetition maximum (1-RM; on separate days); regardless of group assignment, all participants were further characterised at baseline. This involved collection of fasting blood, oral glucose tolerance test (OGTT), muscle ultrasound (Mylab 70; Esaote Biomedica, Italy) of the *m. vastus lateralis* muscle (VL) and a dual-energy X-ray absorptiometry (DXA; Lunar Prodigy II, GE Medical Systems, Little Chalfont, UK) scan. Finally, a unilateral muscle biopsy was taken under rested conditions from the VL. Given the moderately short half-life of testosterone after each injection (i.e. 4-5 days (Nieschlag and Behre 2004)) in the testosterone group, 1-wk, 3-wks, 6-wks biopsies were taken 7, 14, and 7 days after corresponding bi-weekly testosterone injection, respectively. In order to assess rates of MPS, a basal saliva sample was collected before the muscle biopsy, and the first dose of D_2O as a bolus of 3 $ml \cdot kg^{-1}$ body weight was consumed by participants after the biopsy. The initial priming dose of D_2O was followed by daily small-volume “top-

ups" of ~20 mls (calculated from measures of each individual's body water pool turnover). Finally, injection of testosterone or placebo in older groups and zoladex or placebo in younger groups was administered by an unblinded research technician. The fully supervised RET protocol then commenced and continued for the next 6-wks. Additional VL biopsies (~60 min after bouts of RE to obtain temporal acute effects of RE across training) and other tests and samples took place intermittently during these 6-wks (see Figure 2-1). All muscle samples were collected under sterile conditions, using the conchotome biopsy technique (Dietrichson et al. 1987) with 1% $w \cdot v^{-1}$ lidocaine as local anaesthetic. Any fat tissue and connective tissue was rapidly dissected out and muscle was washed in ice-cold phosphate-buffered saline (PBS) and frozen in liquid nitrogen or liquid-nitrogen cooled isopentane, before storage at -80°C. Participants were provided 10 mg (D_3-methyl)-3-Methylhistidine (3MH) in 100ml water on three occasions during the study and returned to the research unit (fasted) after 21 h for blood sampling every 2 h (i.e. at 21, 23, 25 h after 3MH ingestion). A detailed schematic of the study protocol is depicted in Figure 2-1.

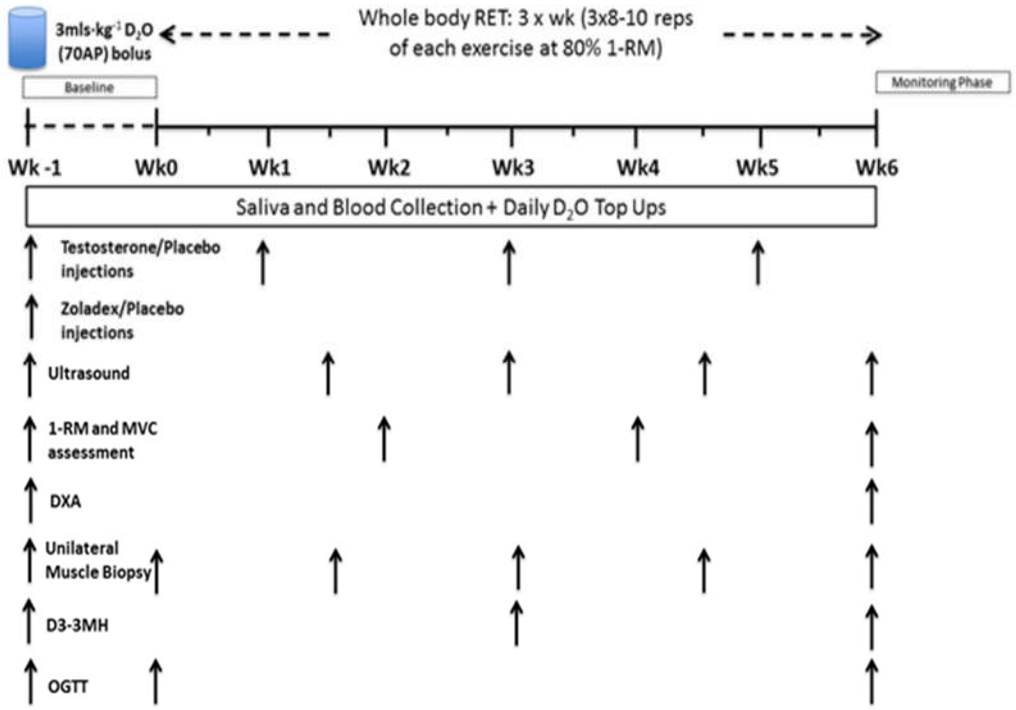


Figure 2-1 Study protocol

2.3 **Physiological and functional measurements**

2.3.1 Resistance training procedures and strength assessments

Participants in both groups performed the same whole-body RET including leg-extension, leg-press, leg-curl, lat-pull-down, shoulder-press and bench-press (all 3-sets of 8-10 reps at 80% 1-RM)) (Sundell 2011) 3-times·wk^{-1} non-consecutively for 6-wks. Individuals' 1-RM (Dohoney et al. 2002) was re-assessed every 2-wks before the corresponding training session to maintain intensity with progression. An isokinetic dynamometer (Isocom; Isokinetic Technologies, Eurokinetics, UK) was

used to assess isometric knee extensor torque during MVC using two knee joint angles (70 and 80°), with full extension corresponding to 0°. Each individual muscle contraction lasted 4 s, with 30 s rest between contractions, and 90 s between knee joint angle assessments. In addition, specific strength (MVC70°·Legs LM^{-1}) was calculated as MVC at 70° divided by legs fat free mass (LM).

2.3.2 Muscle architecture by ultrasound and DXA-derived muscle mass

Every 10 days and immediately before corresponding training session, B-mode ultrasonography (Mylab 70, Esaote Biomedica) with a 100 mm, 10–15 MHz, linear array probe was used for quantification of myo-architecture. Images were obtained at 50% of the VL length and the mid-sagittal line while the participant was lying supine on a couch (Quinlan et al. 2017). To assess fascicle length (Lf), the transducer was aligned with the fascicles to facilitate optimal image capture of the fascicles. The intersection between fascicles and the deep tendon aponeurosis and the perpendicular distance between the superficial and the deep tendon aponeurosis were used to assess pennation angle (PA) and muscle thickness (MT) (Franchi et al. 2015). Finally, extended-field-of-view ultrasonography (EFOV) was used to quantify the cross-sectional area (CSA) of the quadriceps (Noorkoiv, Nosaka, and Blazevich 2010). ImageJ software (ImageJ 1.51h) was used to analyse the images.

Before and after the study, DXA (64752, GE Medical Systems-Lunar Prodigy, US) was used to determine total FFM, legs LM, total fat percentage (TFP), total fat mass (TFM), FFM index (FFM divided by height squared (FFM·height^{-2}), and appendicular FFM (fat free mass of arms and legs in kilograms divided by square of height in metres). Participants were asked to attend overnight fasted having not performed any heavy physical activity 24 h prior to scanning. For the DXA scan, participants wore loose comfortable clothing with no metal or plastic zippers, buttons or snaps. Prior to use on participants, a QA block phantom was used to calibrate the system, to ensure optimal measurement. In addition, spine phantoms were run bi-monthly to assess the reproducibility and accuracy of the system over time.

2.4 Metabolic and biochemical measurements

2.4.1 Oral Glucose Tolerance Test (OGTT)

All participants completed a 2 h OGTT using 75 g unflavoured dextrose dissolved in water 3 times (OGTT1: before injection; OGTT2: ~5 days after first injection; OGTT3: after 6-wks RET) during the study. Venous blood samples were obtained at before and every 15 min (0, 15, 30, 60, 75, 90, 105, and 120 min) after dextrose ingestion to be assayed for glucose and insulin levels. Blood glucose was analysed using glucose analyser machine (YSI 2300 STAT PLUS, UK) and plasma insulin and C-peptide concentrations were measured using high-sensitivity insulin

enzyme linked immunosorbent assays (Insulin: DRG Instruments GmbH, Marburg, Germany; C-peptide: EZHCP-20K, Millipore, US) according to the manufacturer's protocol.

2.4.1.1 Calculation of insulin resistance/sensitivity

$$\mathrm{HOMA-IR} = \frac{G_0 \times I_0}{22.5}$$ (Matthews et al. 1985)

$$\mathrm{Matsuda} = \frac{1000}{\sqrt{G_0 \times I_0 \times G_{mean} \times I_{mean}}}$$ (Matsuda and DeFronzo 1999)

$$\mathrm{HOMA-}\beta = \frac{20 \times C-\mathrm{Peptide}_0}{G_0 - 3.5}$$ (Wallace, Levy, and Matthews 2004)

$$\mathrm{GIR-Index} = \frac{20}{C-\mathrm{Peptide}_0 \times G_0}$$ (Ohkura et al. 2013)

$$\mathrm{Cederholm} = \frac{75000 + (G_0 - G_{120}) \times 1.15 \times 180 \times 0.19 \times \mathrm{Weight}}{120 \times \log(I_{mean}) \times G_{mean}}$$

(Cederholm and Wibell 1990)

Where. G_0 is fasting glucose level, I_0 is fasting insulin level, G_{mean} or I_{mean} are mean of glucose or insulin levels during the OGTT, C-peptide$_0$ is fasting C-peptide level, HOMA-IR is Homeostatic model assessment-insulin resistant, HOMA-β is Homeostatic model assessment-β cell function, Matsuda and Cederholm are indices of insulin sensitivity, GIR-

index is glucose infusion rate-index. In HOMA-IR, insulin levels is used to determine the resistance to insulin action and in HOMA-β C-peptide is used to determine pancreatic insulin secretion (β-cell function) (Matsuda and DeFronzo 1999).

2.4.2 Testosterone ELISA assay

Venous blood samples were collected into EDTA-coated tubes intermittently during the study i.e. before injections and prior to individual RE sessions in the mornings to measure fluctuations in total testosterone concentrations. Blood samples were immediately cold centrifuged at 1750 *g*, with resulting plasma fractions aliquoted and frozen at –80°C until further analysis. An enzyme-linked immunosorbent assay (ELISA; ab108666, Abcam, United Kingdom) competitive technique was used to assess the abundance of total testosterone in the plasma of all participants The intra-assay coefficient of variation was <5%, and the assay sensitivity was 70 $ng \cdot dl^{-1}$; the detection range was 20-1600 $ng \cdot dl^{-1}$.

2.4.3 Muscle immunohistochemistry

Serial 5 μm thick VL muscle cross-sections were cut at -20ºC using a cryostat (Leica, CM 1850, Germany), mounted on glass slides and air-dried at room temperature. Determination of fibre type-specific cross-sectional area was performed using monoclonal antibodies against slow (BA-F8) and fast myosin (SC-71) and laminin (D18) (Developmental

Studies Hybridoma Bank). Visualization of the primary antibodies was achieved by incubation with Alexa Fluor 488 and 568 goat-anti-mouse secondary antibodies (Invitrogen A/S) in situ. Muscle sections were then mounted with Molecular Probes Prolong Gold antifade reagent (Invitrogen A/S). Three major fibre types (I, IIA, IIX) were determined as previously described (Kadi et al. 1998). Fibre area was measured using Sigma Scan Pro 5 software.

2.4.4 Body water and protein-bound alanine myofibrillar protein synthesis (MPS)

To determine the exact volume of D_2O to be consumed for daily "top-ups", each participant provided saliva for the first three days after initial D_2O consumption. These were processed to determine each participant's body water decay rate, and from this, the amount of D_2O needed to maintain a steady-state over the study period could be calculated. Individuals were then provided with stocks of daily D_2O "top-ups" (~10% initial bolus dose) with thrice weekly saliva collection for rest of the study period. Wilkinson et al. (Wilkinson et al. 2014) previously described how body water and muscle protein enrichment were analysed. Briefly, 80-90 µl of saliva was heated in inverted 2 ml autosampler vials for 4 h at 90-100°C to purify fractions of the body water. The vials were then cooled on ice and before the condensed body water was transferred to a clean autosampler vial for injection. A high-temperature conversion elemental analyser (Thermo Finnigan, Thermo Scientific, Hemel Hempstead, UK)

connected to an isotope ratio mass spectrometer (Delta V advantage, Thermo Scientific) was employed to measure deuterium labelling in saliva (0.1 µl). To assess protein bound alanine muscle fraction enrichment, ~40 mg of muscle was homogenised in ice-cold homogenization buffer to isolate myofibrillar proteins (Brook, Wilkinson, Mitchell, et al. 2016). Briefly, 10 min rotary mixing was followed by centrifugation at 11,000 g for 15 min at 4°C, the supernatant (sarcoplasmic fraction) was then collected for immunoblotting and the pellet was re-suspended in 500 µl mitochondrial extraction buffer (MEB) and then homogenised by Dounce, and centrifuged at 1,000g for 5 min at 4° C. Insoluble collagen was separated following centrifugation from myofibrillar proteins that were solubilised in 750 µl NaOH and subsequently precipitated using 1 M perchloric acid (PCA) then pelleted by centrifugation. Following overnight hydrolysis at 110°C in a 0.1 M HCl and Dowex H^+ resin slurry, the amino acids were eluted with 2 M NH_4OH and dried-down. Dried samples were suspended in 60 µl distilled water, 32 µl methanol, and 10 µl of pyridine and 8 µl of methyl chloroformate with intermittent vortex. The n-methoxycarbonyl methyl esters of the amino acids were then extracted after adding 100 µl chloroform. A molecular sieve was added to remove water for ~20 s before being transferred to vials; incorporation of deuterium into the protein bound alanine was determined by gas chromatography-pyrolysis-isotope ratio mass spectrometry (Delta V Advantage, Thermo, Hemel Hempstead, UK) (Brook, Wilkinson, Mitchell, et al. 2016).

2.4.4.1 Calculation of synthetic fractional rate

Myofibrillar MPS was calculated from the deuterium enrichment (APE) in alanine in myofibrillar proteins, using the body water enrichment (APE, corrected for the mean number of deuterium moieties incorporated per alanine, 3.7, and the dilution from the total number of hydrogens in the derivative, i.e. 11) as the precursor labelling between subsequent biopsies. The fractional synthetic rate (FSR) was calculated as follows:

$$\mathrm{FSR}\left(\%\cdot\mathrm{day}^{-1}\right) = -\mathrm{In}\left(\frac{1-\left[\frac{\left(\mathrm{APE}_{\mathrm{Ala}}\right.}{\mathrm{APE}_{\mathrm{P}}}\right]}{\mathrm{t}}\right) \tag{2.1}$$

Where APE_{Ala} is deuterium enrichment of protein-bound alanine, APE_P is mean precursor enrichment of the body water over the period, and t is the time between biopsies. Absolute synthetic rate (ASR) was estimated as:

$$\mathrm{ASR}\left(\mathrm{g}\cdot\mathrm{day}^{-1}\right) = \frac{\mathrm{FSR}}{100} \times Total.FFM \times \frac{12.4}{100} \tag{2.2}$$

where alkali soluble protein of 12.4% total FFM was assumed (MacDonald et al. 2013). Absolute protein breakdown rate (ABR) was estimated as:

$$ABR\left(\mathrm{g}\cdot\mathrm{day}^{-1}\right) = \frac{\mathrm{FBR}}{100} \times Total.FFM \times \frac{12.4}{100} \tag{2.3}$$

Where fractional breakdown rate (FBR) is calculated as FBR=FSR−FGR, with the fractional growth rate (FGR) assumed to be % FFM gain per day over 7-wks derived by DXA. In addition, the net protein turnover was calculated as: ASR-ABR.

2.4.5 Muscle protein breakdown (MPB) measures using D_3-3MH

Precisely 100µl of plasma was aliquoted and deproteinised in 1 ml of ice-cold acetonitrile: methanol (1:1). Following ~1 h of cooling at -20°C, samples were centrifuged at 17,000 g for 20 min at 4°C. The resulting supernatant was dried and resuspended in 100 µl acetonitrile: ddH_2O (1:1). Following centrifugation at 17,000 g, samples were transferred to autosampler vials for the determination of D_3-3MH enrichment by Liquid Chromatography-Mass Spectrometry (LCMS: on a Q Exactive Orbitrap, Thermo Hemel Hempstead, UK). The enrichment decay curves were log transformed to determine the decay constant (k) which represents the fractional rate of myofibrillar muscle protein breakdown (MPB) (Sheffield-Moore et al. 2014).

2.4.6 Muscle RNA, DNA and protein content

Approximately 15 mg wet-weight muscle was used to determine alkaline soluble protein (ASP), RNA and DNA content. Initially, 0.2 M perchloric acid (PCA) was used to homogenize tissue, followed by centrifugation at

11,680 g. Pellets were re-solubilised in 0.3 M NaOH and protein contents quantified by spectrophotometry (NanoDrop Lite, Thermo Scientific). Thereafter, resulting supernatant was used for RNA quantification at 260 nm by spectrophotometry; the pellet was then heated at 70^{o}C for 1h in 2 M PCA to extract the DNA, centrifuged and DNA quantification at 268 nm by spectrophotometry (Brook, Wilkinson, Mitchell, et al. 2016).

2.4.7 Immunoblotting for anabolic/catabolic signalling

Spectrophotometry was used to determine protein concentrations of sarcoplasmic fractions and samples were diluted with 3x Laemmli loading buffer to 1 mg·ml^{-1}, followed by heating at 95°C for 5 min. Precisely 10 µg of samples were loaded onto Criterion XT Bis–Tris–12% SDS-PAGE gels (Bio-Rad) for electrophoresis at 185 V for 45 min. After electrophoresis, as previously described (Bass et al. 2017), samples were transferred onto polyvinylidene difluoride (PVDF) membranes for 45 min at 100 V. Subsequently, 2.5% low-fat milk which was diluted in Tris-buffered saline Tween-20 (TBST) was used to soak and block PVDF membranes for 1 h, at ambient temperature and then incubated in the following primary antibodies overnight at 4°C (1:2000 dilution in 2.5% BSA in TBS-T): rabbit phospho-protein kinase B (Akt)^{Ser473} (#9271), phospho-mechanistic target of rapamycin (mTOR)^{Ser2448} (#2971), phospho-Mitogen-activated protein kinase kinase (MEK1/2)$^{Ser217/221}$ (#9121), phospho-MAP kinase-activated protein kinase 2 (MAPKAPK-2)^{Thr334} (#3007), phospho-ribosomal protein S6 (RPS6)$^{ser235/236}$ (#2211),

phospho-AMP-activated protein kinase (AMPKα)^{Thr172} (#2531), phospho-regulatory associated protein of mTOR (Raptor)^{Ser792} (#2083), phospho-forkhead box O3 (FoxO3a)^{Ser253} (#13129) (all from Cell Signaling Technology, Leiden, The Netherlands), muscle-specific F-box protein (MAFbx) (#AP2041), muscle RING-finger protein-1 (MURF-1) (#101AP) (both from ECM Biosciences, Versailles, KY, USA), mouse oxidative phosphorylation (OxPhos) (Abcam, Cambridge, MA). The mentioned antibodies have covered all main hypertrophic pathways, catabolic factors and mitochondrial energy metabolism. After overnight incubation, membranes were washed 3×5 min in TBS-T, soaked in horseradish peroxidase (HRP)-conjugated secondary antibody (New England Biolabs; 1:2000 in 2.5% bovine serum albumin (BSA) in TBST) for 1 h, before 3×5 min washes in TBS-T. In order to quantify band intensity (Chemidoc MP, Bio-Rad, Hemel Hempstead, UK), membranes were exposed to Chemiluminescent HRP substrate (Millipore Corp., Billerica, MA, USA) for 5 min. Relative arbitrary units (RAU) were normalised to coomassie-stained membranes and to cross gel loading control.

2.4.8 Gene expression analysis of myogenesis, IGF-1 related and testosterone processing

Approximately 10 mg of muscle was homogenized, with one stainless steel bead (Tissue Lyser II, Qiagen, UK), for 2 min at frequency of 30 s^{-1} in 500 µl TRizol (Life Technologies/Thermo Fisher Scientific) to isolate total RNA according to the manufacturer's instructions. A high-capacity

cDNA reverse transcription kit (Life Technologies) was used to reverse-transcribe 500 ng of total RNA for RT-qPCR. Precisely 1 µl of 1:10 diluted cDNA was added in each well of 384-optical well plates (Life Technologies). Exon-exon boundary specific primers were mixed with SYBR Select Master Mix (LifeTechnologies) and RNase-free water and 6 µl of the mixed solution, as well as one µl of each cDNA were added to each well, with samples run in triplicate. The ViiATM 7 Real-Time PCR System (Life Technologies) was used according to the following thermal cycling conditions: 2 min at 50°C; 2 min at 95°C; 40 cycles of 15 s at 95 °C and 60 s at 60°C. The ΔΔCt method was used to quantify target mRNA expression with Peptidylprolylisomerase A levels measured to correct for variations in RNA input/cDNA synthesis (Schmittgen and Livak 2008). Primer sequences for each of the probed genes are listed in Table 2-1.

Table 2-1 Primer sequences used in PCR

	Forward	Reverse
RPL13A	5'-TAAACAGGTACTGCTGGGCCG-3'	5'-CTCGGGAAGGGTTGGTGTTC-3'
AR	5'-GGTGAGCAGAGTGCCCTATC-3'	5'-GCAGTCTCCAAACGCATGTC-3'
SRD5A1	5'-TACGGGCATCGGTGCTTAAT-3'	5'-AATCGCCATTGTACACGCCA-3'
AKR1C3	5'-GGAGAAGCAGCAGCAAACATT-3'	5'-CTTTACTTCTCGGAACCTCTGGA-3'
HSD17B2	5'-GGCTGGCATCTTATGGCTCA-3'	5'-CTGGTGCCTGCGATATTTGTT-3'
HSD17B3	5'-TGTACTCAGCTTCCAAGGCG-3'	5'-TATGGGGTCAGCACCTGGAT-3'
IGF-1Ea	5'-TCAAATGTACTTCCTTCTGGGTC -3'	5'-TAAGGAGGCTGGAGATGTATTGC -3'
IGF-1Ec	5'-AAATCAGCAGTCTTCCAACCC-3'	5'-GTGTGCATCTTCACCTTCAAGAAA-3'
MHC1	5'-ATCTCTACGCCAGGGTCCTTA-3'	5'-TTTCGGAGGAAAGGAGCAGC-3'

MHCIIa	5'-GCCCTTGGAATGAGGCTGAC-3'	5'-TGCTGAACTCAGAGGTCCTTGTT-3'
Myogenin	5'-CCAGGGGATCATCTGCTCACG-3'	5'-GGTTTCATCTGGGAAGGCCA-3'
Myf-5	5'-GATGGCATGCCCGAATGTAAC-3'	5'-GCAATCCAAGCTGGATAAGGA-3'
Myf-6	5'-CAAGAAAATCTTGAGGGTGCGG-3'	5'-TTAGCCGTTATCACGAGCCC-3'
C-Myc	5'-AACCACCACCATCCCTGTTTG-3'	5'-AAGGCCCCCAGACCCATTTC-3'
MyoD	5'-CTCCGACGGCATGATGGACTA-3'	5'-TGGGCGCCTCGTTGTAGTA-3'
MEOX-2	5'-TGAAAGACAGGTGAAAGTCTGG-3'	5'-ACCAGTTCCTTTTCCCGAGC-3'
C-met	5'-ACAGCTGACTTGCTGAGAGG-3'	5'-AGGTTTATCTTTCGGTGCCCA-3'
PAX7	5'-CGGCCAGACTGCTGTTGATTAT-3'	5'-GAGTCCCAGCACAGCAGAGT-3'
MSTN	5'-TGGCTCAAACAACCTGAATCC-3'	5'-AAACGGATTCAGCCCATCTTCT-3'
PGC1-α	5'-GAGTCATACTTGCTCTTGGTG-3'	5'-GATGATGGAGACAGCTATGGT-3'
Tfam	5'-TTCGTCCTCTTTAGCATGCTGA-3'	5'-CACCGCAGGAAAAGCTGAAG-3'

RPL13A: Ribosomal Protein L13A, *AR*: Androgen Receptor, *SRD5A1*: Steroid 5 Alpha-Reductase 1, *AKR1C3*: Aldo-Keto Reductase Family 1 Member C3, *HSD17B3*: Hydroxysteroid 17-Beta Dehydrogenase 3, *IGF-1*: insulin-like Growth Factor-1, *MHC*: Myosin Heavy Chain, *Myf-6*: Myogenic Factor 6, *C-Myc*: MYC Proto-Oncogene, BHLH Transcription Factor, *MEOX-2*: Mesenchyme Homeobox 2, *C-met*: MET Proto-Oncogene, Receptor Tyrosine Kinase, *PAX7*: Paired Box 7, *PGC1-α*: peroxisome proliferator-activated receptor γ co-activator 1-α, *Tfam*: mitochondrial Transcription factor A.

2.4.9 Mitochondrial citrate synthase activity and DNA copy number

Citrate Synthase (CS) activity was measured as described (Trounce et al. 1996). Briefly, after homogenisation of 3-5 mg muscle in 1% Triton-X-100 buffer, samples were centrifuged at 22,000 g for 3 min and the supernatant used for further analysis. Thereafter, 300 µl master mix containing 28% 0.05 M TRIS buffer (pH 7.6), 1.3% one mM 5,5' dithiobis-2-nitrobenzoic acid (DTNB), 7% acetyl-coenzyme A (1.36 $mg \cdot ml^{-1}$), 0.8%

oxaloacetate (9.88 mg·ml^{-1}) and 63 % ddH2O was measured at 412 nm as the blank. Finally, 20 µl of separated supernatant was used to measure the maximum rate of reaction (V max), compared to whole protein content. To quantify relative mitochondrial DNA (mtDNA) copy number, the extraction of genomic and mtDNA from muscle was performed using a Qiagen QIAamp® DNA Mini kit, according to the manufacturer's recommendations. Briefly, the procedure involved tissue lysis in a proteinase K buffer, incubation for 3 h at 56°C to digest myofibrils followed by centrifugation of the lysates through silica-membrane-based nucleic acid purification columns and subsequent elution of the mtDNA and gDNA. The quality and quantity of DNA was assessed by measurement at 260, 280 and 230 nm. The expression level of markers of gDNA and mtDNA used to evaluate their abundance was accomplished by using TaqMan probe real-time PCR. The TaqMan probe design for the detection of gDNA levels was based on interrogation of the intron sequence spanning between exons 3-4 of the genomic hydroxymethylbilane synthase (HMBS) gene to avoid mRNA amplification. The probe design for detection of mtDNA levels was based on interrogation of a stable fragment of the mtDNA loop, namely the mitochondrially encoded NADH:ubiquinone oxidoreductase core subunit 1 (ND1). The $2^{-\Delta Ct}$ formula, where $\Delta=Ct_{ND1}-Ct_{HMBS}$, was used to express relative mtDNA copy number.

2.5 **Statistical analyses**

Data are expressed as mean±SEM, while normality of distribution was examined using the Kolmogorov-Smirnov test. In addition, two-way analysis of variance (ANOVA) and repeated measure ANOVA (time) with one between-subject factor (group) were used to compare the changes during the RET programme both within and between the two (placebo vs. testosterone) groups. In addition, independent t-tests were used for comparing fold change between the two groups. Cohen's effect sizes (ES) were also calculated for significant data. Effect sizes of 0 to <0.20 were considered "trivial", 0.20 to <0.50 were considered ''small'' in magnitude, 0.50 to <0.80 were ''medium'' and ≥0.80 were ''large'' (Lakens 2013). Where significant differences were found using repeated measure ANOVA, a Bonferroni post-hoc test was applied for multiple comparisons. The correlation was assessed using Pearson's product moment correlation coefficient and Intraclass Correlation Coefficient (ICC) was used to test reliability of DXA and ultrasound-related outputs. The significance level was defined as P≤0.05 and all of the statistical analyses were performed using GraphPad Prism 7.01(La Jolla, CA).

Chapter 3

Pharmaceutical depletion of endogenous testosterone blunts resistance exercise-induced hypertrophy in younger men via impairing muscle growth programming

3.1 **Abstract**

Background: The role of endogenous testosterone (T) in mediating hypertrophic responses to resistance exercise training (RET) remains contentious with correlative studies suggesting a limited role, and others a more central role. We thus investigated the physiological and mechanistic effects of chemical endogenous testosterone depletion adjuvant to 6-wks of RET on muscle mass, function, myogenic regulatory factors, muscle anabolic signalling, and protein synthesis in younger men.

Methods: Sixteen non-hypogonadal healthy young men (18-30 y, BMI≤30 kg.m^{-2}, serum T>230 ng·dl^{-1}) were randomised in a double-blinded fashion to receive either: placebo (P, saline n=8) or the GnRH analogue, Goserelin (Zoladex (Z) (3.6 mg, n=8)) injections before undertaking 6-wks of RET (6-exercises, 3-sets, 8-10 reps, 80% 1-RM). Participants underwent dual-energy x-ray absorptiometry, ultrasound of vastus lateralis (VL) muscle architecture, and knee-extensor isometric muscle force tests; VL muscle biopsies were taken to quantify myogenic/anabolic gene expression, anabolic signalling, muscle protein synthesis (D_2O) and breakdown (extrapolated).

Results: Provision of Zoladex during RET suppressed endogenous testosterone (Z: 45.2±4, vs. P: 323±30 ng.dl^{-1}, P<0.001). The Zoladex group exhibited blunted whole-body (Z: 55±3 to 56±3 kg, P=0.6 vs. P: 56±2 to 58±2 kg, P=0.006) and appendicular fat free mass (FFM) (Z: 8.2±0.3 to 8.4±0.4, P>0.9 vs. P: 7.6±0.3 to 8.3±0.3kg·m^{-2}, P=0.07). Augmentations in VL muscle thickness, fascicle length, and quadriceps cross-section area with RET occured to a greater extent in placebo (P<0.05) and composite strength gains (Z: 40±2 vs. P: 50±3%, P=0.03)

increased significantly more in the placebo group. Reductions in body fat percentage were only evident in the placebo group (Z: 23±2 to 24±2%, P=0.2 vs. P: 24±3 to 23±3%, P=0.04). The placebo group exhibited augmented gene expression related to testosterone metabolism (e.g. *Androgen Receptor*: 1.8-fold; *HSD17B3*: 2.8-fold, P<0.05*)*; anabolism/myogenesis (e.g. *IGF-1EA* (3.3-fold), *Myogenin* (2.6-fold), *Myf5* (2.6-fold), *c-Met* (2.7-fold), P<0.05) in addition to RNA/DNA (P:0.5±0.1 to 0.6±0.1, P=0.0003 vs. Z:0.5±0.1 to 0.5±0.1, P=0.3), and RNA/ASP ratio (P:6.5±0.2 to 8.9±1.1, P=0.008 vs. Z:5.8±0.4 to 6.8±0.5, P>0.9). Also, acute RET-induced phosphorylation of AKT^{ser473} (Z: 4.9±1.6-fold, P=0.7 vs. P: 10.1±5.3-fold, P=0.003) and $mTORC1^{ser2448}$ (Z: 2.5±1.3-fold, P>0.9 vs. P: 4.8±2.1-fold, P=0.03) were attenuated in the Zoladex group. Both MPS (P: 2.01±0.1 vs. Z: 1.54±0.1%·day^{-1}, P=0.01) and MPB rates (P: 129.1±13 vs. Z: 93.1±7.8 g·day^{-1}, P=0.04) were higher in placebo group, which led to higher net turnover and protein accretion (P: 3.9±1.1 vs. Z: 1.2±1.1 g·day^{-1}, P=0.04).

Conclusions: Chemical testosterone depletion during RET blunted muscle anabolic signalling, testosterone processing enzyme and pro-myogenic gene expression, perhaps explaining attenuated muscle growth in this group. Thus, decreasing endogenous levels of testosterone coupled to RET attenuates numerous positive adaptations to RET - thereby, demonstrating, at least, permissive importance of endogenous testosterone in regulating muscle hypertrophy.

3.2 Introduction

Skeletal muscles, which comprise up to one-half of the body mass (Lee et al. 2000) and contain ~70% of all body proteins and accounts for ~50% of body protein turnover (Frontera and Ochala 2015) have obvious role in locomotor activity, postural behavior (Lee et al. 2000), basal energy metabolism, storage of the substrates such as amino acids and carbohydrates, the production of heat for the maintenance of core temperature, and the consumption of the majority of oxygen and fuel used during exercise training (Frontera and Ochala 2015). In general, muscle mass is affected by the balance between protein synthesis and degradation and both processes are under influence of factors such as hormonal balance, exercise training, and injury or disease (Frontera and Ochala 2015; Zanou and Gailly 2013). Indeed, there remains a need for identifying the factors (-related mechanisms) regulating muscle cells growth.

In normal conditions, adult skeletal muscle is therefore a stable tissue. This stable tissue with 1–2% of myonuclei replacement per week, has the ability to complete a rapid and extensive regeneration in response to severe damage (e.g. extensive physical activity, especially resistance exercise training (RET)) (Charge and Rudnicki 2004). This remarkable ability of muscles in regenerating newly myofibers from activation of myogenic cells to proliferate, differentiate, and fuse to necrotic fibres (Charge and Rudnicki 2004) via factors such as myogenic regulatory

factors (MRFs) relies on other growth factors (e.g. testosterone) (Zanou and Gailly 2013). In previous studies, decrease in testosterone levels (treatment with gonadotropin-releasing hormone (GnRH)) demonstrated an association between testosterone concentrations and fat free mass (FFM), muscle size and strength (Kvorning et al. 2006a; Ferrando et al. 2002c). However, the mechanisms by which the expression and activity of these factors are regulated (mainly through testosterone) are not completely clarified.

The RET elicits a milieu of physiological responses and adaptations that are critical for increasing muscular hypertrophy and strength (Kraemer et al. 1990). Of primary importance to acute exercise performance and subsequent tissue remodelling is the role(s) played by the neuroendocrine system (Kraemer et al. 1990); especially enhancing testosterone levels after an individual RE in response to RET (Rønnestad, Nygaard, and Raastad 2011). This augmentation seems to be critical, as we already showed older males with lower levels of testosterone had blunted adaptation to RET (Brook, Wilkinson, Mitchell, et al. 2016), and also long term testosterone therapy *per se* positively affected net protein balance (Ferrando et al. 2002c), FFM (Gruenewald and Matsumoto 2003), fat mass (Svartberg et al. 2008), muscle function (Arny A. Ferrando and Randall J 2002) through ill-defined mechanism; nevertheless, short-term testosterone therapy did not improve muscle protein synthesis (MPS), RNA content (Lobley et al. 1990), FFM or appendicular FFM (Ragnarsson et al. 2013), as well as muscle strength (Liu, Swerdloff, and Veldhuis

2004). Conversely, suppression of endogenous testosterone production attenuated the increase in FFM, increased body fat percentage (BFP), and blunted the increase in muscle strength during RET (Kvorning et al. 2006a), but not blunted MyoD, myogenin, IGF, and myostatin expression (Kvorning et al. 2007a) in young men. Thus far, the role of endogenous testosterone in mediating hypertrophic adaptation to RET remains contentious with correlative studies suggesting a limited role (West, Burd, et al. 2009); therefore, interventions that exploring the mechanistic effects of testosterone via manipulating circulating testosterone concentration during RET might be of greater benefit (Hawkins et al. 2008). Hence, in an effort to determine the importance and mechanisms of endogenous testosterone adjuvant to RET, we investigated the cellular mechanisms of short-term RET with or without testosterone suppression in young men, with determining the end-points relating to muscle mass, function, myogenic regulation, anabolic signalling and protein turnover.

3.3 **Methods**

The study protocol and all the methods relating to this section have been previously described in chapter 2.

3.4 **Results**

Physiological characteristics of participants are shown in Table 3-1. Further, total plasma testosterone concentrations in the Zoladex group were significantly lower than in the placebo group at all time-points during RET ($P<0.05$); i.e. 377±31 ng·dl^{-1} at baseline and achieved 287±61 ng·dl^{-1} at 1-wk and 45±4 ng·dl^{-1} at 4-wks and returned to 229±48 ng·dl^{-1} at the end of RET in Zoladex group. Plasma testosterone concentrations during the study are shown in Figure 3-1.

Table 3-1 Participants Characteristics

	Zoladex(n=8)		**Placebo(n=8)**	
	Baseline	**Week 6**	**Baseline**	**Week 6**
Age (y)	22.2(0.8)	—	21.1(1.2)	—
Height (m)	1.71(0.1)	—	1.82(0.1)	—
Weight (kg)	72.9(4.1)	74.1(4.3)	75.7(4.4)	76.7(4.3)
BMI (kg.m^{-2})	23.1(1.1)	23.4(1.1)	22.9(1.4)	23.2(1.4)

Values are means (SEM).

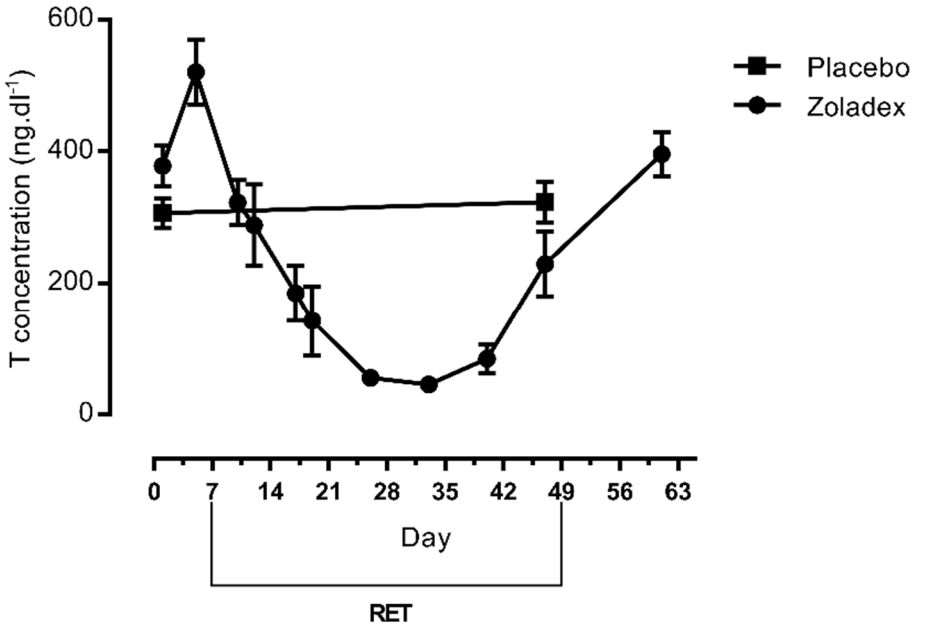


Figure 3-1 Plasma testosterone concentrations during the study

3.4.1 Body composition

The DXA results showed that total FFM (ICC=0.97) increased significantly to a greater extent following the 6-wk RET in placebo (55.9±1.7kg to 57.4±1.7kg, P=0.006, 95% confidence interval (CI)=56-58, ES=0.31, Figure. 3-2.A) than Zoladex (55.4±2.8kg to 55.8±3.1kg, 95%CI=55-57). Nevertheless, placebo study group only showed a trend in leg FFM (P:18.7±0.7 kg to 19.4±0.7 kg (P=0.09) vs. Z:18.9±1.1kg to 19±1.2 kg (P>0.9), Figure 3-2B), appendicular FFM (P:7.6±0.3 to 8.2±0.3 (P=0.07) vs. Z:8.2±0.3 to 8.4±0.3 (P>0.9), Figure 3-2C) and TFM (P:19.1±3.4kg to 18.5±3.3kg (P=0.4) vs. Z:16.7±1.9 kg to 17.4±2.1 kg (P=0.2), Figure 3-2D); indicating lower testosterone level in Zoladex group blunted RET adaptation. Further, both FFM index (17±0.4 $kg.m^{-2}$ to 17.4±0.4 $kg.m^{-2}$,

95%CI=17.4-18 vs. 17.5±0.5 kg.m^{-2} to 17.6±0.6 kg.m^{-2}, P=0.007, ES=0.28, Figure 3-2D) and TFP (24.4±3 % to 23.4±2.9 %, 95%CI=21.7-23.6 vs. 23±2 % to 23.6±2.1 %, P=0.04, ES=0.37, Figure 3-2E) after RET; showing higher testosterone level could boost the RET adaptation.

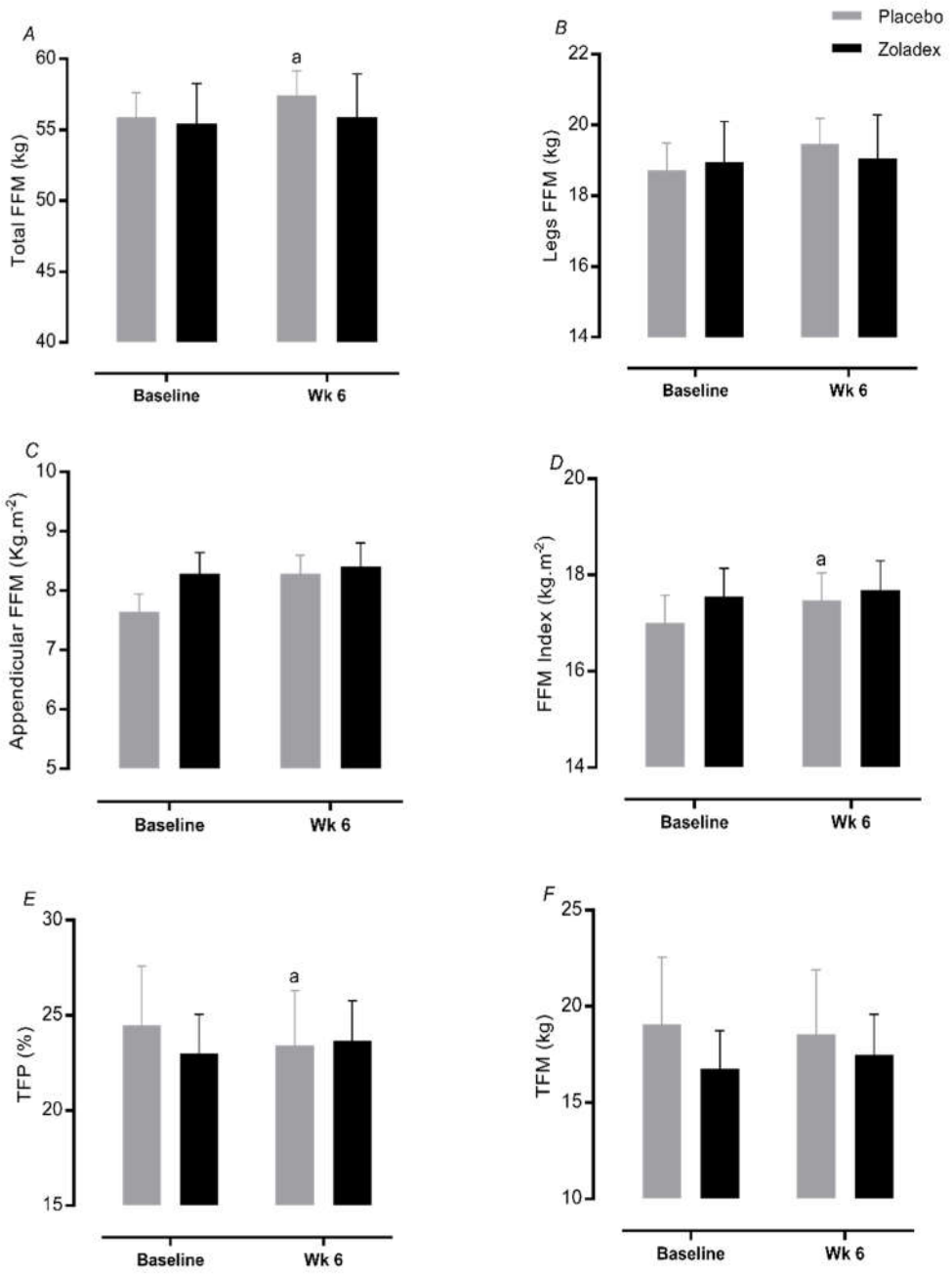


Figure 3-2 **Muscle mass and body composition changes.** Changes in Zoladex and placebo in muscle mass and body composition (A-F), from baseline to wk 6. Values are means ± SEM. **a** Significantly different from baseline, P<0.05; **b** significantly different from placebo, P<0.05 all from two-way ANOVA. FFM: fat free mass; TFP: total fat percentage; TFM: total fat mass.

Over the RET period, placebo increased MT (2.74±0.32 to 2.91±0.32 cm, P<0.0001, 95%CI=2.8-3, ES=0.48, vs. 2.7±0.4 to 2.69±0.36 cm, P>0.99, ICC:0.95, Figure 3-3.A), Lf (7.91±0.87 to 8.44±0.82, P<0.0001, 95%CI=7.8-8.5, ES=0.34 vs. 7.39±1.1 to 7.42±1.1 cm, P>0.99, Figure 3-3.B), PA (22.8±1.8 to 24.3±2.27°, P=0.001, 95%CI=23.8-26.2, ES=0.36 vs. 24.4±4.1 to 25.2±4.1°, P=0.32, Figure 3-3.C), and CSA (79.1±11.8 to 83.8±9.1 cm^2, P=0.005, 95%CI=81.5-87.3, ES=0.45 vs. 80.5±15.4 to 78.8±16.4 cm^2, P>0.99, Figure 3-3.D); illustrating the anabolic/remodelling role of T.

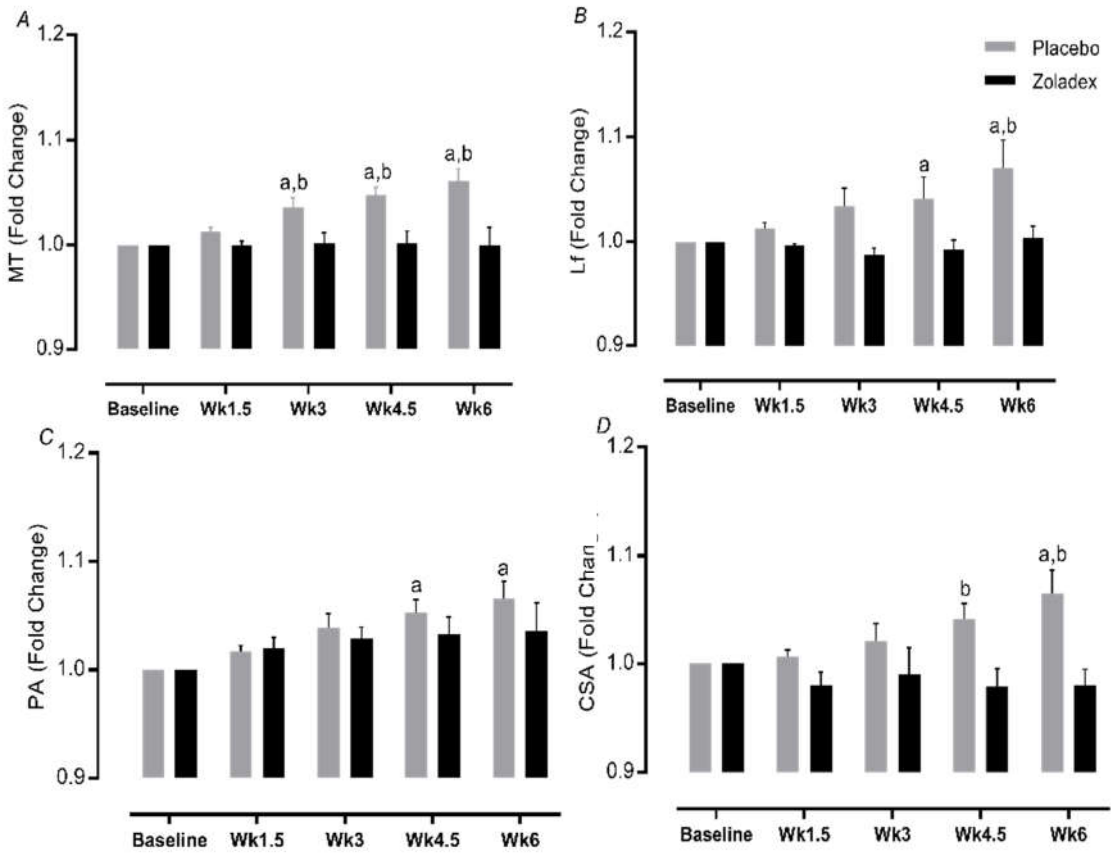
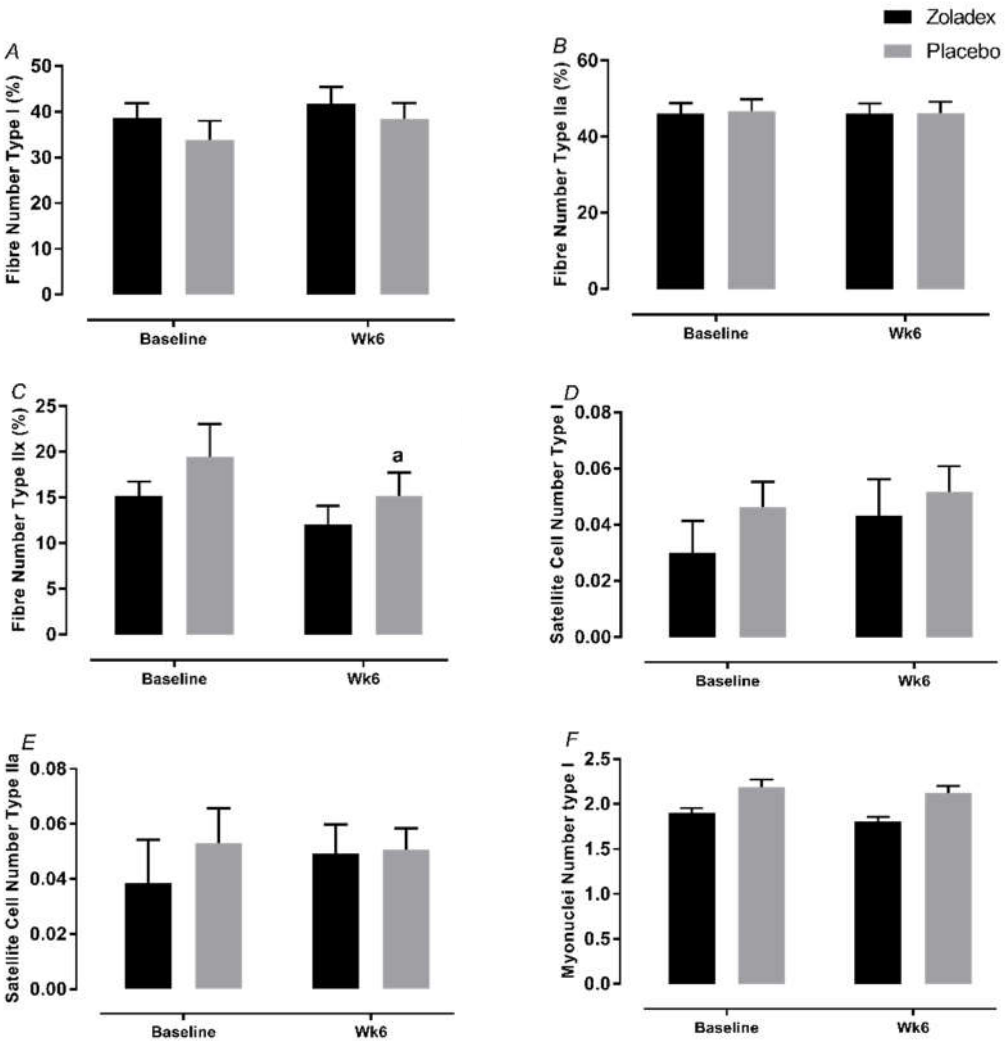


Figure 3-3 **Fold changes in muscle architecture (A-D).** Values are means ± SEM. **a** Significantly different from baseline, P<0.05; **b** significantly different between two groups, P<0.05; all from repeated measure ANOVA.

3.4.3 Fibre type satellite cell, myonuclei number and CSA

The percentage of type IIx number decreased in placebo group (P=0.03), without changing in type I and IIa. Fibre CSA type IIa did not change significantly in any group; but fibre CSA type I increased significantly in the placebo group, with a significant correlation between quadriceps CSA and CSA type I and IIa in placebo group (Figure 3-4 A-K).



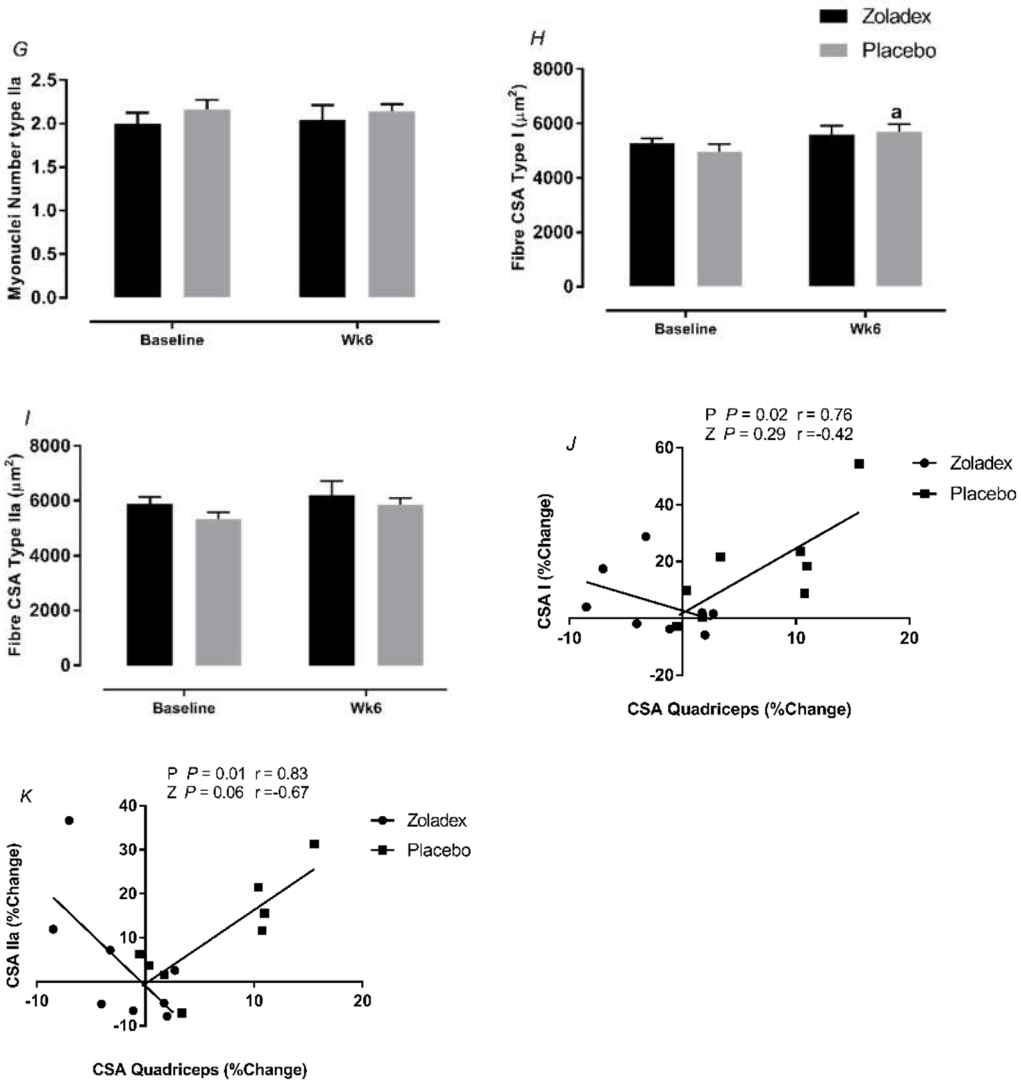


Figure 3-4 Fibre type number (A-C), satellite cell number (D-E), myonuclei number (F-G) and CSA (H-I) and correlation between fibre type and quadriceps CSA (J-K). Values are means ± SEM. a Significantly different from baseline, P<0.05, all from two way ANOVA.

3.4.4 Muscular performance

Dynamic strength (total 1-RM across all six exercises) was augmented in to a greater extent in the placebo group vs. Zoladex (P:49.8±3.3% vs. Z:40±2.3%, 95%CI= P:41.9-49.8 vs. Z:34.5-45.5, P=0.03, ES=1.4, Figure 3-5A). There were similar increases in specific strength, (force per unit area) across the groups (95%CI= P:10.9-11.9; Z:10.4-11.4, ES=T:1.13; Z:0.98, P<0.05, Figure 3-5D). However, there were no significant differences in static strength in both groups; e.g. MVC at 70° (275±11.4 to 306.3±20.6 Nm vs. 288.3±31.3 to 320.7±35 Nm, P=0.22, Figure 3-5 B,C).

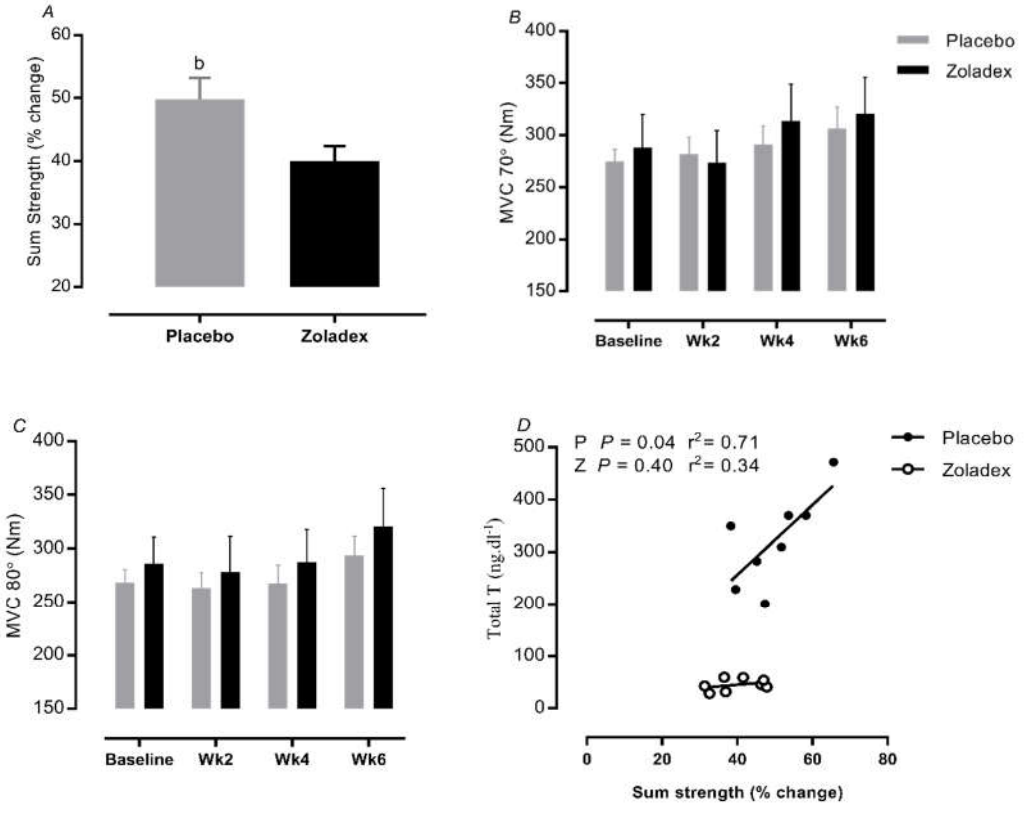


Figure 3-5 Time course of changes in Total strength (A) and muscle strength (B-D), and correlation between FFM and total strength (E). Values are means ± SEM. a Significantly different from baseline, P<0.05; b significantly different between two groups, P<0.05. A: independent t-tests, B-D: two-way ANOVA.

3.4.5 Muscle protein synthesis and [extrapolated] breakdown

There was a significant augmentation in cumulative myofibrillar MPS in placebo vs. Zoladex till 3-wks RET (P:1.5±0.12 to 2±0.15 %·day^{-1} vs. Z:1.45±0.11 to 1.5±0.06 %·day^{-1}, 95%CI=P:1.7-2.2 vs. Z:1.3-1.7, P=0.01, ES=0.97); in line with findings of elevated FGR in placebo compared to Zoladex (0.05±0.01 %·day^{-1} vs. 0.01 %·day^{-1}, 95%CI=0.02-0.09 vs. -0.02-0.05, P=0.04, ES=0.43) (Figure 3-6 A-B). Furthermore, ASR was significantly increased as a result of RET in placebo (104.6±10.1 to 133.1±13.9 g·day^{-1}, 95%CI=109.9-153.5, P=0.03, ES=0.32), but not in Zoladex (99.9±9.5 to 94.3±7.8 g·day-1, 95%CI=73.9-117.5, P>0.05). In addition, estimated ABR was significantly higher in placebo than Zoladex during RET (129.1±13.8 vs. 93.16±7.8 g·day^{-1}, 95%CI=105-153 vs 69-117, P=0.04, ES=0.92). Finally, when comparing ASR and ABR, net protein turnover in the placebo group was higher than the Zoladex group (3.9±1.1 g·day-1 vs. 1.2±1.1 g·day-1, 95%CI=1.6-6.3 vs. -1.1-3.5, P=0.04, ES=0.95, Figure 3-6 C-E).

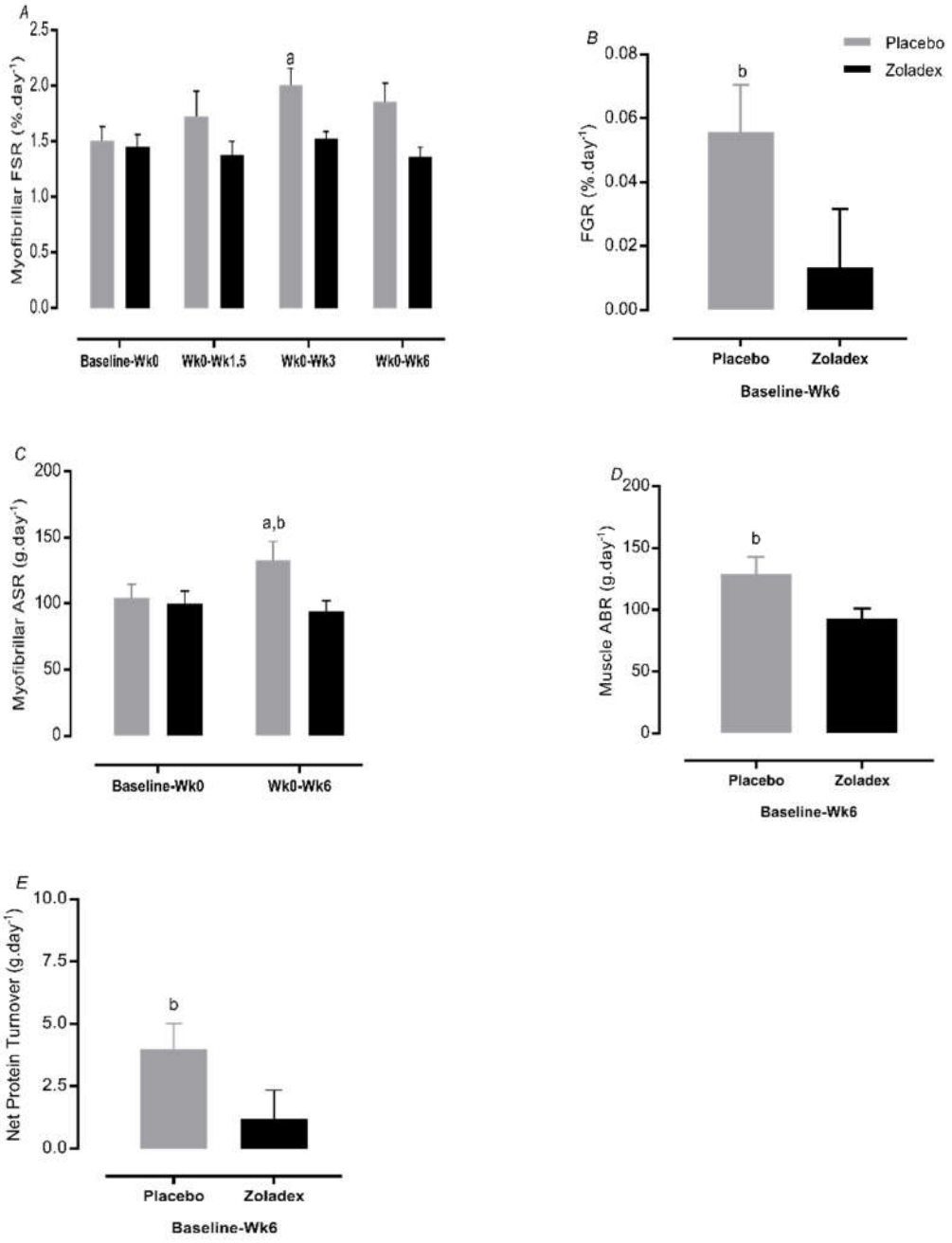


Figure 3-6. Muscle protein synthesis, fractional growth rate, absolute synthetic rate, absolute break-down rate and protein turnover. Values are means ± SEM. a Significantly different from baseline, $P<0.05$; b significantly different between two groups, $P<0.05$. B, D, E: independent t-tests, A and C: two-way ANOVA.

3.4.6 Muscle ASP, DNA and RNA content

There were no changes in ASP or DNA concentrations per dry weight (dw) muscle ($\mu g \cdot mg^{-1}$) in Zoladex or placebo with RET; nonetheless, total RNA content (95%CI=P:4.1-6.3; Z:2.9-5.2, P<0.0001, ES=0.75) and RNA:DNA ratio, a reflection of ribosomal capacity for protein synthesis, only increased in the testosterone group (95%CI=P:0.55-0.72; Z:0.44-0.62, P=0.003, ES=0.72). Similarly, RNA:ASP ratio, primarily a measure of ribosomal capacity (Brook, Wilkinson, Mitchell, et al. 2016), increased in placebo (95%CI= 7-10.8, P=0.008, ES=0.63) but not Zoladex (P>0.99, 95%CI=4.8-8.7) over the RET phase. Finally, the ratio of ASP:DNA, a measure of cell size did not change in either group (Table 3-2).

Table 3-2 Muscle protein, RNA, DNA and related ratios

	P				Z			
	Baseline	**WK1**	**WK3**	**WK6**	**Baseline**	**WK1**	**WK3**	**WK6**
ASP Content (μg·mg^{-1} dw)	528.1(21.6)	522.6(32.9)	564.6(42)	595.8(25.4)	592.4(24.3)	548(28.7)	581.5(31.2)	598.2(24.5)
RNA Content (μg·mg^{-1} dw)	3.4(0.1)	3.8(0.1)	4.1(0.1)	5.2(0.5)[a,b]	3.4(0.1)	3.5(0.3)	3.5(0.3)	4.1(0.4)
DNA Content (μg·mg^{-1} dw)	6.8(0.1)	6.9(0.3)	7.2(0.2)	8.1(0.3)	7.4(0.4)	7.5(0.2)	7.4(0.4)	7.7(0.7)
RNA:DNA (Ratio)	0.5(0.01)	0.55(0.01)	0.57(0.01)	0.64(0.04)[a,b]	0.47(0.03)	0.46(0.04)	0.47(0.03)	0.53(0.03)
RNA:ASP (Ratio)	6.5(0.2)	7.4(0.4)	7.5(0.4)	8.9(1.1)[a]	5.8(0.4)	6.5(0.7)	6.1(0.4)	6.8(0.5)
ASP:DNA (Ratio)	77.2(3.2)	75.6(4.4)	78(4.6)	74.1(4.2)	81.1(4.3)	72.7(3.1)	78.3(1.8)	81.2(8.1)

Values are means (SEM). a Significantly different from baseline, P<0.05; b significantly different between two groups, P<0.05. all from repeated measure ANOVA.

3.4.7 Intramuscular signalling pathways

Acute, exercise-induced anabolic signalling was quantified 60 min after the first (1-wk), 9th (3-wk), and 18th (6-wk) resistance exercise session. In doing so, we showed there was significant increases in placebo study group compared to Zoladex, in phosphorylation of protein kinase B (AKT^{Ser473}) (95%CI=1.7-18.6, P=0.003, ES=0.54), mammalian target of rapamycin (mTOR^{Ser2448}) (95%CI=0.98-8.6, P=0.03, ES=0.53), adenosine monophosphate-activated protein kinase (AMPKα^{Thr172}) (95%CI=3.3-10.9, P=0.02, ES=0.68) and regulatory-associated protein of mTOR (Raptor^{Ser792}) (95%CI=1.1-2.5, P=0.001, ES=0.59) acutely after 6-wk RET. Further, the Zoladex group increased activation ribosomal protein S6 (RPS6$^{ser240/244}$) (95%CI=22-59, P=0.001, ES=0.79) acutely after first RE, In contrast, phosphorylation levels of forkhead box O3 (FoxO3a^{Ser253}) and Tuberin/TSC2^{Thr1462} did not change at any time (P>0.05) (Figure 3-7).

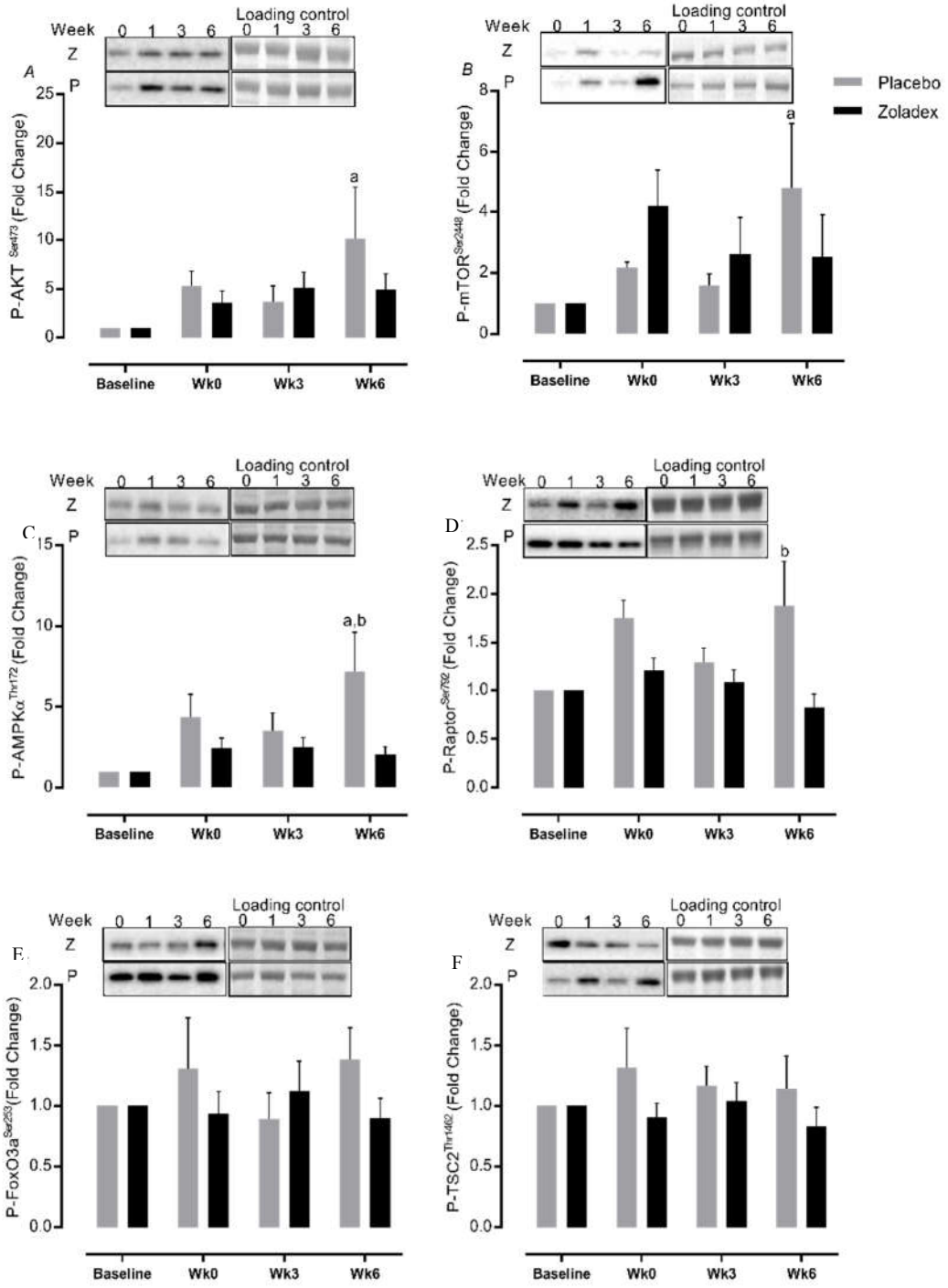
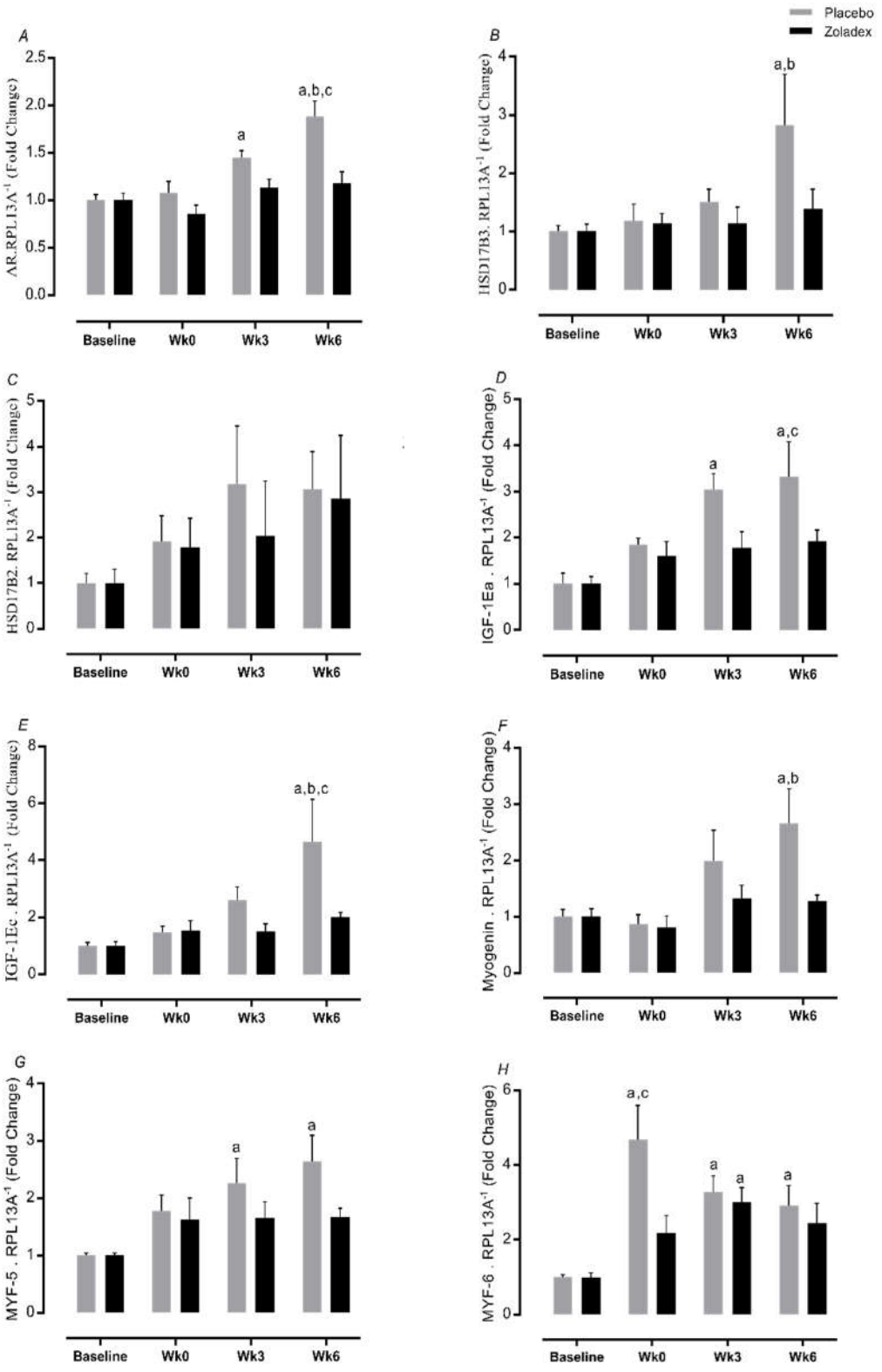


Figure 3-7 Intramuscular anabolic signalling pathways. Values are means ± SEM. a Significantly different from baseline, P<0.05; b significantly different between two groups P<0.05. all from repeated measure ANOVA.

3.4.8 Muscle gene expression

Acute, exercise-induced increase in Testosterone metabolism-related gene expression was augmented in placebo compared to Z. Accordingly, androgen receptor (*AR*) (1.8±0.1-fold change, 95%CI=1.5-2.1, P<0.0001, ES=0.85), HSD17β3 (2.8±0.8-fold change, 95%CI=1.4-4.2, P=0.007, ES=1.04) and increased after 6-wk RET only in placebo (Figure 3-8 A-B). In addition, *IGF-1Ea* (3.3±0.7-fold change, 95%CI=2.1-4.5, P=0.0005, ES=0.72), *IGF-1Ec* (4.6±1.4-fold change, 95%CI=2.3-6.9, P=0.0005, ES=0.68) expression increased only in placebo after 6-wk RET (Figure 3-8.D-F). While mRNA expression of select myogenesis-related genes i.e. *Myogenin* (2.6±0.6-fold change, 95%CI=1.7-3.6, P=0.002, ES=0.72), *Myf-5* (2.6±0.4-fold change, 95%CI=1.9-3.3, P=0.0001, ES=0.81), *Myf-6* (2.9±0.5-fold change, 95%CI=1.7-4, P=0.01, ES=0.79) increased in placebo, *C-met* (2.7±0.4 vs. 1.9±0.2 fold change, 95%CI=P:1.8-3.5, Z:1.1-2.8, ES= P:0.82, Z:0.77) and *C-Myc* (10.6±3.2 vs. 11.6±3.5 fold change, 95%CI=P:3.2-17.9, Z:4.2-18.9, ES=P:0.74, Z:0.74) increased in both groups (P<0.05); with insignificant changes in MYOD and PAX-7 (P>0.05) (Figure 3-8.G-M). Nevertheless, a measure of myostatin (MSTN), displayed a trend to increase by 3-wk in placebo (2.2 ± 0.7-fold change, 95%CI=1.1-3.3, P = 0.01, ES=0.55), whilst there was no significant changes by 6-wk in both groups (P>0.05) (Figure 3-8.N).


Placebo
Zoladex
A
AR.RPL13A-1 (Fold Change)
a
a,b,c
B
HSD17B3. RPL13A-1 (Fold Change)
a,b
C
HSD17B2. RPL13A-1 (Fold Change)
D
IGF-1Ea . RPL13A-1 (Fold Change)
a
a,c
E
IGF-1Ec . RPL13A-1 (Fold Change)
a,b,c
F
Myogenin . RPL13A-1 (Fold Change)
a,b
G
MYF-5 . RPL13A-1 (Fold Change)
a
a
H
MYF-6 . RPL13A-1 (Fold Change)
a,c
a
a
a
Baseline
Wk0
Wk3
Wk6

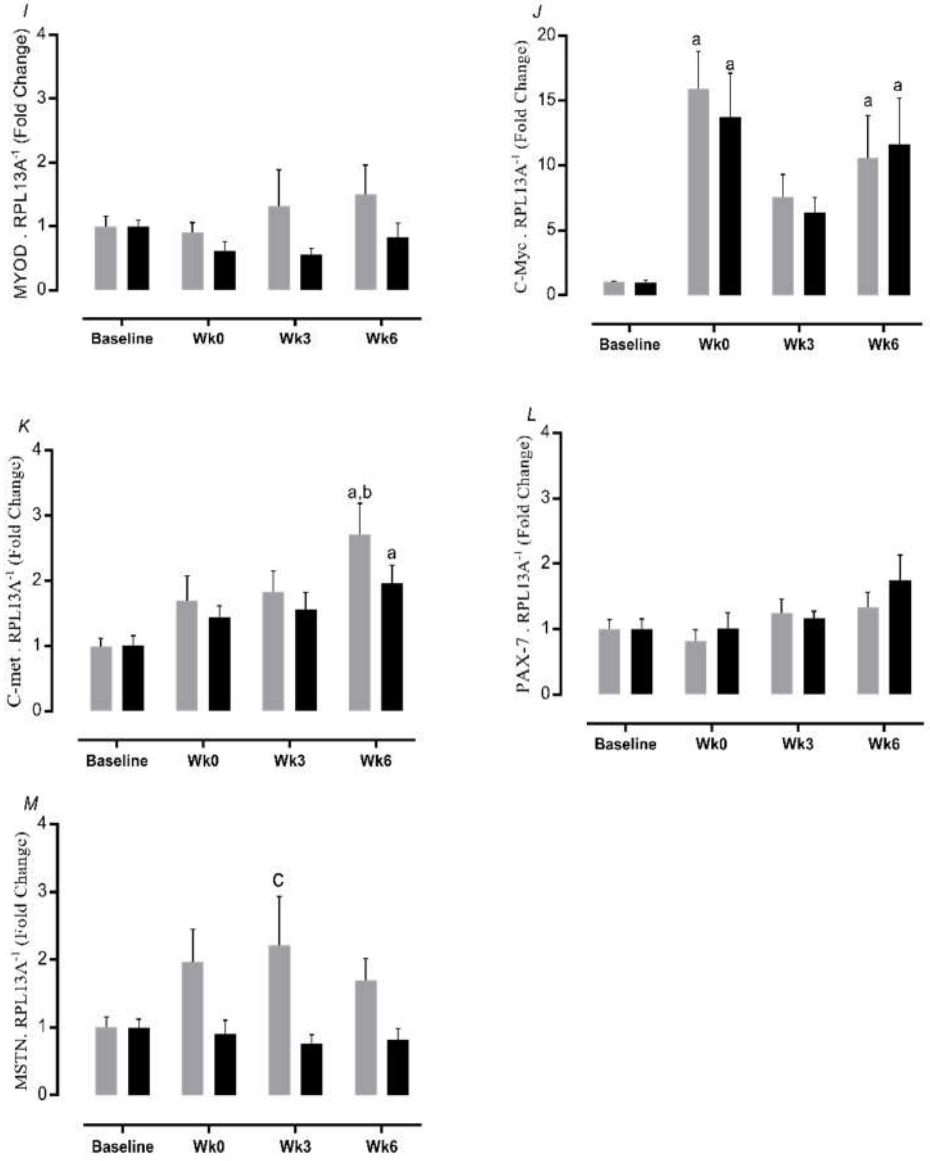
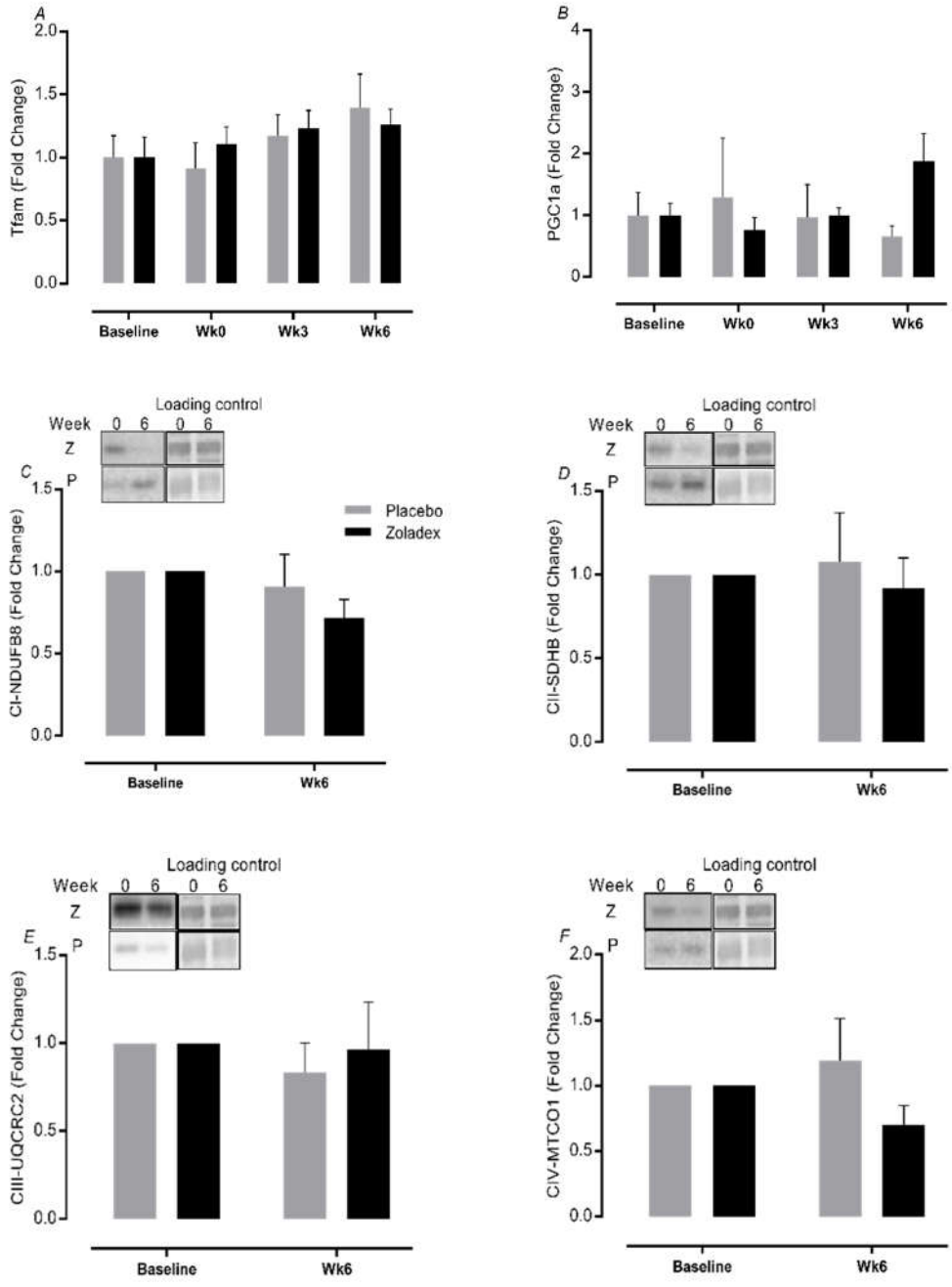


Figure 3-8 Muscle gene expression Changes in T and P in T metabolism gene expression (A-C), anabolism (D-F), and myogenic (G-L) and myogenesis inhibitor (M) from baseline to wk 6. Values are means ± SEM. a Significantly different from baseline, P<0.05; b significantly different between two groups, P<0.05. all from repeated measure ANOVA.

3.4.9 Mitochondrial oxidative phosphorylation capacity

There was no significant differences between and within groups in gene expression of mitochondrial transcription factor A (*Tfam*), peroxisome

proliferator-activated receptor γ co-activator-1α (PGC-1α), and levels of oxidative phosphorylation (OxPhos) proteins in either of groups (P>0.05).



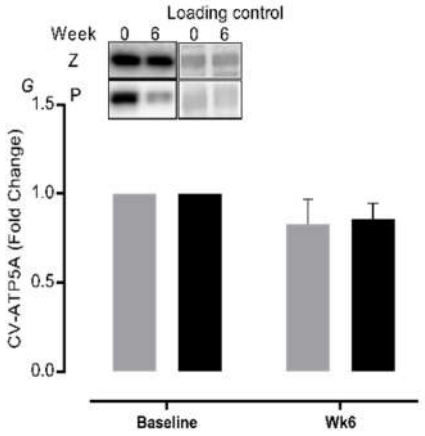


Figure 3-9 . Mitochondrial oxidative phosphorylation capacity markers. Values are means ± SEM. a Significantly different from baseline, P<0.05; b significantly different between two groups, P<0.05.

3.5 **Discussion**

There is a great deal of interest in identifying the interventions-related mechanisms that will explain the effects of androgens on muscle programming. Herein, we elucidated that chemical endogenous testosterone depletion coupled with 6-wks of RET significantly blunted/suppressed augmentation in FFM, function, MRFs, muscle anabolic signaling, protein synthesis in adaptive responses to RET in younger men.

The mechanism and the role of testosterone in regulating muscle gains when coupled with RET remains somewhat contentious. For instance, Ahtiainen et al. (Ahtiainen et al. 2015) reported that 12-months RET increased FFM without altering endogenous testosterone levels, concluding that changes in endogenous testosterone levels did not

impact RET-induced muscular adaptations (Ahtiainen et al. 2015). However, 10-wks endogenous testosterone suppression decreased FFM (Mauras et al. 1998); and in another study, suppression of endogenous testosterone blunted muscle adaptations and increased TFP to 8-wks RET in younger men (Kvorning et al. 2006b) - suggesting that endogenous testosterone levels are, at very least, permissive in regulating hypertrophic adaptations to RET. Therefore, given established links between testosterone levels and muscle mass (Griggs et al. 1989), we postulated that short-term testosterone declines adjuvant to RET blunts adaptations/responses to RET. Consistent with this thesis and the marked effect of short term (8-wk) RET on FFM (Kon et al. 2014), it was previously reported that endogenous testosterone is of paramount importance to the adaptation to RET; i.e. suppressed endogenous testosterone attenuated FFM and strength gains while increased TFP (Kvorning et al. 2006b). Our present albeit *shorter-term* data also showed that RET adjuvant to testosterone suppression led to attenuated increases in whole-body and; moderately, appendicular FFM. Moreover, RET-induced gains in MT, Lf, PA, and CSA in VL and quadriceps and with a decrease in fibre type IIx after RET (Andersen and Aagaard 2000) in placebo but not in Zoladex group; remodeling also is associated with T-specific hypertrophy of type I fibres and related correlation (i.e. type I and IIa) with quadriceps CSA, illustrating that suppression of endogenous testosterone attenuated local thigh muscle remodeling. Thus, in spite of some previous studies (Wilkinson et al. 2007a; West, Kujbida, et al. 2009), our data suggest that endogenous testosterone in healthy younger

men can be considered as of paramount importance for the muscular adaptation to RET.

Since muscular performance is the culmination of neural and muscle structural elements, and is the most important attribute to maintaining mobility, we determined the role of endogenous testosterone in relation to aspects of muscle strength. In doing so, we established that the placebo group developed greater total dynamic (1-RM) strength gains (Kon et al. 2014) which also is strongly correlated with testosterone levels (Figure 3-5.E) (Häkkinen and Pakarinen 1994). To support, it has reported 8-wks testosterone suppression decreased strength in younger men (Mauras et al. 1998; Kvorning et al. 2006a). Interestingly, previous work has shown that there may be a neural component to the effects of testosterone e.g. influencing neurotransmitter synthesis (Hakkinen et al. 2000), leading to reduced force fluctuations (Tracy, Byrnes, and Enoka 2004) and inducing recruitment of larger/faster motor units (fast twitch fibres) (Bellew 2002). Nonetheless, given our total strength·FFM^{-1} 'specific force' data illustrate that muscle mass gains were related to strength-related improvements; we can thus conclude RET-induced FFM gains were a major component of improved muscle performance in both groups.

In order to assess the mechanisms underlying the ergogenic effects of normal endogenous testosterone levels adjuvant to RET in younger men, we quantified cumulative rates of myofibrillar protein synthesis over the 6-wks of RET. In doing so, we demonstrated that, in opposition to Zoladex

group, placebo group with normal testosterone levels augmented MPS during RET while concomitantly increasing [estimated] fractional breakdown rates (FBR) (Pivarnik, Hickson, and Wolinsky 1989) and crucially, with net balance calculations illustrating MPS>MPB. MPB, is an important metabolic component of muscle remodelling and protein turnover, increase with RET but to a lesser extent than MPS (Tipton, Hamilton, and Gallagher 2018). The logical extrapolation of a sustained increase in net muscle protein deposition after RET is an increase in FFM and strength (Ferrando et al. 2002c), entirely in line with our mass and strength-gain data. Links between testosterone and MPS have been previously established. For instance, decreased muscle mass with castration-induced androgen withdrawal suppressed myofibrillar protein synthesis in mice (White et al. 2013b). In addition, castration and testosterone replacement for 8-wks in mice indicated testosterone as a key regulator of muscle protein turnover (Haren et al. 2011); with increased efficiency of reutilization of amino acids from MPB during testosterone replacement (Ferrando et al. 1998b). Interestingly, it was suggested exogenous testosterone may improve net protein balance via decreasing MPB, rather than an increasing MPS (Nader et al. 2014). Further, 8-wks testosterone suppression per se decreased whole body MPS without any change in plasma amino acid concentrations (Mauras et al. 1998), with an early stage of increase in protein degradation rather than ubiquitin-proteasome process (Haren et al. 2011). However, we show that, in opposition to Zoladex group, normal endogenous testosterone levels coupled to RET increased net protein accretion

despite elevation of both MPS and MPB, because increases in MPS>MPB, indicating the effects of endogenous testosterone in myofibrilar MPS (Morgan et al. 1993). Further, Factors control intracellular protein turnover (i.e. protein translation, available mRNA, and transcriptional capacity) seem to have large effect on the rate of muscle hypertrophy (Van der Meer, Jaspers, and Degens 2011).

In order to explore the mechanisms underpinning increases in MPS, we investigated aspects regulating the translational 'efficiency' of ribosomes (Van der Meer, Jaspers, and Degens 2011) corresponding to the translational rate per ribosome (Chaillou 2017). To evaluate this, we determined acute RE-induced phosphorylation within mTORC1 pathways (Brook, Wilkinson, Mitchell, et al. 2016), across the duration of the 6-wks RET. It is noteworthy from past work that activation of many of these pathways are blunted in low testosterone levels conditions (Williamson et al. 2003; Parkington et al. 2004; Brook, Wilkinson, Mitchell, et al. 2016; Haren et al. 2011) and that in the present study, depletion of testosterone in younger men attenuated the hypertrophy-related protein phosphorylation. Consistently, there is a general blunted activation of mTORC1 (Parkington et al. 2004), ERK1/2 (Williamson et al. 2003), MAPKs (Williamson et al. 2003) presumably due to lesser bio-availability of testosterone (Bennett et al. 2010; White et al. 2013b). In support of this, exogenous testosterone augmented IGF-1/Akt/mTOR signaling-activity (which regulates MPS (White et al. 2013b)), presumably explaining enhanced mass and functional gains and suggesting that testosterone

levels, both exogenous and endogenous, at least permissively, regulate muscle adaptations. In addition to increases in translational efficiency, another facet that is under influence of testosterone and potentially impacts 'capacity' for increasing protein synthesis and net protein balance and is critical for anabolic potential and hypertrophy is ribosomal RNA content (Wilborn et al. 2009). In accordance, it is reported testosterone depletion decreased transcriptional activity and cell size (Morgan et al. 1993). In the present study, despite testosterone suppression group, normal testosterone level coupled with RET increased total RNA content per 'cellular unit' (RNA:DNA ratio), an indicator of ribosomal abundance (Kemp et al. 2013), and total RNA:ASP ratio, as an index of synthetic capacity - thereby indicating the central role of endogenous testosterone in transcriptional activity during RET.

No study has previously examined the effect of RET adjuvant to depletion of testosterone in relation to muscle steroid-metabolism enzyme expression in younger human muscle. In our study, testosterone suppression attenuated AR mRNA and steroidogenic perhaps resulting in lower muscle testosterone processing capacity - presumably due to lower infiltration of testosterone from the circulation (Vingren et al. 2008). In our placebo group, the AR (Bhasin et al. 2003), apparently alters mRNA expression of thousands of target genes (Jiang et al. 2009), but is also associated with fibre type CSA increases (Roberts, Haun, et al. 2018), as well as triggering anabolic kinase signalling; i.e. Akt (Bennett et al. 2010), suggesting transcriptional links to augmented MPS, FFM and

strength in the present study and others (Roberts, Haun, et al. 2018; Sato et al. 2014; Willoughby and Taylor 2004; Brook, Wilkinson, Smith, et al. 2016). However, it has been reported depletion of endogenous testosterone has no effect on regulation of expression of AR, anabolic factors and myogenesis during RET (Kvorning et al. 2007b). Nevertheless, in addition to increases in testosterone metabolism-related mRNA expression, we demonstrated that myogenic and anabolic-related gene expression was blunted with RET with lower testosterone levels, but augmented with normal testosterone levels. Specifically, *IGF-1Ec* up-regulation is purported to correlate with transcriptional activity (Philippou et al. 2009) and initiation of satellite cell proliferation (Hameed et al. 2003), while *IGF1-Ea* expression is correlated with increased translation (Philippou et al. 2009) and promotion of myogenic differentiation (Hameed et al. 2003). Both intramuscular IGF-1 and testosterone independently stimulate AR and thereafter expression of other anabolic genes (Bennett et al. 2010; Bhasin et al. 2003; Jiang et al. 2009); therefore it is highly likely that an IGF-1 signaling axis played a role in the efficacy of T; and testosterone suppression likely downregulates this axis (Mauras et al. 1998). Here we also show suppression of testosterone was associated with attenuation of augmented expression of myogenic regulatory factors (MRFs) which is in line with lower whole body hypertrophy. This supports previous work (Bhasin et al. 2003; Singh et al. 2003a) demonstrating a pro-myogenic role for testosterone e.g. testosterone increased the transformation of pluripotent precursor cells down the myogenic lineage. It has been shown that single bouts of RE or

short-term RET are sufficient to increase the abundance of MRFs mRNA species in young participants (Vingren et al. 2008; Wilborn et al. 2009). However, in the present study, in line with past work in older age (Hameed et al. 2003; Kvorning et al. 2007b), in the Zoladex group, these regulatory genes did not change either after an acute bout of RE or after 6-wks RET with the exception of *C-met* and *C-myc* (which controls cell growth and ribosomal biogenesis (Brook, Wilkinson, Mitchell, et al. 2016)). We speculate this contributed to blunted adaptations to RET in Zoladex group with lower testosterone levels (Brook, Wilkinson, Mitchell, et al. 2016) while helping explain greater muscle mass accretion with normal testosterone levels group.

Despite the blunted adaptation to RET in Zoladex group compared to placebo group, we showed in our study groups, short term RET with/without testosterone depletion did not change mitochondrial adaptation considerably. In accordance, high load RET did not alter mitochondrial content in young untrained individuals (Wilkinson et al. 2008a), but 12-wk RET moderately increased protein levels of complex-I and II in younger men (Porter et al. 2015a). Further, despite increasing in FFM and strength after 8-wks RET in young men, PGC1-α levels and expression and mitochondrial content did not alter (Kon et al. 2014). Conversely, it has been reported that RET stimulated mitochondrial biogenesis, albeit to a relatively lesser extent than endurance training and depending on training principles (Wilkinson et al. 2008a; Vissing, McGee, Farup, Kjolhede, et al. 2013). 12-wks RET augmented mitochondrial

content (Balakrishnan et al. 2010), volume (Roberts, Romero, et al. 2018) and respiratory capacity (Porter et al. 2015b) in younger participants. In summary, no differences between placebo and Zoladex group in our study reveal lack of effects of short-term RET on mitochondrial adaptations and yielding insubstantial effects of short term endogenous testosterone deplation as well.

We conclude that short-term (6-wks) chemical testosterone depletion during RET blunted the responsiveness of younger muscle to RET. Short-term testosterone suppression via one-off GnRh analogue injection was also well-tolerated and could demonstrate the impact of lack of testosterone on attenuated muscle hypertrophy programming via blunted muscle anabolic signalling, testosterone processing enzyme, pro-myogenic gene expression and MPS. Nevertheless, it is important to be cognisant of potential adverse effects. GnRH analogue injection has been associated by (non-significant) increases in some disorders; e.g. gonadal development, Epilepsy and fertility problems (Tonini and Lazzerini 2000), albeit without any effect on systolic and diastolic hypertension over 6 months injections (Karamizadeh et al. 2013). In addition, no systemic side effects or major local allergic reactions have been recorded with GnRh analogue (Karamizadeh et al. 2013). In line with this, no adverse events were noted during or after completion of the present study in younger males. Finally, we reveal the likely mechanisms underlying the effect of testosterone in relation to aspects elevating protein turnover (with greater increases in MPS), enhancing translational efficiency and capacity, and

inducing pro-myogenic and testosterone handling gene regulation-thereby, demonstrating the, at least, permissive importance of endogenous testosterone in regulating muscle hypertrophy.

Published Chapter: Journal of Cachexia, Sarcopenia and Muscle (2019)

Chapter 4

Testosterone therapy induces molecular programming augmenting physiological adaptations to resistance exercise in older men

4.1 **Abstract**

Background: The andropause is associated with declines in serum testosterone (T), loss of muscle mass (sarcopenia) and frailty. Two major interventions purported to offset sarcopenia are anabolic steroid therapies and resistance exercise training (RET). Nonetheless, the efficacy, and physiological and molecular impacts of T therapy adjuvant to *short-term* RET remain poorly defined.

Methods: Eighteen non-hypogonadal healthy older men, 65-75 y, were assigned in a random double-blinded fashion to receive, bi-weekly, either placebo (P, saline, n=9) or T (Sustanon 250 mg, n=9) injections over 6-weeks whole-body RET (3-sets of 8-10 reps at 80% 1-RM). Subjects underwent dual-energy x-ray absorptiometry, ultrasound of vastus lateralis (VL) muscle architecture, and knee-extensor isometric muscle force tests; VL muscle biopsies were taken to quantify myogenic/anabolic gene expression, anabolic signalling, muscle protein synthesis (D_2O) and breakdown (extrapolated).

Results: T adjuvant to RET, augmented total fat free mass (FFM) (P=0.007), legs fat free mass (P=0.02), and appendicular FFM (P=0.001) gains, while decreasing total fat mass (P=0.02). Augmentations in VL muscle thickness, fascicle length, and quadriceps cross-section area with RET occured to a greater extent in T (P<0.05). Total strength (P=0.0009) and maximal voluntary contract (e.g. knee extension at 70°) (P=0.002) increased significantly more in the T group. Mechanistically, both muscle protein synthesis rates (T: 2.13±0.21%·day^{-1} vs. P: 1.34±0.13%·day^{-1}, P=0.0009) and absolute breakdown rates (T: 140.2±15.8 vs. P: 90.2±11.7g·day^{-1}, P=0.02) were elevated with T therapy, which led to higher net turnover and protein accretion in the T group (T: 8.3±1.4g·day^{-1} vs. P: 1.9±1.2 g·day^{-1}, P=0.004). Increases in ribosomal

biogenesis (RNA:DNA ratio); mRNA expression relating to T metabolism (*Androgen Receptor*: 1.4-fold; *Srd5a1*: 1.6-fold; *AKR1C3*: 2.1-fold; *HSD17β3*: 2-fold*)*; IGF-1-signalling (*IGF-1Ea* (3.5-fold), *IGF-1Ec* (3-fold) and myogenic regulatory factors (MRF); as well the activity of anabolic signalling (e.g. mTOR, AKT, RPS6; $P<0.05$) were all upregulated with T therapy. Only T up-regulated mitochondrial citrate synthase activity ($P=0.03$) and transcription factor A (*Tfam*) (1.41±0.2-fold, $P=0.0002$), in addition to PGC1-α mRNA (1.19±0.21-fold, $P=0.037$).

Conclusions: Administration of T adjuvant to RET enhanced skeletal muscle mass and performance, while upregulating myogenic gene programming, myocellular translational efficiency and capacity - collectively resulting in higher protein turnover, and net protein accretion. T coupled with RET is an effective short-term intervention to improve muscle mass/ function in older non-hypogonadal men.

4.2 Introduction

Age-related declines in muscle mass and strength (sarcopenia) (Mitchell, Williams, et al. 2012) are associated with mitochondrial abnormalities (Groennebaek and Vissing 2017), fatigue, lack of energy, loss of libido, decreased sexual performance (Stanworth and Jones 2008) and work capacity (Borst and Yarrow 2015). Many of these symptoms are accompanied by declines in testosterone (T) bioavailability – the "andropause" (Ferrando et al. 2002c). Moreover, hypogonadal males exhibit a 3-fold increase in mortality and a 5-fold increased risk of cancer-related deaths (Hackett 2016). Thus, as the ageing populace grows, there remains a need for therapies to counteract the andropause and associated muscle wasting and dysfunction.

Clinically, older males with a total testosterone of <231 ng.dl-1 may be offered testosterone therapy (Hackett 2016) due to established positive effects on fat-free mass (Gruenewald and Matsumoto 2003), fat mass (Svartberg et al. 2008), muscle function (Ferrando et al. 2002c) and mitochondrial biogenesis (Usui et al. 2014). Intramuscular testosterone injections offers a safer more efficacious treatment than oral or transdermal testosterone (Hackett 2016). Indeed, dose-dependent increases in type I and type II fibre areas were found in relation to testosterone injection titrations (25-600 $mg \cdot wk^{-1}$) in men >65 y (Sinha-Hikim et al. 2006a). In addition, 6-months testosterone injection (~210-mg biweekly) enhanced net protein balance (Ferrando et al. 2002c), muscle protein synthesis (MPS) (Brodsky, Balagopal, and Nair 1996), fat free

mass (FFM) (Brodsky, Balagopal, and Nair 1996; Ferrando et al. 2002c) and muscular performance (Ferrando et al. 2002c) (albeit through ill-defined mechanisms). However, the therapeutic efficacy of testosterone injection in older men remains controversial, and likely, non-beneficial with short-term physiological dosing (at least in the absence of co-existing countermeasures). For example, ≤6-weeks (wks) testosterone therapy did not improve MPS, RNA content (Lobley et al. 1990), FFM (transdermal 50 mg·day-1) (Ragnarsson et al. 2013), or strength (200 mg biweekly for 12-wks) (Liu, Swerdloff, and Veldhuis 2004; Clague, Wu, and Horan 1999). Besides administering longer-term pharmacologic doses of exogenous testosterone, interventions that boost endogenous sex hormones i.e. exercise, might be of greater benefit (Hawkins et al. 2008).

In relation to strategies to overcome age-related muscle dysfunction, the effects of resistance exercise training (RET) on skeletal muscle are well defined. RET promotes muscle anabolism (even in old age (Ribeiro et al. 2017; Phillips et al. 2017)) and stimulates mitochondrial adaptations (Groennebaek and Vissing 2017); these effects are also associated with enhanced endogenous testosterone in response to acute bouts of RE (Rønnestad, Nygaard, and Raastad 2011). Nonetheless, hypertrophic responses to RET are blunted in older age (vs. younger cohorts), so called “anabolic resistance”, suggesting the need for strategies beyond, or adjuvant to RET (Rønnestad, Nygaard, and Raastad 2011), in order to maximise efficacy. We previously showed that acute RET-induced systemic induction of endogenous testosterone was related to ensuing muscle hypertrophy in younger men (Brook, Wilkinson, Mitchell, et al.

2016), suggesting a link between testosterone and muscle mass gains with RET. Moreover, in keeping with a role for testosterone as an adjuvant therapy to RET in older age, 50-100 mg·day-1 transdermal testosterone therapy, enhanced RET-induced muscle mass gains over 12-wks (Glintborg et al. 2013), while supra-physiological doses (600 mg·week-1) of testosterone adjuvant to RET for 10-wks increased FFM and strength (in younger men (19-40 y) (Bhasin et al. 1996a). Such 'medium-term' use of testosterone therapy is indicated for minimising side-effects (Wentzensen and Trabert 2015) of longer-term testosterone therapy e.g. ≥24-wks (Rolf and Nieschlag 1998) and may be useful for pre-habilitation/rehabilitation (e.g. from bed-rest). That said, RET interventions of ~12-wks still engender a major time burden from the perspective of physiotherapy, and engender minimal anthropometrical benefits in the absence of adjuvant therapy, even after 24-wks in older men (Glintborg et al. 2013).

We have previously shown that the majority of muscle mass gains with RET occur early into RET (Brook et al. 2015; Brook, Wilkinson, Mitchell, et al. 2016; Brook, Wilkinson, Smith, et al. 2016), albeit that this is blunted in older individuals. Therefore, in an effort to identify a more time- and clinically-efficient strategy of testosterone therapy, and to determine the mechanisms of testosterone therapy adjuvant to RET in relation to anabolic resistance, we investigated the efficacy and cellular mechanisms of short-term RET, coupled to adjuvant testosterone therapy in older men. In a double-blind investigation into the effects 6-wks of RET with or without

testosterone therapy, we determined end-points relating to muscle mass, function, myogenic regulation, anabolic signalling and protein turnover.

4.3 **Methods**

The study protocol and all the methods relating to this section have been previously described in chapter 2.

4.4 **Results**

Physiological characteristics of participants are shown in Table 4-1. In accordance, only the testosterone study group significantly increased weight (P=0.006, 95% confidence interval (CI)=81-83, ES=0.19) and BMI (P=0.006, 95%CI=26-26.6) after 6-wks of RET; primarily through significant gains in FFM in the testosterone group vs. baseline (see body composition section below). Further, total plasma testosterone concentrations in the testosterone therapy group were significantly higher than in the placebo group at all time-points after baseline (P<0.05); i.e. 234.9±7 $ng.dl^{-1}$ at baseline and achieved 973±54 at 1-wk and 1149±75 $ng.dl^{-1}$ after 5-wks in testosterone group (95%CI=1044-1232). Plasma testosterone concentrations during the study are shown in Figure 4-1.

Table 4-1 Participants Characteristics

	T(n=9)		P(n=9)	
	Baseline	**Week 6**	**Baseline**	**Week 6**
Age (y)	69.7 (0.8)	—	69.5 (1.3)	—
Height (m)	1.75 (0.02)	—	1.76 (0.02)	—
Weight (kg)	78.6 (3.1)	80.4 (3.2)[a]	81.8 (4.3)	82.4 (4.1)
BMI (kg.m^{-2})	25.7 (1)	26.2 (1)[a]	26.5(1.1)	26.7 (1.1)

Values are means (SEM). **a** Significantly different from baseline, P<0.05.

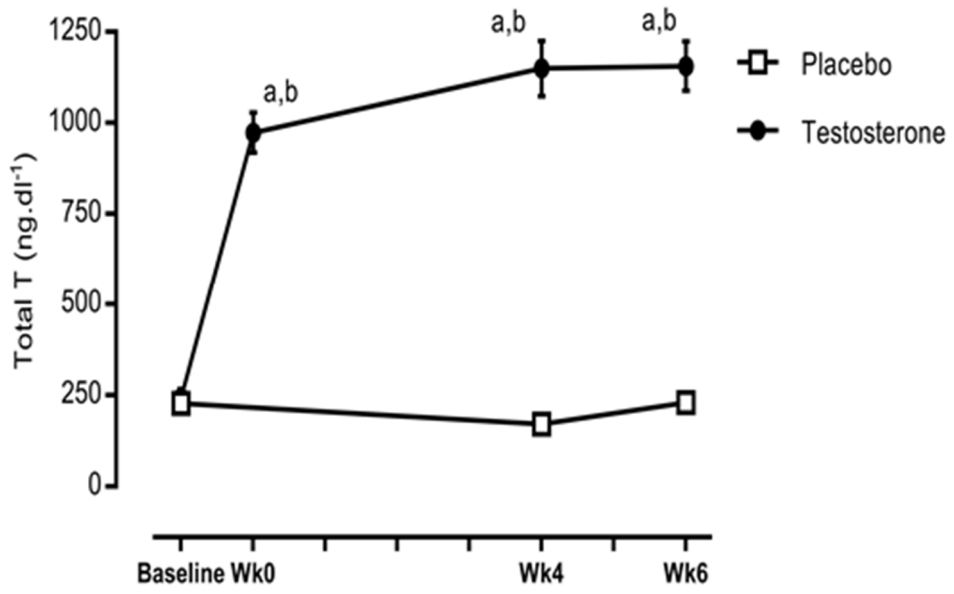


Figure 4-1 Schematic diagram of the study protocol

4.4.1 Body composition

Total FFM (ICC=0.94) increased significantly following 6-wks RET in testosterone (53.0±1.7 to 56.0±5.2 kg, P=0.007, 95%CI=55.5-57.6, ES=0.45, Figure 4-2A), but not in placebo (54.1±6.3 kg to 54.8±5.8 kg, 95%CI=53.2-55.4). Likewise, only testosterone augmented legs fat free mass (17.2±0.7 kg to 18.1±0.8 kg, 95%CI=17.8-18.6 vs. 17.4±0.8 kg to 17.6±0.7 kg, 95%CI=17.1-17.9, P=0.02, ES=0.30, ICC=0.96, Figure 4-2B), appendicular FFM (7.8±0.3 to 8.3±0.3, 95%CI=8.2-8.5 vs. 7.9±0.3 to 8.0±0.3, 95%CI=7.7-8.1, P=001, ES=0.54, ICC=0.94, Figure 4-2D) and FFM index (17.2±0.4 kg.m-2 to 18.2±0.4 kg.m-2, 95%CI=18.1-18.7 vs. 17.5±0.6 kg.m-2 to 17.8±0.5 kg.m-2, 95%CI=17.3-18.1, P=005, ES=0.42, ICC=0.92, Figure 4-2C) after RET. In addition, both TFP (29.7±1.7 % to 27.5±1.4 %, 95%CI=27-28.8 vs. 30.6 % to 30.2 %, 95%CI=29-30.7, P=0.004, ICC=0.96, ES=0.43) and TFM (22.5±2.0 kg to 21.3±1.8kg, 95%CI=21.6-23.1 vs. 24.7±2.8kg to 24.5±2.7kg, 95%CI=22.8-24.2, P=0.02, ICC=0.97, ES=0.29) decreased significantly in testosterone, but not in placebo (Figure 4-2E-F).

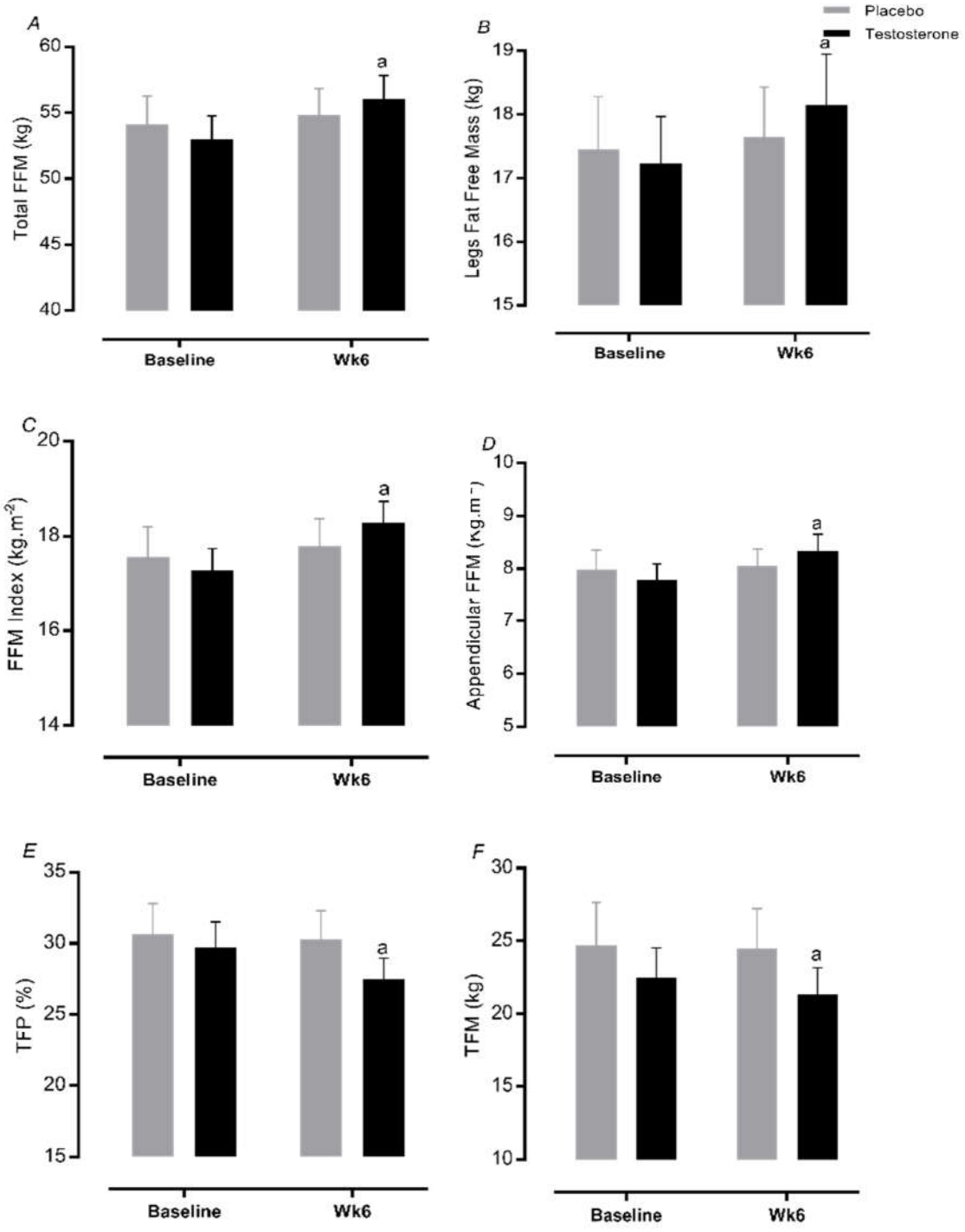


Figure 4-2 Muscle mass and body composition changes. (A–F). Values are means ± standard error of the mean. a Significantly different from baseline, $P < 0.05$. all from two-way. FFM, fat-free mass; TFP, total fat percentage; TFM, total fat mass.

4.4.2 Muscle architecture

Over the RET period, testosterone and placebo both exhibited increases in MT (2.3±0.07 to 2.6±0.04 cm, 95%CI=2.5-2.6, P<0.0001, ES=0.83 vs. 2.3±0.1 to 2.4±0.09 cm, 95%CI=2.4-2.5, P<0.0001, ICC=0.81, ES=0.47), Lf (7.1±0.2 to 7.9±0.2 cm, 95%CI=7.9-8.2, P<0.0001, ES=0.84 vs. 7.7±0.1 to 8.1±0.1 cm, 95%CI=7.7-8, P<0.0001, ES=0.73) PA (20.0±0.8 to 23.1±0.7°, 95%CI=21.2-22.6, P<0.0001, ES=1.2 vs. 17.0±1.2 to 20.3±0.9°, 95%CI=20.7-22.1, P<0.0001, ES=0.88) and CSA of the quadriceps (61.4±3.1 to 69.9±2.6 cm2, 95%CI=66.2-70.9, P<0.0001, ES=0.93 vs. 58.8±4.1 to 64±4.8 cm2, 95%CI=62.9-67.7, P<0.0001, ES=0.41, ICC=0.89). The majority of increases were to a greater extent in the testosterone group, and with significant differences between groups in MT (P=0.01), Lf (P=0.001), and CSA (P=0.04) (Figure 4-3A-C).

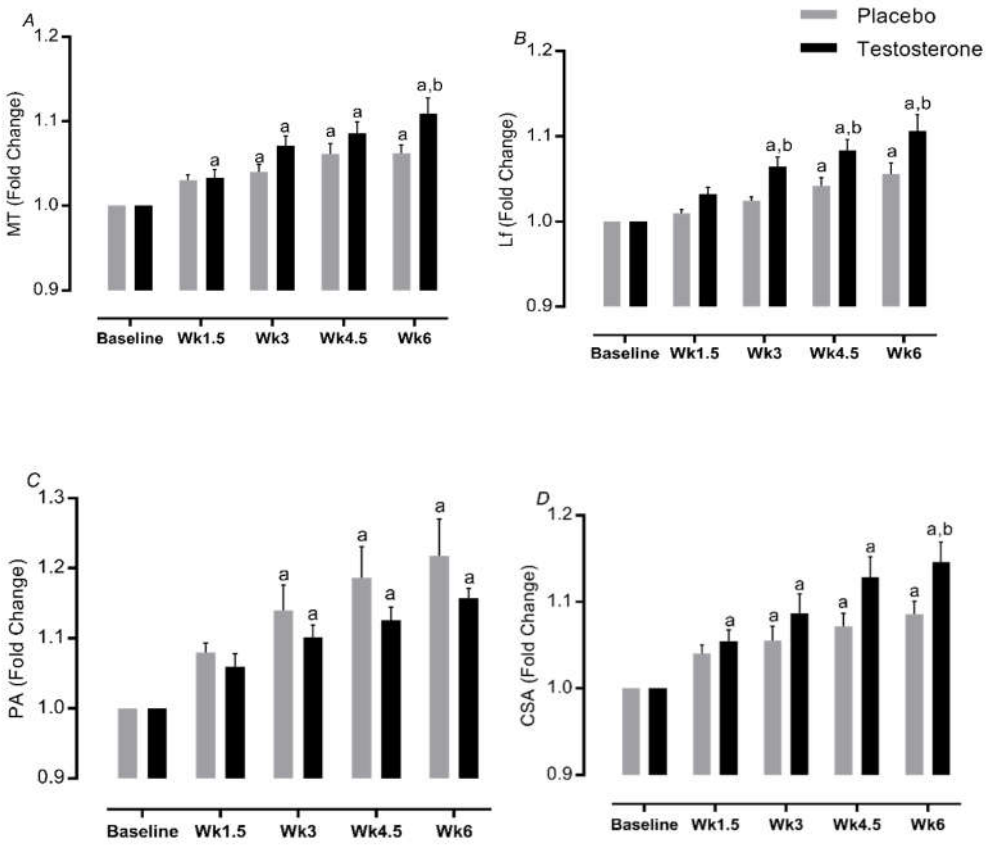


Figure 4-3 (A–D) Fold change in muscle architecture. Values are means ± standard error of the mean. a Significantly different from baseline, $P < 0.05$; b significantly different between two groups, $P < 0.05$. all from repeated-measure ANOVA. CSA, cross-section area; Lf, fascicle length; MT, muscle thickness; PA, pennation angle.

4.4.3 Fibre type CSA

Type I and IIa CSA increased in both testosterone and placebo groups after RET (P<0.05), but only testosterone augmented type IIx (3455±187 to 4576±338 μm2, 95%CI=3814-5659 vs. 3751±478 to 3797±767 μm2, 95%CI=2560-4630, P=0.02, ES=0.64, Figure 4-4A-C).

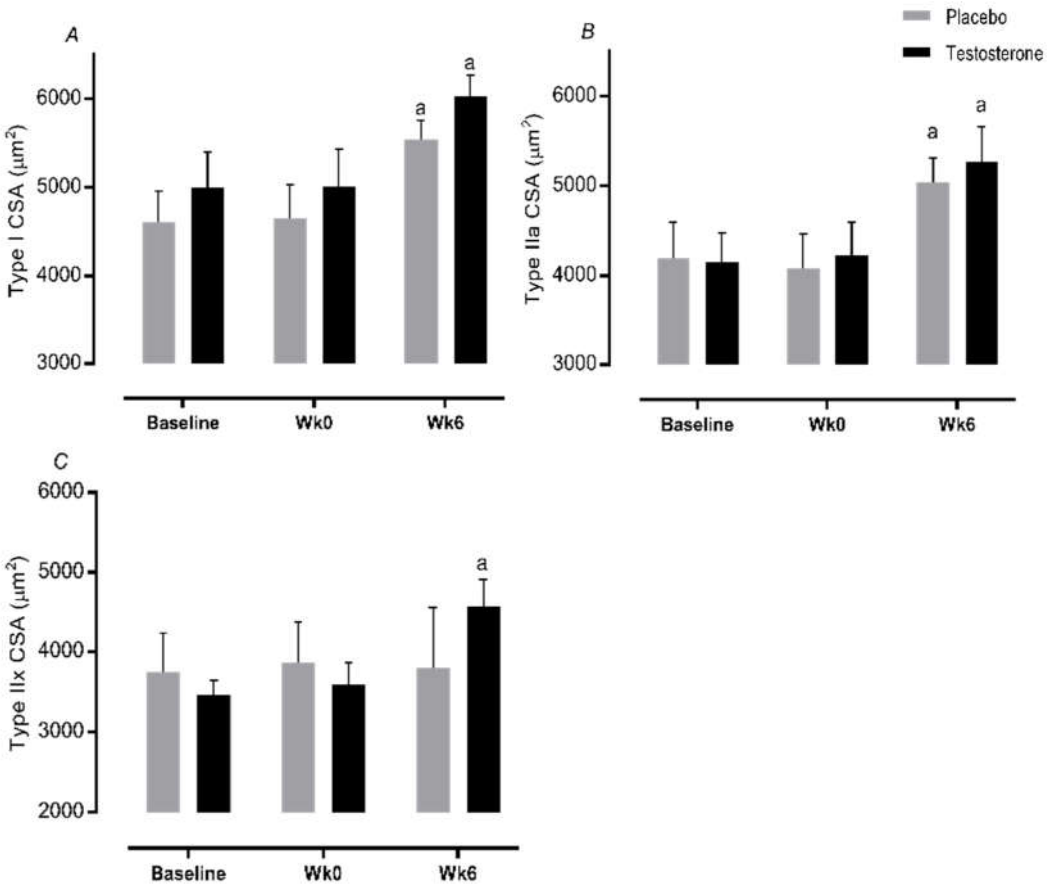


Figure 4-4 (A–C) Time course of changes in fibre-type cross-section area (CSA). Values are means ± standard error of the mean. a Significantly different from baseline, P < 0.05; b significantly different between two groups, P < 0.05. all from repeated measure ANOVA.

4.4.4 Muscular performance

In line with higher levels of testosterone (P<0.0001, Figure 2), only the testosterone group showed a greater increase in static strength after RET e.g. MVC at 70° (169.9±9.6 Nm to 209±11.8 Nm, 95%CI=11.5-66.6, P=0.002, ES=1.3, Figure 4-5B) and 80° (169.4±7.3 Nm to 191.7±12.5 Nm, 95%CI=3.8-40.5, P=0.01, ES=1.01, Figure 4-5C) vs. baseline. Furthermore, dynamic strength (total 1-RM across all six exercises) was augmented in to a greater extent in the testosterone group vs. placebo (60.8±3.6% vs. 43.25±2.4%, 95%CI= T: 54.2-67.7 vs. P: 36.7-49.7, P=0.0009, ES=0.96, Figure 4-5A). There were similar increases in specific strength, (force per unit area) across the groups (95%CI= T: 10.4-12.4; P: 10.1-11.9, ES=T: 0.88; P: 0.65, P<0.05, Figure 4-5D). Finally, FFM gains were correlated with total strength gains in the testosterone study group; but not in placebo group (Figure 4-5E).

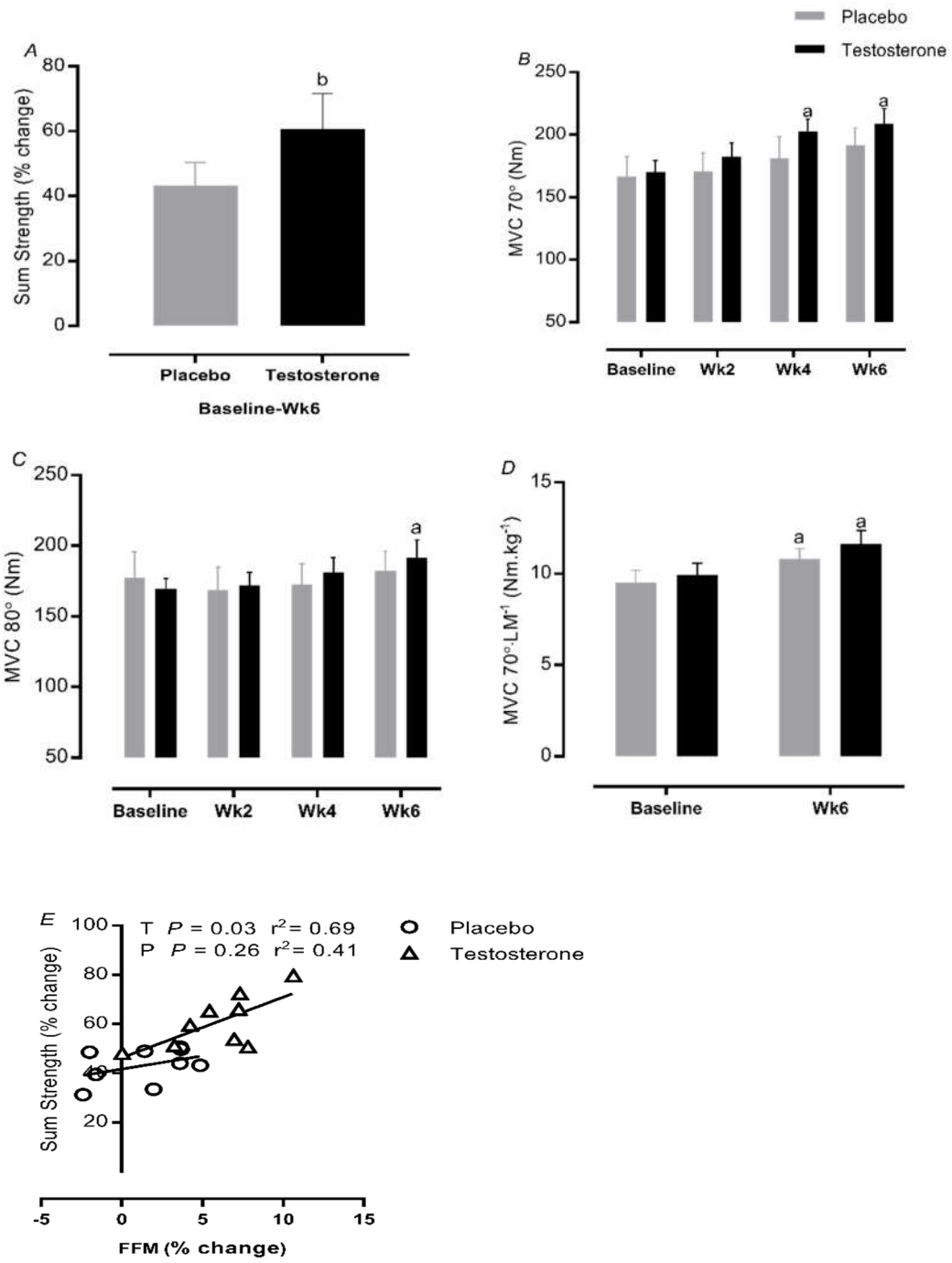


Figure 4-5 Time course of changes in (A) sum strength and (B–D) muscle strength and (E) correlation between fat-free mass (FFM) and sum strength. Values are means ± standard error of the mean. a Significantly different from baseline, $P < 0.05$; b significantly different between two groups, $P < 0.05$. LM, legs fat-free mass; MVC, maximal voluntary contraction. A: independent t-test, B and C: repeated measure and D: two-way ANOVA.

4.4.5 Muscle protein synthesis and [extrapolated] breakdown

There was a significant difference in cumulative myofibrillar MPS between testosterone and placebo during RET (2.13±0.21 %·day−1 vs. 1.34±0.13 %·day−1, 95%CI=1.7-2.4 vs. 1.1-1.8, P=0.0009, ES=0.75); in line with findings of elevated FGR in testosterone compared to placebo (0.12±0.02 %·day−1 vs. 0.03±0.01 %·day−1, 95%CI=0.07-0.16 vs. -0.12-0.07, P=0.005, ES=0.85) (Figure 4-6A-B). Furthermore, ASR was significantly increased as a result of RET in testosterone (87.8±5.1 to 148.5±15.3 g·day-1, 95%CI=120-176, P=0.02, ES=0.31), but not in placebo (84.7±4.7 to 92.2±11.3 g·day-1, 95%CI=72-128, P>0.05). In addition, estimated ABR was significantly higher in testosterone than placebo during RET (140.2±15.8 testosterone vs. 90.2±11.7 g·day-1, 95%CI=112-168 vs 70-126, P=0.02, ES=0.55). Finally, when comparing ASR and ABR, net protein turnover in the testosterone group was higher than the placebo group (8.3±1.4 g·day-1 vs. 1.9±1.2 g·day-1, 95%CI=5.4-11.2 vs. -0.9-4.8, P=0.004, ES=0.86, Figure 4-6C-E). Using D3-3-MH, there was no significant difference in MBP between testosterone and placebo at 6-wks over the 4 h sampling period (T: 0.043±0.012 vs. P: 0.032±0.004 k·h-1, 95%CI= T:0.02-0.06 vs. P:0.01-0.05, P=0.5) (Figure 4-6F).

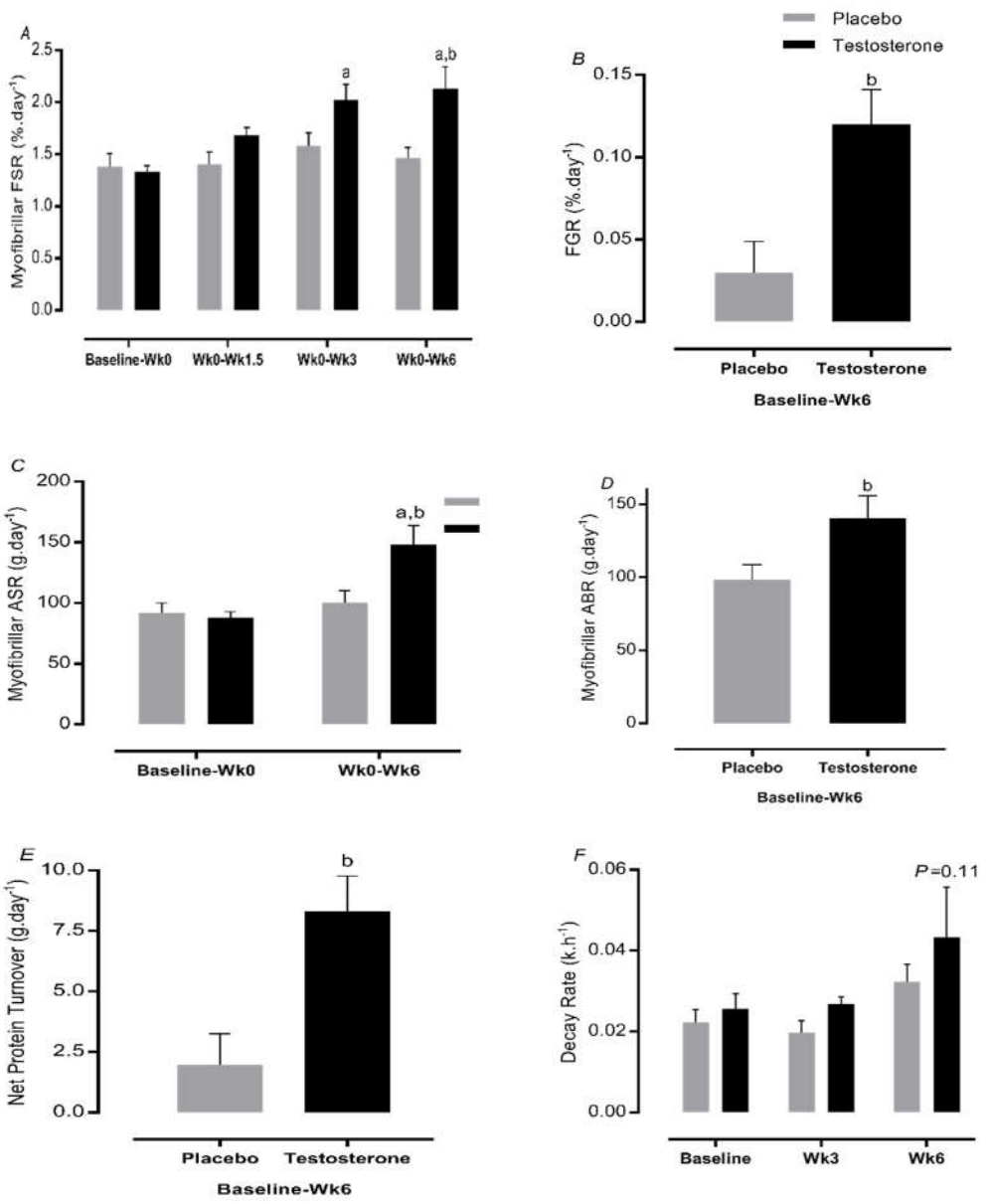


Figure 4-6 Muscle protein synthesis, fractional growth rate (FGR), absolute synthetic rate (ASR), absolute breakdown rate (ABR), and protein turnover. Values are means ± standard error of the mean. a Significantly different from baseline, $P < 0.05$; b significantly different between two groups, $P < 0.05$; $P < 0.11$ Week 6 vs. baseline in testosterone group. B, D, E: independent t-test, A, C, F: two-way ANOVA. FSR, fractional synthetic rate.

4.4.6 Muscle ASP, DNA and RNA content

There were no changes in ASP or DNA concentrations per wet weight muscle (μg·mg-1) in testosterone or placebo with RET; nonetheless, total RNA content (95%CI=T:2.1-2.5; P;1.6-2.1, P=0.002, ES=0.78) and RNA:DNA ratio, a reflection of ribosomal capacity for protein synthesis, only increased in the testosterone group (95%CI=T:1.1-1.2; P;0.7-1, P=0.002, ES=0.83). Similarly, RNA:ASP ratio, primarily a measure of ribosomal capacity (Brook, Wilkinson, Mitchell, et al. 2016), increased in testosterone (95%CI=17.8-23.5, P=0.002, ES=0.24) but not placebo (P=0.6, 95%CI=15.2-20.9) over the RET phase. Finally, the ratio of ASP:DNA, a measure of cell size did not change in either group (Table 4-2).

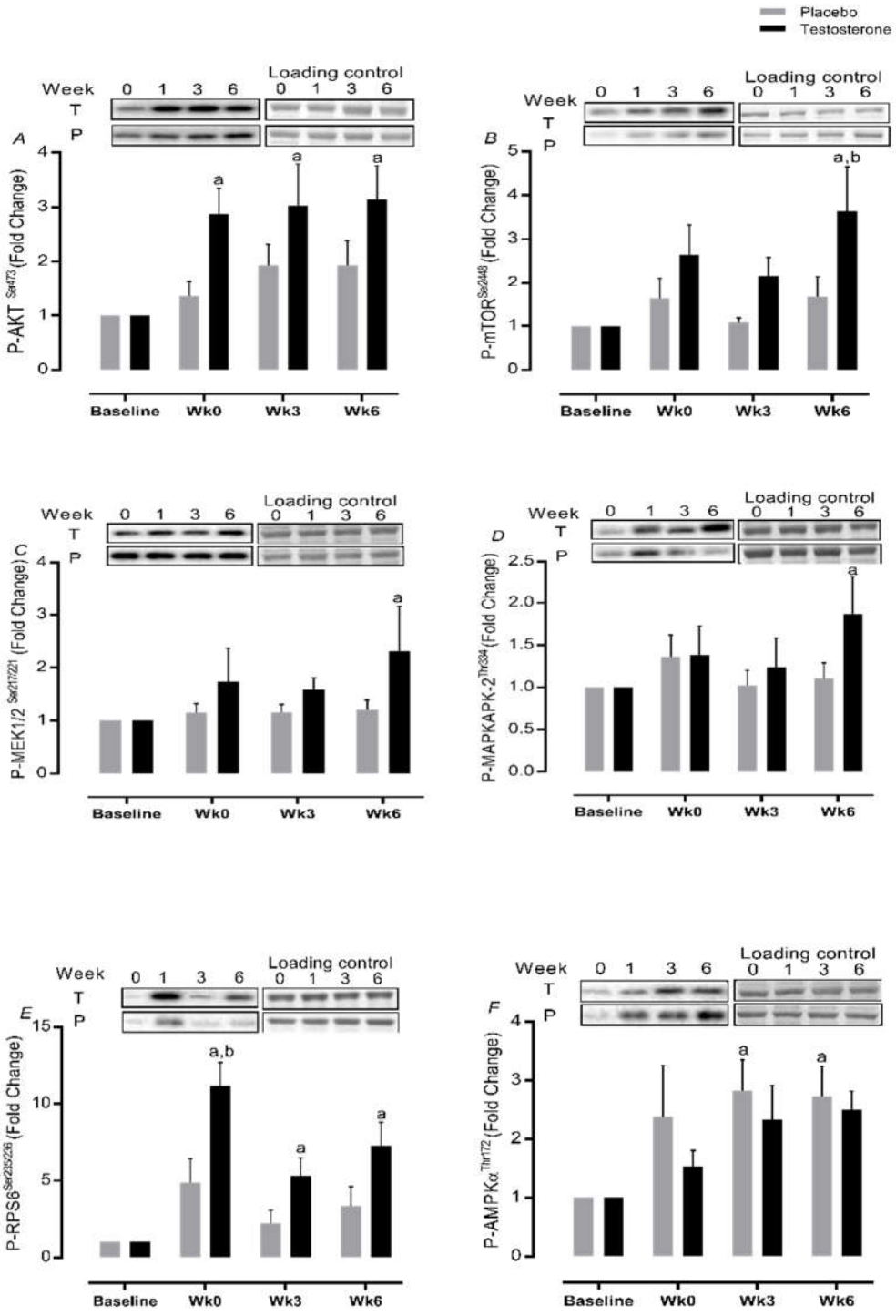
Table 4-2 Muscle protein, RNA, DNA and related ratios

	Placebo				**Testosterone**			
	Baseline	WK0	WK3	WK6	Baseline	WK0	WK3	WK6
ASP Content (μg·mg^{-1}ww)	110.5(3.9)	108.5(2.8)	107.4(4.9)	106.9(4.5)	118.2(3.7)	115.3(3.4)	118.2(2.7)	118.5(4.6)
RNA Content (μg·mg^{-1}ww)	1.6(0.1)	1.8(0.1)	2.1(0.1)	1.9(0.1)	1.7(0.1)	2.1(0.1)	1.8(0.1)	2.3(0.1)[a]
DNA Content (μg·mg^{-1}ww)	2.2(0.1)	2.3(0.1)	2.2(0.1)	2.2(0.1)	2.2(0.1)	2.2(0.1)	2.1(0.1)	2.1(0.1)
RNA:DNA (Ratio)	0.7(0.1)	0.7(0.1)	0.9(0.1)	0.8(0.1)	0.7(0.1)	0.9(0.1)	0.8(0.1)	1.1(0.1)[a]
RNA:ASP (Ratio)	15.3(0.5)	17.1(0.8)	19.9(1.7)	18.1(0.9)	14.6(0.9)	17.8(1.5)	15.5(0.6)	20.7(1.5)[a]
ASP:DNA (Ratio)	50.7(3.6)	46.1(1.7)	49.2(3.8)	47.9(3.1)	54.2(2.4)	52.6(2.4)	56.5(2.4)	56.6(5.2)

Values are means (SEM). ww: wet weight. **a** Significantly different from baseline, $P<0.05$; all from repeated measure ANOVA.

4.4.7 Intramuscular signalling pathways

Acute exercise-induced anabolic signalling was quantified 60 min after the first (1-wk), 9th (3-wks), and 18th (6-wks) resistance exercise sessions. We observed significant increases in the acute phosphorylation of AKT^{Ser473} (95%CI=2-4.2 vs. 0.7-3, P=0.0006, ES=0.66), $mTOR^{Ser2448}$ (95%CI=1.9-5.3 vs. 0.1-3.3, P=0.001, ES=0.0.64), $MEK1/2^{Ser217/221}$ (95%CI=1.1-3.6 vs. -0.1-2.5 , P=0.02, ES=0.43), $MAPKAPK\text{-}2^{Thr334}$ (95%CI=1.1-2.6 vs. 0.3-1.8, P=0.03, ES=0.32), and $RPS6^{ser240/244}$ (95%CI=4.2-10.2 vs. 0.3-6.3, P=0.001, ES=0.44) in testosterone (6-wks vs. baseline), but not in P. In contrast to this, the placebo group increased activation of $AMPK\alpha^{Thr172}$ (95%CI=1.5-3.4 vs 1.8-3.6, P=0.02, ES=0.25) and $Raptor^{Ser792}$ (95%CI=0.24-1.6 vs. 1.1-2.4, P=0.01, ES=0.84) after 6-wks RET. Furthermore, $FoxO3a^{Ser253}$ phosphorylation (P=0.01) was increased only after the first exercise bout in the placebo group. The relative abundance of MAFbx and MURF-1 was unchanged throughout the study (Figure 4-7).


Placebo
Testosterone
Week 0 1 3 6 Loading control 0 1 3 6
T
P
A
P-AKT Ser473 (Fold Change)
B
P-mTOR Ser2448 (Fold Change)
C
P-MEK1/2 Ser217/221 (Fold Change)
D
P-MAPKAPK-2 Thr334 (Fold Change)
E
P-RPS6 Ser235/236 (Fold Change)
F
P-AMPKα Thr172 (Fold Change)
Baseline
Wk0
Wk3
Wk6
a
a,b

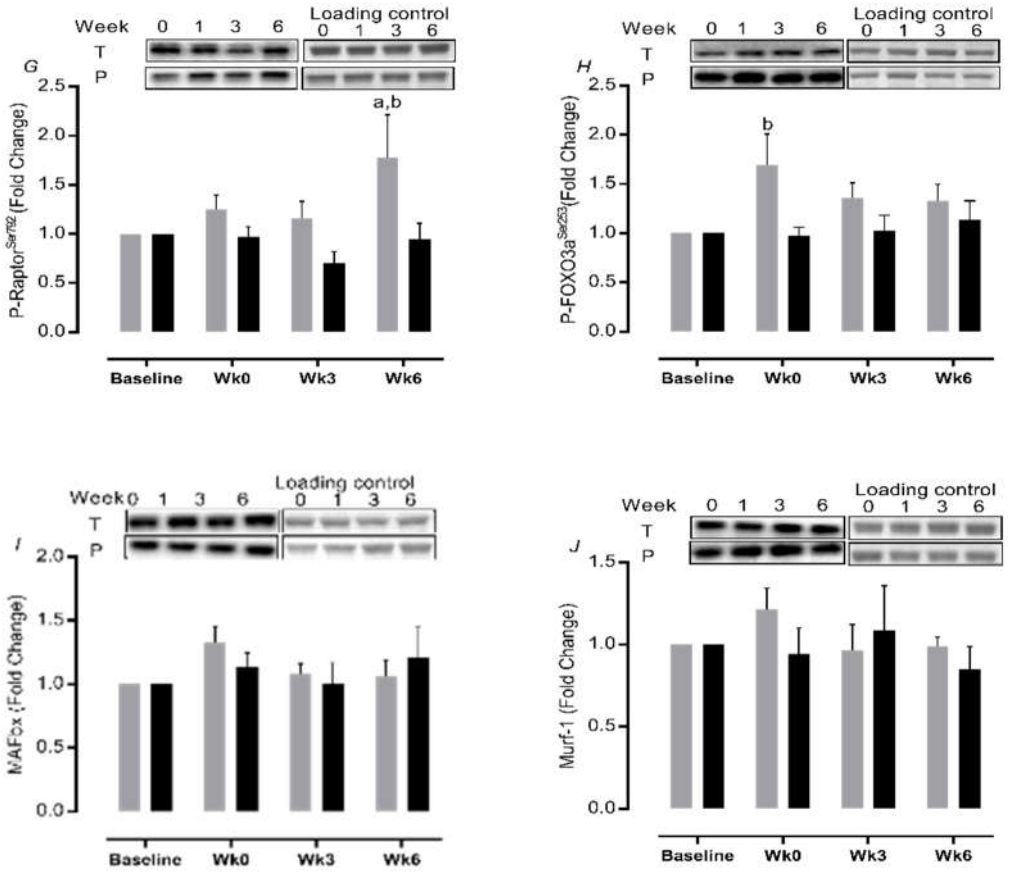
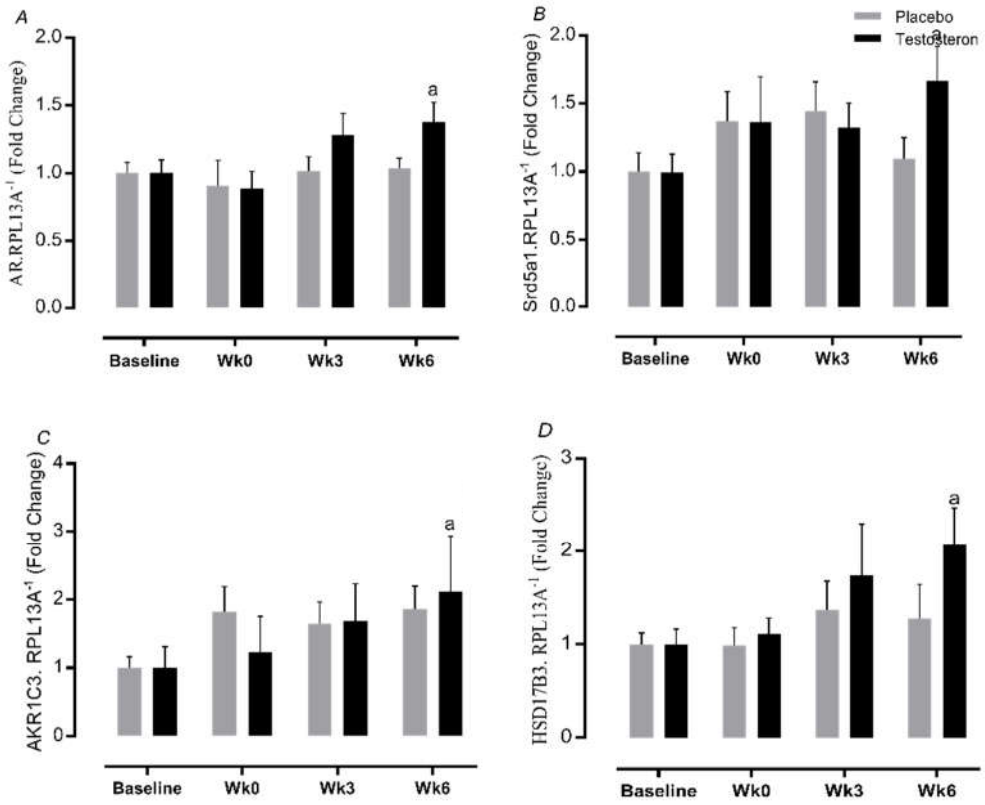


Figure 4-7 Intramuscular signalling pathways. Values are means ± standard error of the mean. a Significantly different from baseline, $P < 0.05$; b significantly different between two groups, $P < 0.05$. all from repeated measure ANOVA.

4.4.8 Muscle gene expression

The expression of a number of genes involved in testosterone metabolism was augmented in testosterone vs. baseline, but not in the placebo group, i.e. androgen receptor (AR) (1.4±0.1-fold change, 95%CI=1.2-1.5 vs 0.84-1.2, P=0.01, ES=0.71), *Srd5a1* (1.6±0.2-fold change, 95%CI=1.3-1.9 vs. 0.76-1.4, P=0.01, ES=0.7), HSD17β3 (2±0.4-fold change, 95%CI=1.3-2.7 vs. 0.5-1.9, P=0.03, ES=0.33) and AKRIC3 (2.1±0.8-fold change, 95%CI=1.2-3.1 vs. 0.9-2.7, P=0.01, ES=0.14, Figure 4-8 A-D) increased

after 6-wks RET only in T. In addition, IGF-1Ea (3.5±0.07-fold change, 95%CI=2.3-4.7 vs. 0.4-2.8, P<0.0001, ES=0.63), IGF-1Ec (3±0.4-fold change, 95%CI=2.3-3.7 vs. 0.8-2.2, P<0.0001, ES=0.83) expression increased only in testosterone after 6-wks RET (Figure 4-8E-F). While mRNA expression of MHCI decreased in both groups (P<0.05), the expression of MHCIIa was augmented only in testosterone (1.72±0.3-fold change, 95%CI=1.2-2.2 vs. 0.6-1.7, P=0.02, ES=0.33) (Figure 4-8G-H). testosterone coupled with RET augmented myogenesis-related gene expression i.e. Myogenin (2.6±0.7-fold change, 95%CI=1.3-4.1 vs. 0.1-2.7, P=0.02, ES=0.53), C-Myc (7.2±1.1-fold change, 95%CI=5.3-9.1 vs. 4.3-8.3, P=0.04, ES=0.28), Myf-6 (3.9±0.5-fold change, 95%CI=3.1-4.7 vs. 1.1-2.8, P<0.0001, ES=0.92), MEOX-2 (2.1±0.4-fold change, 95%CI=1.3-2.8 vs. 0.7-2.2, P=0.01, ES=0.19), C-met (1.7±0.1-fold change, 95%CI=1.4-1.9 vs. 0.8-1.4, P=0.003, ES=0.89), but not PAX-7 (p>0.05) as a marker of satellite cell proliferation (Figure 4-8I-N).



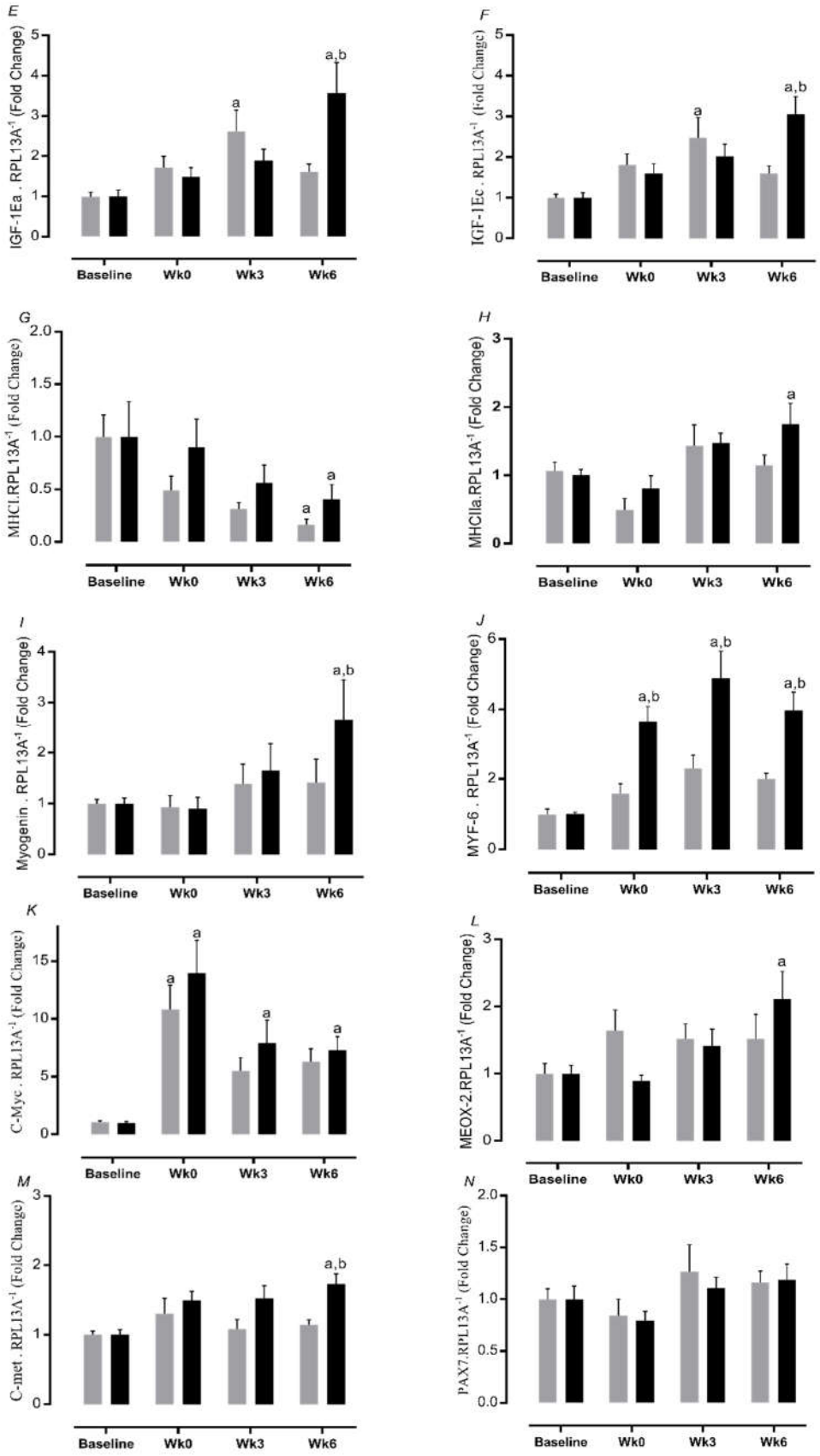
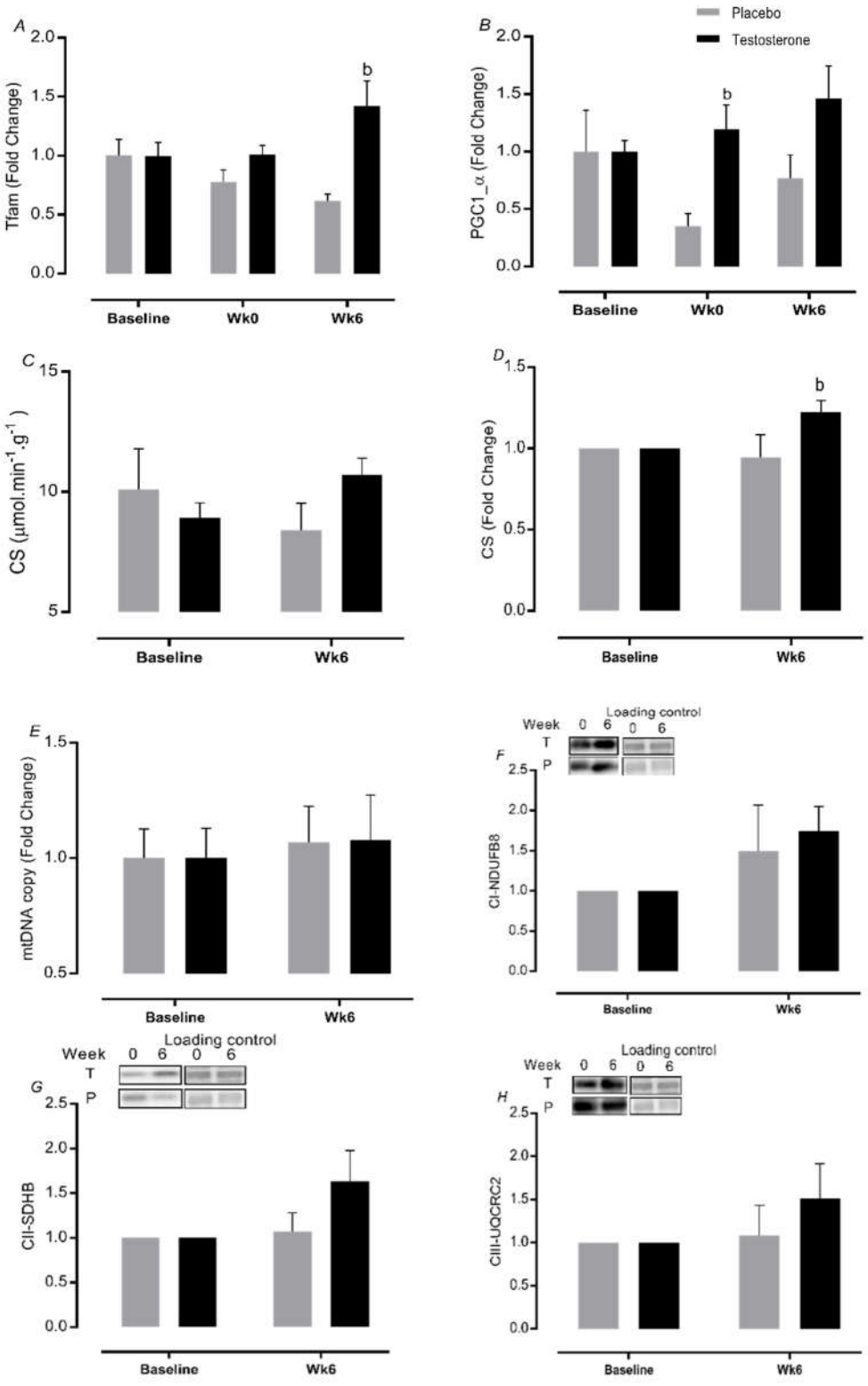


Figure 4-8 Muscle gene expression. Changes in testosterone and placebo in (A–D) testosterone metabolism gene expression, (E, F) anabolism, (G, H) fibre type, and (I–N) myogenic from baseline to Week 6. Values are means ± standard error of the mean. a Significantly different from baseline, $P < 0.05$; b significantly different between two groups, $P < 0.05$. all from repeated measure ANOVA.

4.4.9 Mitochondrial oxidative phosphorylation capacity

T augmented mitochondrial transcription factor A (Tfam) expression at 6 weeks (1.4±0.2-fold change, 95%CI=1.1-1.7 vs. 0.2-0.9, P=0.0002, ES=0.91), whereas expression of peroxisome proliferator-activated receptor γ co-activator 1-α (PGC1-α) was increased only after the first bout of exercise (1.2±0.2-fold change, 95%CI=1.1-1.8 vs. 0.3-1.1, P=0.037, ES=0.7, Figure 4-9A-B). In addition to increased mitochondrial density (assessed by relative change of CS activity) in the testosterone group (1.22±0.07-fold change, 95%CI=0.9-1.4 vs. 0.7-1.1, P=0.03, ES=0.29, Figure 4-9D), only the testosterone group showed enhanced protein levels of OxPhos complex (C-) IV (95%CI=1.2-2.6 vs. 0.3-1.7, P=0.02, ES=0.41) and V (95%CI=0.9-2.2 vs. 0.1-1.3, P=0.04, ES=0.23), while there was a trend in C-II (P=0.08), but not in C-I (P=0.24) or -III (P=0.3) (Figure 4-9F-J). There was no impact of RET or testosterone therapy upon mtDNA copy number (P>0.05, Figure 4-9E).


A
Tfam (Fold Change)
2.0
1.5
1.0
0.5
0.0
b
Baseline
Wk0
Wk6
B
Placebo
Testosterone
PGC1_α (Fold Change)
b
C
CS (µmol.min-1.g-1)
15
10
5
D
CS (Fold Change)
b
E
mtDNA copy (Fold Change)
F
Loading control
Week 0 6 0 6
T
P
CI-NDUFB8
G
CII-SDHB
H
CIII-UQCRC2

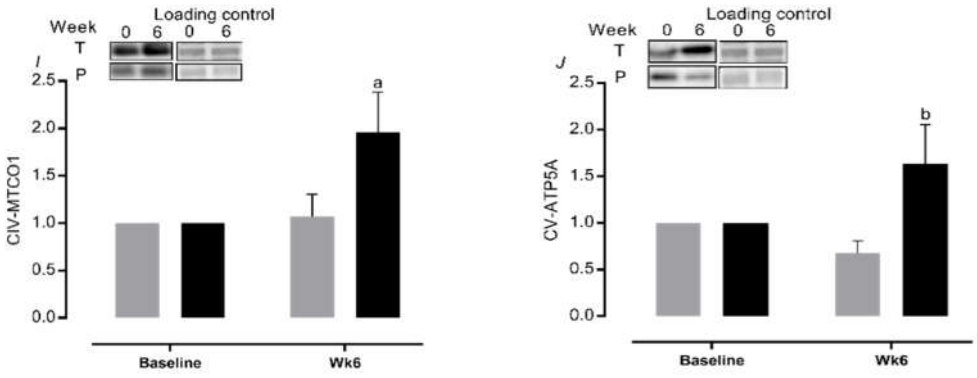


Figure 4-9 Mitochondrial oxidative phosphorylation capacity markers. Values are means ± standard error of the mean. a Significantly different from baseline, $P < 0.05$; bsignificantly different between two groups, $P < 0.05$. all from two-way ANOVA

4.5 **Discussion**

There is a great deal of interest in identifying interventions that will combat muscle wasting in older age. Herein, we elucidated that just 6-wks whole-body RET coupled with the exogenous provision of testosterone had significant positive effects on FFM, and muscular performance via inducing net protein accretion through anabolic pathways - thus offsetting age-related deficits in adaptive responses to RET (Brook, Wilkinson, Mitchell, et al. 2016).

The role of testosterone in regulating muscle gains when coupled with RET remains somewhat contentious. For instance, Ahtiainen et al. (Ahtiainen et al. 2015) reported that 12-months RET increased FFM without altering endogenous testosterone levels, concluding that changes in endogenous testosterone levels did not impact RET-induced muscular adaptations (Ahtiainen et al. 2015). However, in another study,

suppression of endogenous testosterone using Zoladex blunted muscle adaptations to 8-wks RET in younger men (Kvorning et al. 2006b) - suggesting that endogenous testosterone levels are, at very least, permissive in regulating hypertrophic adaptations to RET. Therefore, given established links between ageing and muscle loss, declines in testosterone responses to acute RE (Brook, Wilkinson, Mitchell, et al. 2016), and age-related anabolic resistance to RET (Brook, Wilkinson, Mitchell, et al. 2016), we postulated that short-term testosterone adjuvant to RET would be of benefit. Consistent with this thesis, it was previously reported that exogenous testosterone therapy adjuvant to RET for longer periods (10-12 wk) led to greater muscle mass gains (Sullivan et al. 2005; Casaburi et al. 2004) than with RET alone. Our present albeit shorter-term data also showed that RET adjuvant to testosterone led to significant increases in whole-body and appendicular FFM. Moreover, RET-induced gains in MT, Lf, PA, and CSA in VL and quadriceps, illustrate that testosterone therapy augmented local thigh muscle remodelling, mainly by increasing Lf (number of sarcomeres in series (Atkinson et al. 2010)) rather than increases in PA; remodelling also associated with T-specific hypertrophy of type IIX fibres. Previous studies in younger adults have shown that the hypertrophic response to testosterone occurs in both slow and fast muscle fibres (Kadi et al. 1999; Kadi 2008; Sinha-Hikim et al. 2002a) (although these studies were over longer periods). Considering previously described preferential loss of fast fibres during ageing (Kovacheva et al. 2010; Deschenes 2004a), our data suggest that exogenous provision of testosterone to healthy non-hypogonadal older

men can be considered as a therapy preventing atrophy - and perhaps loss - of these muscle fibres.

Since muscular performance is the culmination of neural and muscle structural elements, and is the most important attribute to maintaining mobility, we determined the role of exogenous testosterone in relation to aspects of muscle function. In doing so, we established that the testosterone group developed greater static (MVC) and total dynamic (1-RM) strength gains (in line with greater muscle mass gains which strongly correlated with strength improvement). Interestingly, previous work has shown that there may be a neural component to the effects of testosterone e.g. influencing neurotransmitter synthesis (Hakkinen et al. 2000), leading to reduced force fluctuations (Tracy, Byrnes, and Enoka 2004) and inducing recruitment of larger/faster motor units (fast twitch fibres) (Bellew 2002). Nonetheless, our MVC.LM-1 ‘specific force’ data illustrate that muscle mass gains were related to strength-related improvements; given the strong correlation between FFM gains and strength improvement in testosterone group, we can thus conclude RET-induced FFM gains were a major component of improved muscle performance in both groups. Physiological endogenous testosterone levels are correlated with RET-induced gain of FFM and strength (Häkkinen and Pakarinen 1994); perhaps explaining blunted adaptation to RET in older vs. younger men (Vingren et al. 2010; Brook, Wilkinson, Mitchell, et al. 2016). Dose-response studies reported that higher doses (supra-physiological doses; i.e. 600 mg weekly) of testosterone result in graded increments in FFM and strength than lower doses (physiological doses) in men, indicating

efficiency of the testosterone therapy is associated with the higher testosterone doses (Bhasin et al. 2001a; Bhasin et al. 1996b; Storer et al. 2003; Bhasin et al. 1996a). Thus, the 'moderately' supra-physiological testosterone levels (Travison et al. 2017) in our study reveals that short-term testosterone supplementation was able to ‘overcome’ previously observed anabolic resistance to RET (Brook, Wilkinson, Mitchell, et al. 2016) in relation to muscle growth. However, further studies are needed to fully address the physiological vs. supra-physiological pre-requisites to efficacy.

In order to assess the mechanisms underlying the ergogenic effects of testosterone therapy adjuvant to RET in older men, we for the first time, quantified cumulative rates of myofibrillar protein synthesis over the 6-wks of RET. In doing so, we demonstrated that testosterone augmented MPS during RET while concomitantly increasing [estimated] fractional breakdown rates (FBR) (Pivarnik, Hickson, and Wolinsky 1989) (which is supported by the 3MH data) and crucially, with net balance calculations illustrating MPS>MPB. MPB, is an important metabolic component of muscle remodelling and protein turnover, increase with RET but to a lesser extent than MPS (Tipton, Hamilton, and Gallagher 2018). The logical extrapolation of a sustained increase in net muscle protein deposition after RET is an increase in FFM and strength (Ferrando et al. 2002c), entirely in line with our mass and strength-gain data. Links between testosterone and MPS have been previously established. For instance, decreased muscle mass with castration-induced androgen withdrawal suppressed myofibrillar protein synthesis in mice (White et al.

2013b). In addition, 6-months (Brodsky, Balagopal, and Nair 1996) and 4-wks (Urban et al. 1995a) of testosterone administration increased MPS measured acutely in older men. Further, testosterone administration led to increases in MPS without concomitant increases in inward amino acid transport (Ferrando et al. 1998b), indicating increased efficiency of reutilization of amino acids from MPB with testosterone (Ferrando et al. 1998b). Interestingly, it was suggested testosterone administration may improve net protein balance via decreasing MPB, rather than an increasing MPS (Nader et al. 2014). However, we show that testosterone coupled to RET increased net protein accretion despite elevation of both MPS and MPB, because increases in MPS>MPB.

In order to explore the mechanisms underpinning increases in MPS, we investigated aspects regulating the translational 'efficiency' of ribosomes (Van der Meer, Jaspers, and Degens 2011) corresponding to the translational rate per ribosome (Chaillou 2017). To evaluate this, we determined acute RE-induced phosphorylation within MAPKAPK-2 (target of MAPK pathway), MEK1/2 (upstream components in ERK1/2 cascade) (Williamson et al. 2003), and mTORC1 (Brook, Wilkinson, Mitchell, et al. 2016) pathways, across the duration of the 6-wks RET. It is noteworthy from past work that activation of many of these pathways are blunted in older age (Williamson et al. 2003; Parkington et al. 2004; Brook, Wilkinson, Mitchell, et al. 2016) and that in the present study, testosterone administration could reverse these impairments. Consistently, there is a general blunted activation of mTORc1 (Parkington et al. 2004), ERK1/2 (Williamson et al. 2003), MAPKs (Williamson et al. 2003) presumably due

to lesser bio-availability of testosterone (Bennett et al. 2010; White et al. 2013b). In support of this, testosterone therapy augmented IGF-1/Akt/mTOR signaling-activity (which regulates MPS (White et al. 2013b)), presumably explaining enhanced mass and functional gains and suggesting that testosterone levels, at least permissively, regulate muscle adaptations. In addition to increases in translational efficiency, another facet that is blunted with age (Brook, Wilkinson, Mitchell, et al. 2016) and potentially impacts 'capacity' for increasing protein synthesis and net protein balance and is critical for anabolic potential and hypertrophy is ribosomal RNA content (Wilborn et al. 2009). In the present study, exogenous testosterone therapy coupled with RET increased total RNA content per 'cellular unit' (RNA:DNA ratio), an indicator of ribosomal abundance (Kemp et al. 2013), and total RNA:ASP ratio, as an index of synthetic capacity - thereby offsetting age-related deficits (Saunders, Steciw, and Kline 1962). Although we observed only subtle (non-significant) increases in ASP:DNA ratio, an indicator of myonuclear domain size (Kemp et al. 2013), despite there being robust increases in muscle mass (DXA, ultrasound, histology), this is to be expected due to the maintenance of the myonuclear domain size beyond which additional hypertrophy can only be realised by addition of myonuclei (Van der Meer, Jaspers, and Degens 2011).

No study has previously examined the effect of RET adjuvant to testosterone in relation to muscle steroid-metabolism handling in older human muscle. In our study, testosterone therapy augmented AR mRNA and steroidogenic enzyme expression perhaps resulting in higher muscle

testosterone processing capacity - presumably due to infiltration of testosterone from the circulation (Vingren et al. 2008) and processing by 5-alpha reductase. Furthermore, the AR (Bhasin et al. 2003) not only alters mRNA expression of thousands of target genes (Jiang et al. 2009), but is also associated with fibre type CSA increases (Roberts, Haun, et al. 2018), as well as triggering anabolic kinase signalling; i.e. ERK, PI3K, Akt (Bennett et al. 2010), suggesting transcriptional links to augmented MPS, FFM and strength in the testosterone group in the present study and others (Roberts, Haun, et al. 2018; Sato et al. 2014; Willoughby and Taylor 2004; Brook, Wilkinson, Smith, et al. 2016). In addition to increases in testosterone metabolism-related mRNA expression, we demonstrated that myogenic and growth factor-related gene expression was enhanced with T. Specifically, IGF-1Ec up-regulation is purported to correlate with transcriptional activity (Philippou et al. 2009) and initiation of satellite cell proliferation (Hameed et al. 2003), while IGF1-Ea expression is correlated with increased translation (Philippou et al. 2009) and promotion of myogenic differentiation (Hameed et al. 2003). Both intramuscular IGF-1 and testosterone independently stimulate AR and thereafter expression of other anabolic genes (Bennett et al. 2010; Bhasin et al. 2003; Jiang et al. 2009); therefore it is highly likely that an IGF-1 signaling axis played a role in the efficacy of testosterone therapy. Here we also show augmented expression of myogenic regulatory factors (MRFs) and fast fibre type specific mRNA expression (i.e. MHCIIa expression (Hameed et al. 2003)) in line with fast fibre, lower body and whole body hypertrophy with T. This supports previous work (Bhasin et al. 2003; Singh et al. 2003a)

demonstrating a pro-myogenic role for testosterone e.g. testosterone increased the transformation of pluripotent precursor cells down the myogenic lineage. It has been shown that single bouts of RE or short-term RET are sufficient to increase the abundance of MRFs mRNA species in young participants (Vingren et al. 2008; Wilborn et al. 2009). However, in the present study, in line with past work in older age (Hameed et al. 2003; Kvorning et al. 2007b), in the placebo group, these regulatory genes did not change either after an acute bout of RE or after 6-wks RET with the exception of C-myc (which controls cell growth and ribosomal biogenesis (Brook, Wilkinson, Mitchell, et al. 2016)). We speculate this contributed to blunted adaptations to RET in placebo (Brook, Wilkinson, Mitchell, et al. 2016) while helping explain greater muscle mass accretion with testosterone therapy.

In addition to declines in muscle mass and function with age, muscle mitochondria are also subject to age-related remodelling. For instance, it was reported that ageing is associated with decreases in PGC1-α mRNA (a main regulator of mitochondrial biogenesis and oxidative capacity (Wilkinson et al. 2008c)), OxPhos capacity, mitochondria enzyme activity (e.g. CS), mtDNA content and increases in oxidative stress; which all result in an impaired mitochondrial function (Mosaferi Ziaaldini, Attarzadeh Hosseini, and Fathei 2017)). Crucially, exercise, including RET, is known to induce mitochondrial adaptations. For instance, 12-wks RET augmented mitochondrial content (Balakrishnan et al. 2010), volume (Roberts, Romero, et al. 2018) and respiratory capacity (Porter et al. 2015b), in younger participants. Further, 6 months RET increased

mitochondrial mass in older women (Manfredi et al. 2013) and mitochondrial function/transcriptome activity in older men (Melov et al. 2007a). As we showed in our placebo study group, short term RET did not augment mitochondrial CS activity in older males; but similar to short term (i.e. 7-9-wks) endurance training adjuvant to testosterone therapy in mice (van Breda et al. 1992; Guo et al. 2012), we showed RET coupled to testosterone therapy augmented PGC1-α/Tfam mRNA, mitochondrial CS activity (Groennebaek and Vissing 2017)) and protein levels of complex IV (cytochrome c oxidase subunit (Guo et al. 2012)) and V (ATP synthase subunit) (Guo et al. 2012) (not mtDNA copy number). Possible mechanisms include heightened activation of IGF-1/MAPK pathways (Mosaferi Ziaaldini, Attarzadeh Hosseini, and Fathei 2017; Petersson et al. 2014) and augmented MRFs mRNA (e.g. Myogenin) (Chang et al. 2006) during RET, as observed in the testosterone study group, which consequently mediate mitochondrial gene expression (Mosaferi Ziaaldini, Attarzadeh Hosseini, and Fathei 2017; Chang et al. 2006). Furthermore, since mitochondrial gene expression (i.e. PGC1-α) is regulated by Akt/mTOR signalling (Cunningham et al. 2007; Traish, Abdallah, and Yu 2011), which were all upregulated with testosterone, this may also explain our observations of greater CS activity with adjuvant testosterone therapy. Moreover, sustained muscle growth and MPS during RET in the testosterone group likely required greater increases in mitochondrial function or volume due to the increased energy demands of intracellular protein accretion (Roberts, Romero, et al. 2018). Previous work has also shown that the AR (Traish, Abdallah, and Yu 2011) and systemic levels

of testosterone, were positively associated with cytochrome c oxidase (Koenig, Goldstone, and Lu 1980; Traish, Abdallah, and Yu 2011), CS (Costello and Franklin 1993) activity, mitochondrial protein synthesis, in addition to Tfam and PGC1-α expression (Traish, Abdallah, and Yu 2011); culminating in enhanced mitochondrial biogenesis, respiratory capacity and function (Koenig, Goldstone, and Lu 1980). Conversely, AR and testosterone deficiency, has been associated with lower levels of PGC1-α expression (Fan et al. 2005) resulting in decreases in OxPhos capacity (Pitteloud, Mootha, et al. 2005b). In summary, greater MPS, myofibrillar protein accretion and consequent muscle hypertrophy alongside greater mitochondrial adaptations (Groennebaek and Vissing 2017) in the testosterone therapy group demonstrates the efficacy of adjuvant testosterone in yielding positive hypertrophic and energetic adaptations.

We conclude that administration of testosterone coupled to RET is an effective short-term (6-wks) intervention to overcome age-related deficits in the responsiveness of older muscle to resistance exercise training. Short-term RET adjuvant to testosterone was also well-tolerated and could be useful as both pre- and rehabilitation interventions in elective surgery and other clinical procedures in older populations. Nevertheless, it is important to be cognisant of potential adverse effects. testosterone therapy has been associated by (non-significant) increases in some disorders; e.g. cardiovascular risk (Fernández-Balsells et al. 2010) and/or prostate hypertrophy (Osterberg, Bernie, and Ramasamy 2014). While randomized controlled trials have been insufficiently powered to detect differences in the rates of adverse events (Loo et al. 2019), some have

reported an increased risk of stroke and/or myocardial infarction (Vigen et al. 2013) whereas others have reported to be uncertain of such effects (Loo et al. 2019; Sih et al. 1997; Kenny et al. 2010; Rhoden and Morgentaler 2004). No adverse events were noted during or after completion of the present study in older males and a major benefit of our approach is the short-term efficacy of therapy. Finally, we reveal the likely mechanisms underlying the effect of testosterone therapy in relation to 'overcoming' aspects of age-related anabolic resistance; vis-à-vis, elevating protein turnover (with greater increases in MPS), enhancing translational efficiency and capacity, and inducing pro-myogenic and testosterone handling gene regulation. Given links between declining testosterone and muscle ageing in women (Häkkinen and Pakarinen 1993), similar studies in women are likely to deliver similar benefits. Short-term testosterone administration may have a role in the treatment of frailty in older males without inducing any adverse side effects; however, older men receiving testosterone therapy should be carefully monitored because of its potential risks.

Chapter 5 A

The role of endogenous testosterone (T) in regulating resistance exercise-induced modulation of glycaemic control in young men

5.1 **Abstract**

Introduction: Insulin resistance is a risk factor for many metabolic diseases, coincident with the established pandemic of obesity, worldwide. The main behavioural strategies to overcome insulin resistance remain diet and exercise training. However, the “andropause”, so-called due to age-related testosterone (T) depletion has been associated with impaired glycaemic control – leading to investigations of the role of testosterone in regulating insulin sensitivity which is unclear. Therefore, the aim of this study was to investigate the role of endogenous testosterone during 6-wks of resistance exercise training (RET), in relation to glycaemic control in healthy young men.

Methods: 16 healthy non-hypogonadal men (~18-30y, BMI≤30kg.m^{-2}, serum T>230 ng·dl^{-1}) were recruited with the aim of determining the role of endogenous suppression of testosterone in relation to RET-induced improvements in glycaemic control in younger men. To do so, the participants were assigned in a randomised double-blinded fashion to receive injection of either: placebo (P; saline, n=8) or the GnRH analogue, Zoladex 3.6mg (Z; n=8). All subjects underwent 6-wks whole body RET (3-sets, 8-10 reps at 80% 1-RM) with 75g oral glucose tolerance testing (OGTT baseline, ~5 days after injection and after RET), and Dual-energy X-ray Absorptiometry (DXA) to assess glycaemic control and body composition, respectively.

Results: Provision of Zoladex significantly reduced total testosterone in Zoladex (vs. placebo) throughout RET. Total fat free mass (FFM) increased significantly following 6-wks RET in placebo (55.9±1.7 to 57.4±1.7kg, P=0.006), but not in Zoladex (55.4±2.8 to 55.8±3.1kg). Over 6-wks of RET, placebo group showed

decreased (2381±1013 $mU^{-1}.2h^{-1}$, P=0.009) insulin area under curve (AUC). Further, measures of C-Peptide highlighted a decreasing trend in placebo compared to Zoladex (551±57 $ng.ml^{-1}$ vs. 789±116 $ng.ml^{-1}$, P=0.08). Additionally, Cederholm index of peripheral insulin sensitivity in placebo showed an increase after RET (61.7±4.7 vs. 74.5±3.7 $mg{\cdot}L^{2}{\cdot}mmol{\cdot}L^{-1}{\cdot}mU^{-1}{\cdot}min^{-1}$, P<0.05). Finally, Matsuda index of insulin sensitivity in placebo yielded significant increases based on fold-change from baseline OGTT to OGTT after RET (9.9±2.4 vs 12.8±3.2, P<0.05).

Conclusion: Suppressed endogenous testosterone (Zoladex), attenuated insulin sensitivity (supported by c-peptide) in young men, during RET. Thus, endogenous testosterone is a permissive regulator of RET-induced improvements in insulin sensitivity and glycaemic control.

5.2 Introduction

Obesity and inactivity are associated with an increase in metabolic syndrome in the general population; i.e. increase in risk of endocrine disorders (Kelly and Jones 2013), decrease in muscle mass and strength (Mitchell, Williams, et al. 2012), insulin sensitivity (Bandeen-Roche et al. 2006), and prevalence of type 2 diabetes (Feldman et al. 2002; Harman et al. 2001; Morley et al. 1997). Reduced insulin sensitivity is the central biochemical defect associated with diabetes (Kelly and Jones 2013). However, many of these symptoms are accompanied by declines in testosterone (T) bioavailability – the “andropause” (Ferrando et al. 2002c; Dandona and Dhindsa 2011). In accordance, hypogonadal men exhibit a 3-fold increase in mortality (Hackett 2016); with the prevalence of diabetes of ~40% (Chandel et al. 2008; Dhindsa et al. 2018); and associated diabetes per se carried a hazard ratio of ~15% for sudden death (Balkau et al. 1999). However, the mechanisms by which testosterone regulates metabolism and glycaemic control are ill-defined not fully clear in men.

It is suggested there is an inverse association between testosterone and fasting insulin levels in men (Phillips 1977). In male rats, acute castration induces significant increase in insulin resistance(Holmäng and Björntorp 1992). Further, total whole-body glucose disposal was positively associated with testosterone levels in healthy middle-aged men (Haffner et al. 1994) and men with diabetes have lower testosterone levels (Barrett-Connor 1992). Conversely, it is reported that free testosterone levels might not have correlation with insulin sensitivity (Birkeland et al. 1993;

Abate et al. 2002). In accordance, the role of testosterone in prevalence of diabetes is ill-defined; i.e. only <19% of men suffered from type 2 diabetes had total testosterone <231 ng.dl-1 (Grossmann 2014b). However, moderately short-term (i.e. 12-wks) (Smith, Lee, and Nathan 2006a) and long term (i.e. 24-months) (Haidar et al. 2007) androgen-deprivation therapy in men decreased muscle Insulin sensitivity and increased TFM which were associated with an increased risk for diabetes (Kintzel et al. 2008). Therefore, considering the positive effects of testosterone on FFM (Gruenewald and Matsumoto 2003), fat mass (Svartberg et al. 2008), and perhaps glycaemic control (Dhindsa et al. 2016), interventions that boost FFM and endogenous testosterone i.e. resistance exercise training (RET), might be of greater benefit (Hawkins et al. 2008).

It is well-defined that RET promotes muscle mass and associated muscle programming (Ribeiro et al. 2017; Phillips et al. 2017) and stimulates muscle glycaemic control (Lee, Kim, and Kim 2017). These effects are also associated with enhanced endogenous testosterone in response to acute bouts of RE (Rønnestad, Nygaard, and Raastad 2011). We previously showed that acute RE-induced systemic induction of endogenous testosterone was related to ensuing muscle hypertrophy in younger men (Brook, Wilkinson, Mitchell, et al. 2016), suggesting a link between testosterone and muscle mass gains with RET. Nevertheless, withdrawal of testosterone led to significant decrements in insulin sensitivity within 2 weeks without changing FFM and TFM, suggesting

testosterone may exert direct effects on metabolic regulators or signalling, rather than by effecting changes in body composition (Yialamas et al. 2007). In contrast, it is reported testosterone deprivation for 4-wks did not change glycaemic control in younger men (Rabiee et al. 2010). Thus, glycaemic control improvement during RET cannot be ascribed to a mere increase in FFM and likely via factors independent of changes in FFM; perhaps via endogenous androgen hormones.

Therefore, in an effort to identify the role of endogenous testosterone adjuvant to RET, we investigated the role of endogenous testosterone suppression coupled to short-term RET in younger men. In a double-blind investigation into the effects of 6-wks of RET with or without testosterone suppression, we aimed to identify whether endogenous testosterone is an important factor in glycaemic control.

5.3 **Methods**

The study protocol and all the methods relating to this section have been previously described in chapter 2.

5.4 **Results**

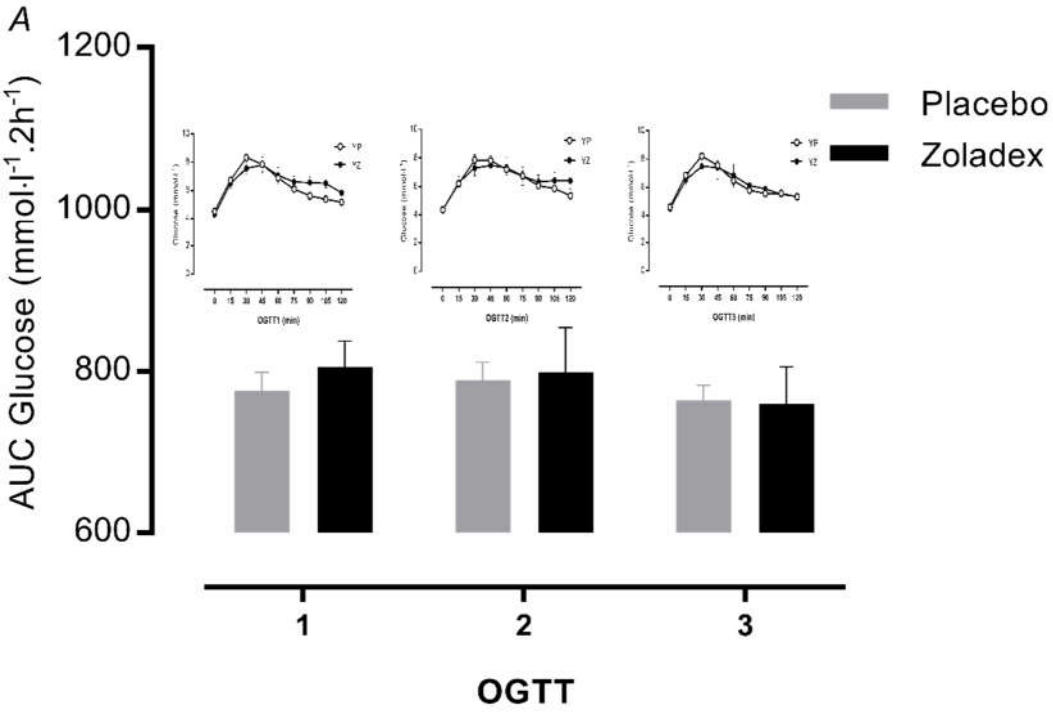
Physiological characteristics of participants are shown in Table 3-1 in chapter 3. Further, total testosterone concentrations in the Zoladex group in Figure 3-1 in chapter 3.

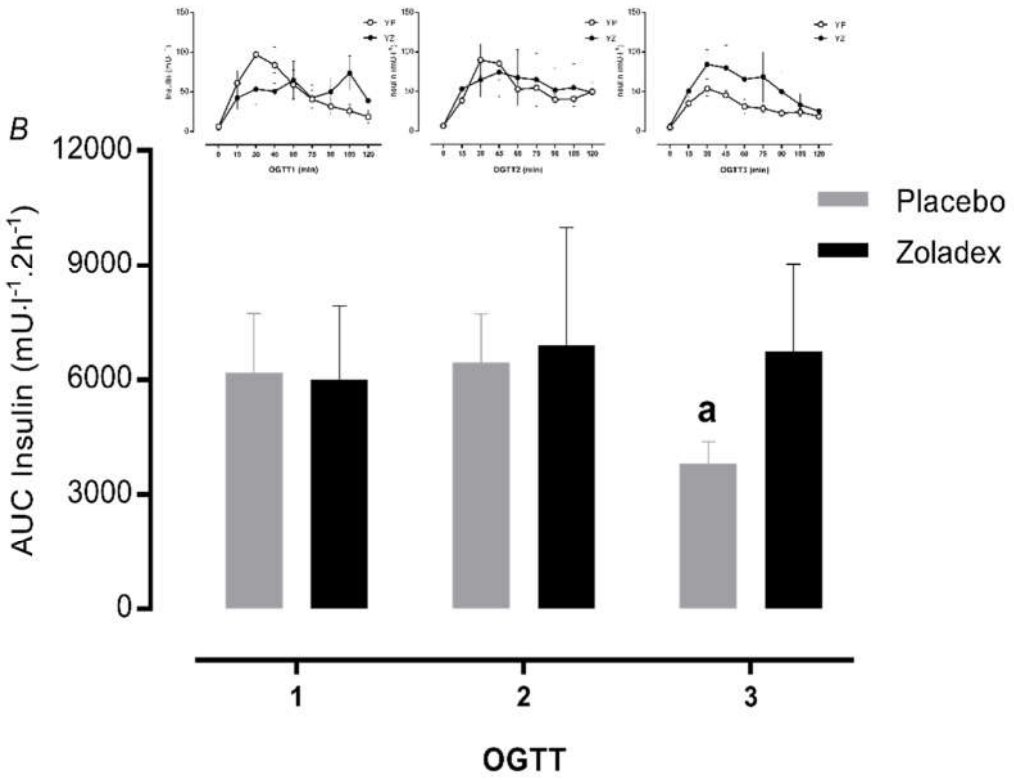
5.4.1 Body composition

The body composition data in Zoladex and placebo groups are shown in Figure 3-2.

5.4.2 Glucose, insulin and C-peptide levels

Over the RET, placebo study group decreased AUC insulin, decreasing 2381 ± 1013 $mU^{-1}.2h^{-1}$, P = 0.009. Further, measures of C-Peptide showed a trend in placebo group compared to Zoladex group (551±57 vs. 789±116, P=0.08). However, by 6 weeks of RET there was no changes in glucose AUC in both groups.





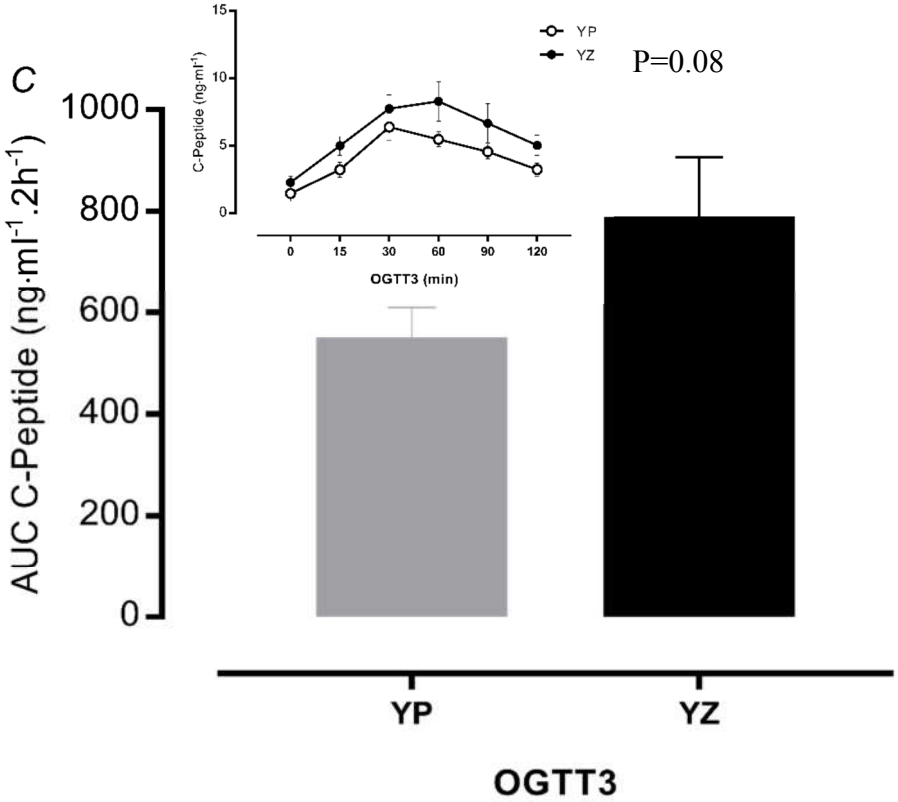


Figure 5-1 changes in insulin, glucose and C-peptide (A-F). Values are means ± SEM. a Significantly different from baseline, P<0.05; b significantly different between two groups, P<0.05. all from repeated measure ANOVA. AUC: area under curve.

Insulin resistance and sensitivity

Over the RET, HOMA-β and HOMA-IR did not change significantly ($P<0.05$). However, GIR, Matsuda and Cederholm indices increased significantly in placebo ($P<0.05$, Table 5-1), compared to Zoladex group.

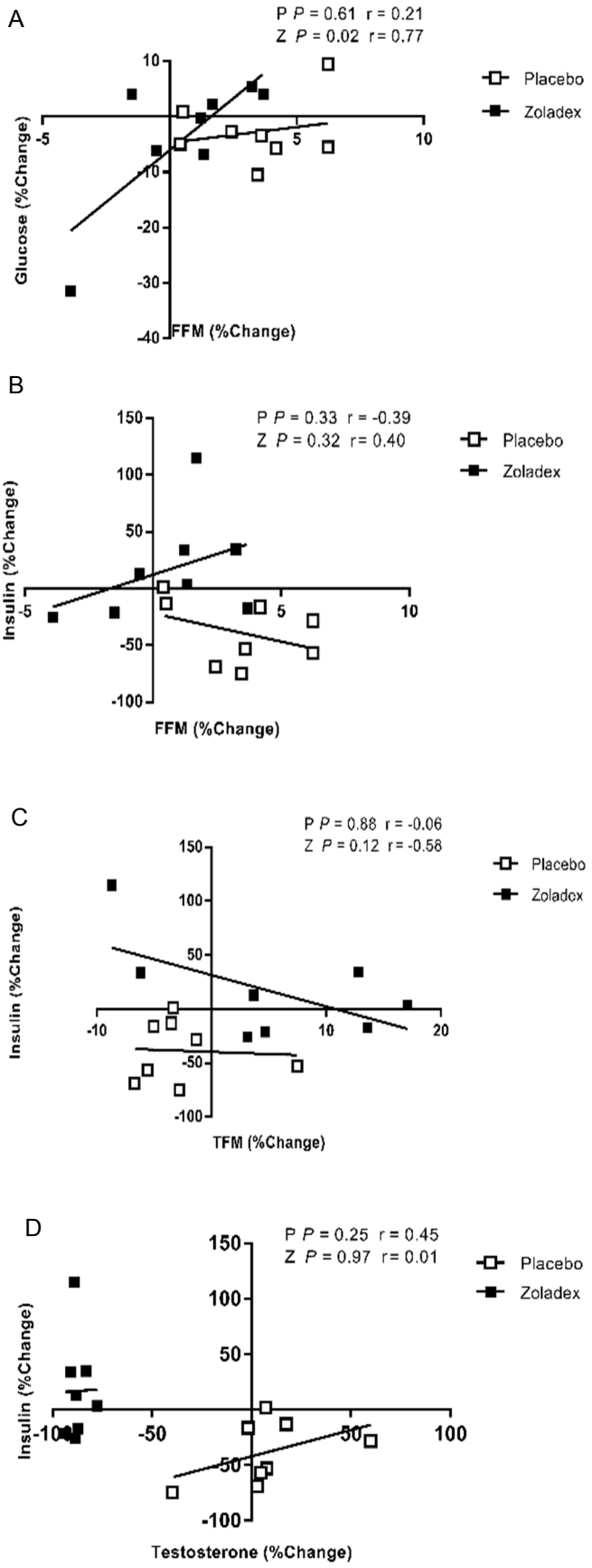
Table 5-1 Glycaemic control-related calculations.

Index	Group	OGTT1	OGTT2	OGTT3
HOMA-β	YP(n=8)	----------	----------	27.8±10.5
	YZ(n=8)	----------	----------	48.9±8.5
HOMA-IR	YP(n=8)	1.28±0.3	1.27±0.2	0.99±0.1
	YZ(n=8)	0.91±0.2	1.36±0.4	1.36±0.3
GIR-Index	YP(n=8)	----------	----------	8.2±2.5[d]
	YZ(n=8)	----------	----------	2.5±0.4
Matsuda	YP(n=8)	9.9±2.4	7.6±1.2	12.8±3.2[c]
	YZ(n=8)	12.4±2.6	10.1±1.7	8.4±1.1
Cederholm	YP(n=8)	68.1±5.1	61.7±4.7	74.5±3.7[b]
	YZ(n=8)	65.7±6.1	66.1±6.9	68.1±5.3

a significantly different from OGTT1, b significantly different from OGTT2, c significantly different based on fold changes, d significantly different between groups. HOMA-IR: homeostasis model assessment for insulin resistance; HOMA-B: homeostasis model assessment for beta cell function; GIR-index: Glucose infusion rate index.

5.4.3 Correlations and ratios

Increase in FFM was correlated with increase in glucose levels in Zoladex group with lower testosterone levels (Figure 5-2 A). However, there was no association between FFM and insulin levels (Figure 5-2 B). Nevertheless, there was a trend in negative correlation between insulin sensitivity and FFM (Figure 5-2 E). Further, insulin levels per unit of FFM decreased in placebo with higher testosterone levels during 6-wks RET, compared to Zoladex group (Figure 5-2 G). Similarly, insulin to glucose ratio decreased in placebo group significantly in comparison with Zoladex (Figure 5-2 H).


A
P P = 0.61 r = 0.21
Z P = 0.02 r = 0.77
Placebo
Zoladex
Glucose (%Change)
FFM (%Change)
B
P P = 0.33 r = -0.39
Z P = 0.32 r = 0.40
Placebo
Zoladex
Insulin (%Change)
FFM (%Change)
C
P P = 0.88 r = -0.06
Z P = 0.12 r = -0.58
Placebo
Zoladex
Insulin (%Change)
TFM (%Change)
D
P P = 0.25 r = 0.45
Z P = 0.97 r = 0.01
Placebo
Zoladex
Insulin (%Change)
Testosterone (%Change)

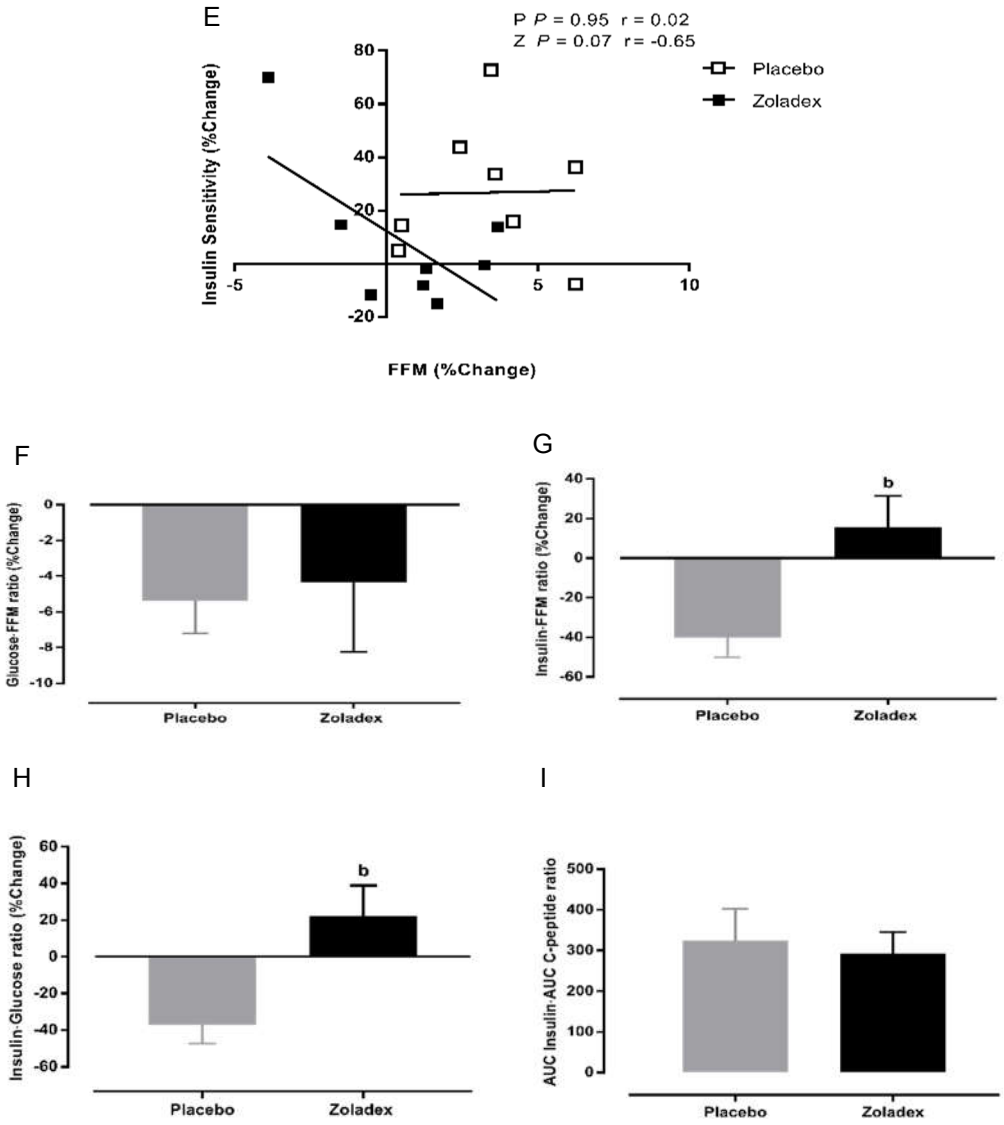


Figure 5-2 Correlations (A-E) and ratios (F-I). Values are means ± SEM. b Significantly different from placebo, P<0.05; G and H from independent t-test. AUC: area under curve.

5.5 **Discussion**

There is a great need within the scientific field to identify the role of endogenous testosterone and interventions that alter glucose metabolism. Herein, we elucidated that just 6-wks whole-body RET coupled with the suppression of endogenous testosterone in younger men had significant effects; i.e. Zoladex was associated with blunted decrease in AUC insulin (vs. baseline).

To support our results showing the positive effects of RET on glycaemic control (Lee, Kim, and Kim 2017), it is reported that 16-wks (Shaibi et al. 2006; Ibañez et al. 2005), 20-wks (Smutok et al. 1993) and 3-month (Eriksson et al. 1997) RET augmented insulin sensitivity similar to those observed following endurance training. Indeed, increase in FFM enhanced the available glucose storage area, thereby facilitating the clearance of glucose from the circulation and reducing the amount of insulin required to maintain a normal glucose tolerance (Ibañez et al. 2005). Thus, augmented FFM is associated with improving glycaemic control after RET in men (Eriksson et al. 1997); albeit without altering the intrinsic capacity of the muscle to respond to insulin (Poehlman et al. 2000a; Poehlman et al. 2000b; Miller, Sherman, and Ivy 1984). In the present study, we showed the group had augmented FFM gain (i.e. placebo group) represented attenuated insulin levels and augmented insulin sensitivity after 6-wks RET. However, in line with previous studies (Takala, Nuutila, Knuuti, Luotolahti, and Yki-Jarvinen 1999; Takala,

Nuutila, Knuuti, Luotolahti, and Yki-Järvinen 1999), RET did not change insulin-stimulated glucose uptake per kilogram of FFM after 6-wks RET, and as increased glycaemic control represents a true long-term adaptation to RET (Ebeling et al. 1993), it seems failure to show increase in insulin sensitivity in Zoladex group was probably due to short-term (6-wks) RET (Takala, Nuutila, Knuuti, Luotolahti, and Yki-Järvinen 1999). Nevertheless, 12-wks combined RET and aerobic training had no effect on glycaemic control (i.e. glucose and insulin levels) in men with prostate cancer undergoing androgen deprivation therapy (>2-months) (Galvão et al. 2009). Further, 16-wks heavy RET with/without growth hormone provision increased FFM but did not change glucose, insulin, and C-peptide levels in healthy older men (Zachwieja et al. 1996b). Therefore, considering a positive correlation between FFM gain (YZ: 0.6%) and glucose levels in Zoladex group, the positive effects of RET on glycaemic control in placebo group cannot be ascribed to a mere increase in FFM (Andersen et al. 2003) and likely via factors independent of changes in FFM (Shaibi et al. 2006).

While the relationship of hypogonadism with the development of metabolic syndrome is not well-defined (Laaksonen et al. 2004), low testosterone levels *per se* are associated with metabolic syndrome (i.e. increased insulin resistance (Grossmann 2014a; Wang et al. 2016), decreased insulin sensitivity (Keating, O'Malley, and Smith 2006) and type 2 diabetes, probably via decrease in FFM and increase in TFM (Wang et al. 2016). Indeed, to support our results showed lower testosterone levels in Zoladex group during RET was associated with attenuated glycaemic

control adaptation, it is postulated that higher testosterone levels and FFM gain were associated with up-regulated glucose metabolism in healthy men (Hou et al. 2015; Pitteloud, Mootha, Dwyer, Hardin, Lee, Eriksson, Tripathy, Yialamas, Groop, Elahi, et al. 2005). Indeed, short-term (12-wks) (Smith, Lee, and Nathan 2006a) and long term (24-months) (Haidar et al. 2007) androgen-deprivation therapy in men with prostatic cancer decreased Insulin sensitivity and increased TFM which were associated with an increased risk for diabetes (Kintzel et al. 2008). In addition, lower endogenous testosterone levels were associated with increase in C-peptide levels, indicating impaired pancreatic β-cell function and increased insulin secretion (Tsai et al. 2000). In our study, we assessed C-peptide levels to measure endogenous insulin secretion, GIR and β-cell function (Jones and Hattersley 2013). Both C-peptide and insulin are secreted in equimolar amounts from the beta cell as the reliable surrogate for assessing pancreatic β-cells function and GIR (Chailurkit et al. 2007; Ohkura et al. 2013). However, C-peptide is only minimally extracted or metabolized by the liver with longer half-life than insulin (20–30 min vs. 3–5 min) (Jones and Hattersley 2013). In our study, younger group with higher endogenous testosterone than Zoladex group had lower levels of C-peptide after 6-wks RET, showing decreasing secretion of insulin in both groups with higher peripheral insulin sensitivity and GIR. Therefore, testosterone had permissive role in improvement of glycaemic control during short-term RET. However, previous studies reported contentious results about the effects of long term RET on glycaemic control in men with lower endogenous T; e.g. 16-wks RET did not improve glycaemic

control (involving C-peptide levels) In older men (Zachwieja et al. 1996b), but 12-wks (Iglay et al. 2007) and 4-months (Hansen et al. 2012) RET decreased fasting C-peptide levels but not estimated insulin sensitivity and insulin levels in middle aged healthy men. In accordance, we showed testosterone deprivation during RET blunted glycaemic control improvement in younger men (vs. RET with normal testosterone levels). Nevertheless, it seems RET diminished worsened glycaemic control-induced testosterone suppression (Smith, Lee, and Nathan 2006a). It seems changes in FFM and TFM are associated with attenuation in insulin sensitivity within 12-wks of initiating androgen-deprivation therapy (Smith, Lee, and Nathan 2006b). Interestingly, only 2-wks testosterone suppression led to decrements in insulin sensitivity without changing body composition in younger men (Yialamas et al. 2007).

In conclusion, non-suppressed endogenous testosterone (P group), enhanced insulin sensitivity (supported by reduced c-peptide) during RET in young men (vs. Zoladex group), showing endogenous testosterone is a permissive mediator of RET-induced improvements in glycaemic control.

Chapter 5 B

The role of exogenous testosterone (T) therapy in regulating resistance exercise-induced modulation of glycaemic control in older men

5.1 **Abstract**

Introduction: Insulin resistance is a risk factor for many metabolic diseases, coincident with the established pandemic of obesity, worldwide. The main behavioural strategies to overcome insulin resistance remain diet and exercise training. However, the “andropause”, so-called due to age-related testosterone (T) depletion has been associated with impaired glycaemic control – leading to investigations of the role of testosterone in regulating insulin sensitivity. Recently, testosterone therapies have been evaluated in humans with variable effects, such that the role and efficacy of testosterone therapy in relation to glycaemic control across age remains unclear. The aim of this study was therefore to investigate the therapeutic role of testosterone during 6-wks of resistance exercise training (RET), in relation to glycaemic control in groups of healthy older men.

Methods: 18 healthy non-hypogonadal men were recruited (~60-75y, BMI≤30kg.m^{-2}, serum T>230 ng·dl^{-1}) with the aim of determining the role of provision of exogenous testosterone therapy adjuvant to short-term RET in older men. Thus, older men received placebo (P; saline, n=9) or testosterone esters 250 mg (T; n=9). All subjects underwent 6-wks whole body RET (3-sets, 8-10 reps at 80% 1-RM) with 75g oral glucose tolerance testing (OGTT baseline, ~5 days after injection and after RET), and Dual-energy X-ray Absorptiometry (DXA) to assess glycaemic control and body composition, respectively.

Results: testosterone group elicited marked enhanced testosterone levels (both P<0.05) that were sustained throughout RET. Total fat free mass (FFM) increased significantly following 6-wks RET in testosterone (53.0±1.7 to 56.0±5.2

kg, P<0.0001), but not in placebo (54.1±6.3 to 54.8±5.8 kg). Over 6-weeks of RET, testosterone group showed decreased (1367±572 $mU^{-1}.2h^{-1}$, P=0.03) insulin area under curve (AUC). Further, measures of C-Peptide highlighted decreases in testosterone compared to placebo (OT: 601±46 $ng.ml^{-1}$ vs. OP: 830±77 $ng.ml^{-1}$, P=0.02). Additionally, Cederholm index of peripheral insulin sensitivity in testosterone, showed a significant increase from baseline to OGTT after RET (57.3±5.1 vs 68.6±5.3 $mg{\cdot}L^{2}{\cdot}mmol{\cdot}L^{-1}{\cdot}mU^{-1}{\cdot}min^{-1}$, P<0.05).

Conclusion: testosterone therapy enhanced insulin sensitivity (supported by reduced c-peptide) in older men, coupled with RET. Thus, testosterone therapy adjuvant to pre/re-habilitation RET may be of value to overcome the burgeoning trend of worsening insulin resistance with aging.

5.2 Introduction

Ageing is associated with a decrease in muscle mass and strength (sarcopenia) (Mitchell, Williams, et al. 2012), insulin sensitivity (Bandeen-Roche et al. 2006), and prevalence of type 2 diabetes (Feldman et al. 2002; Harman et al. 2001; Morley et al. 1997). Many of these are accompanied by declines in testosterone (T) bioavailability – the "andropause" (Ferrando et al. 2002c; Dandona and Dhindsa 2011). Moreover, hypogonadal men exhibit a 3-fold increase in mortality (Hackett 2016) and low testosterone is associated with diabetes which per se is responsible of ~15% for sudden death in older population (Balkau et al. 1999). Thus, as the ageing populace grows, there remains a need for investigating the role of testosterone in glycaemic control as well as therapies to counteract the andropause and associated glycaemic disorders.

Clinically, older males with a total testosterone of <231 ng.dl-1 may be offered testosterone therapy (Hackett 2016) due to established positive effects of testosterone on fat-free mass (FFM) (Gruenewald and Matsumoto 2003), fat mass (Svartberg et al. 2008), and glycaemic control (Dhindsa et al. 2016). Pitteloud et al. reported that men with hypogonadal testosterone levels were twice as insulin resistant as their eugonadal counterparts, confirming a positive correlation between testosterone level and insulin sensitivity across full spectrum of glucose tolerance (Pitteloud, Mootha, et al. 2005a); indicating this is an effective strategy in men with persistent insulin resistant state and hypogonadism to combat attenuated

FFM and insulin sensitivity, and increase in total fat mass (TFM) (Dhindsa et al. 2016). However, the role of testosterone in prevalence of diabetes is ill-defined; i.e. only <19% of men suffered from type 2 diabetes had total testosterone <231 ng.dl-1 (Grossmann 2014b). Nevertheless, provision of exogenous testosterone (200 mg, every 2-wks for 3-monts (Kapoor et al. 2006) or oral 160 mg/day for 6-months) (Emmelot-Vonk et al. 2008) has been shown to reduce insulin resistance and improves glycaemic control in hypogonadal men with type 2 diabetes and healthy older men, respectively. Controversially, it is reported testosterone therapy for 40-wks (1000 mg injection, every ~2 month) did not improve insulin resistance and glycaemic control in hypogonadal men with type 2 diabetes (Gianatti et al. 2014). In addition, moderately short-term (i.e. 12-wks) (Smith, Lee, and Nathan 2006a) and long term (i.e. 24-months) (Haidar et al. 2007) androgen-deprivation therapy in men decreased Insulin sensitivity and increased TFM which were associated with an increased risk for diabetes (Kintzel et al. 2008). Thus, besides administering long-term exogenous testosterone, interventions that boost endogenous sex hormones i.e. exercise, might be of greater benefit (Hawkins et al. 2008).

Ageing and exercise training related changes in glucose handling and insulin resistance involve several tissues, but skeletal muscle metabolic changes leads to particularly large impact on whole body glucose metabolism (Tsintzas et al. 2013). Further, age related decline in skeletal muscle mass and strength (sarcopenia) (Mitchell, Atherton, et al. 2012) is

associated with increases in muscle insulin resistance (Cleasby, Jamieson, and Atherton 2016). It is well-defined that resistance exercise training (RET) promotes muscle mass and anabolism (even in older men (Ribeiro et al. 2017; Phillips et al. 2017)) and stimulates muscle glycaemic control (Lee, Kim, and Kim 2017); these effects are also associated with enhanced endogenous testosterone in response to acute bouts of RE (Rønnestad, Nygaard, and Raastad 2011). Nonetheless, hypertrophic and glycaemic responses to RET are blunted in older age (vs. younger cohorts), so called “anabolic resistance” (Zachwieja et al. 1996a), suggesting the need for strategies beyond, or adjuvant to RET (Rønnestad, Nygaard, and Raastad 2011), in order to maximise efficacy on muscle mass and glycaemic control. We previously showed that acute RE-induced systemic induction of endogenous testosterone was related to ensuing muscle hypertrophy in younger men (Brook, Wilkinson, Mitchell, et al. 2016), suggesting a link between testosterone and muscle mass gains with RET. Moreover, in keeping with a role for testosterone as an adjuvant therapy to RET in older age, 50-100 mg·day-1 transdermal testosterone therapy, enhanced RET-induced muscle mass gains over 12-wks (Glintborg et al. 2013), while supra-physiological doses (600 mg·week-1) of testosterone adjuvant to RET for 10-wks increased FFM and strength (in younger men (19-40 y) (Bhasin et al. 1996a). However, the effect of ‘medium-term’ or even “short-term” use of testosterone therapy adjuvant to RET on glycaemic control is poor-defined.

Therefore, in an effort to identify the effect of exogenous testosterone therapy adjuvant to RET in relation to anabolic resistance, we investigated the role and efficacy of short-term RET, coupled to testosterone therapy in older men. In a double-blind investigation into the effects 6-wks of RET with or without testosterone increase, we aimed to identify that low testosterone is a consequence of insulin resistance, or simply a biomarker.

5.3 **Methods**

The study protocol and all the methods relating to this section have been previously described in chapter 2.

5.4 **Results**

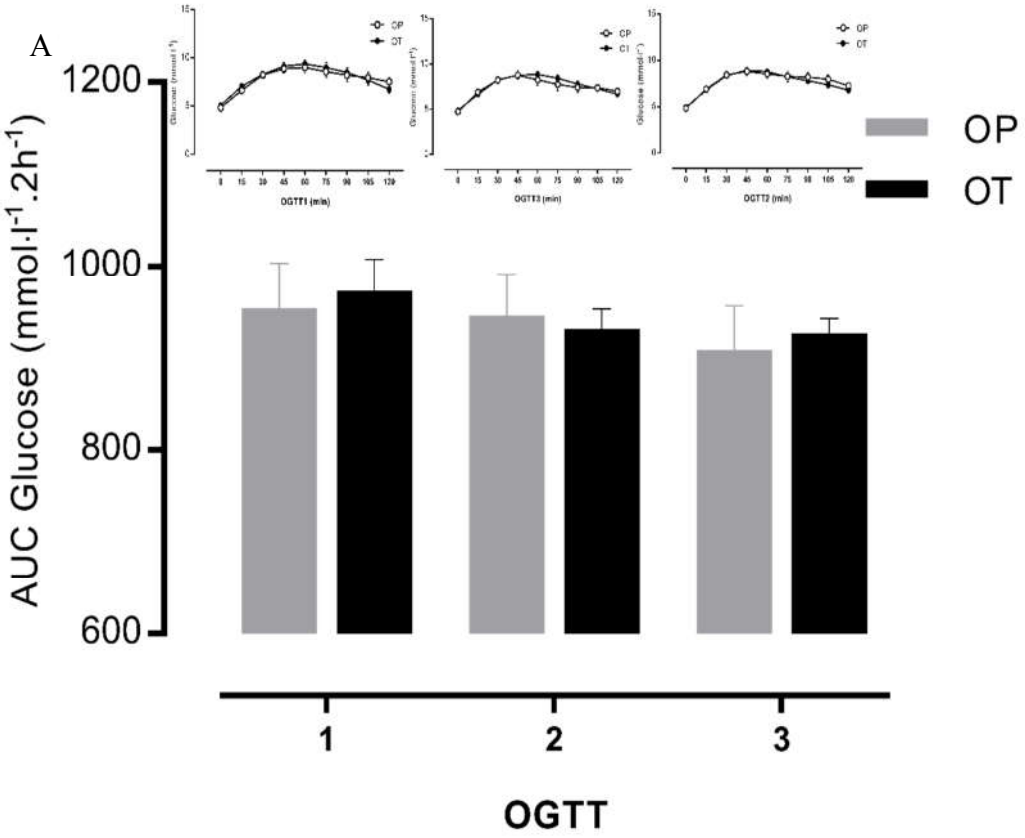
Physiological characteristics of participants are shown in Table 4-1 in chapter 4. Further, total testosterone concentrations in the testosterone group in Figure 4-1 in chapter 4.

5.4.1 Body composition

The body composition data in testosterone and placebo groups are shown in Figure 4-2.

5.4.2 Glucose, insulin and C-peptide levels

Over the RET, AUC insulin decreased in testosterone group in greater extent than placebo group (1367 vs. 433 $mU^{-1}.2h^{-1}$ decrease, P=0.03). Further, measures of C-Peptide showed decreases in testosterone compared to placebo (testosterone: 601±46 vs. placebo: 830±77, P=0.02). However, by 6 weeks of RET there was no changes in glucose AUC in all groups.



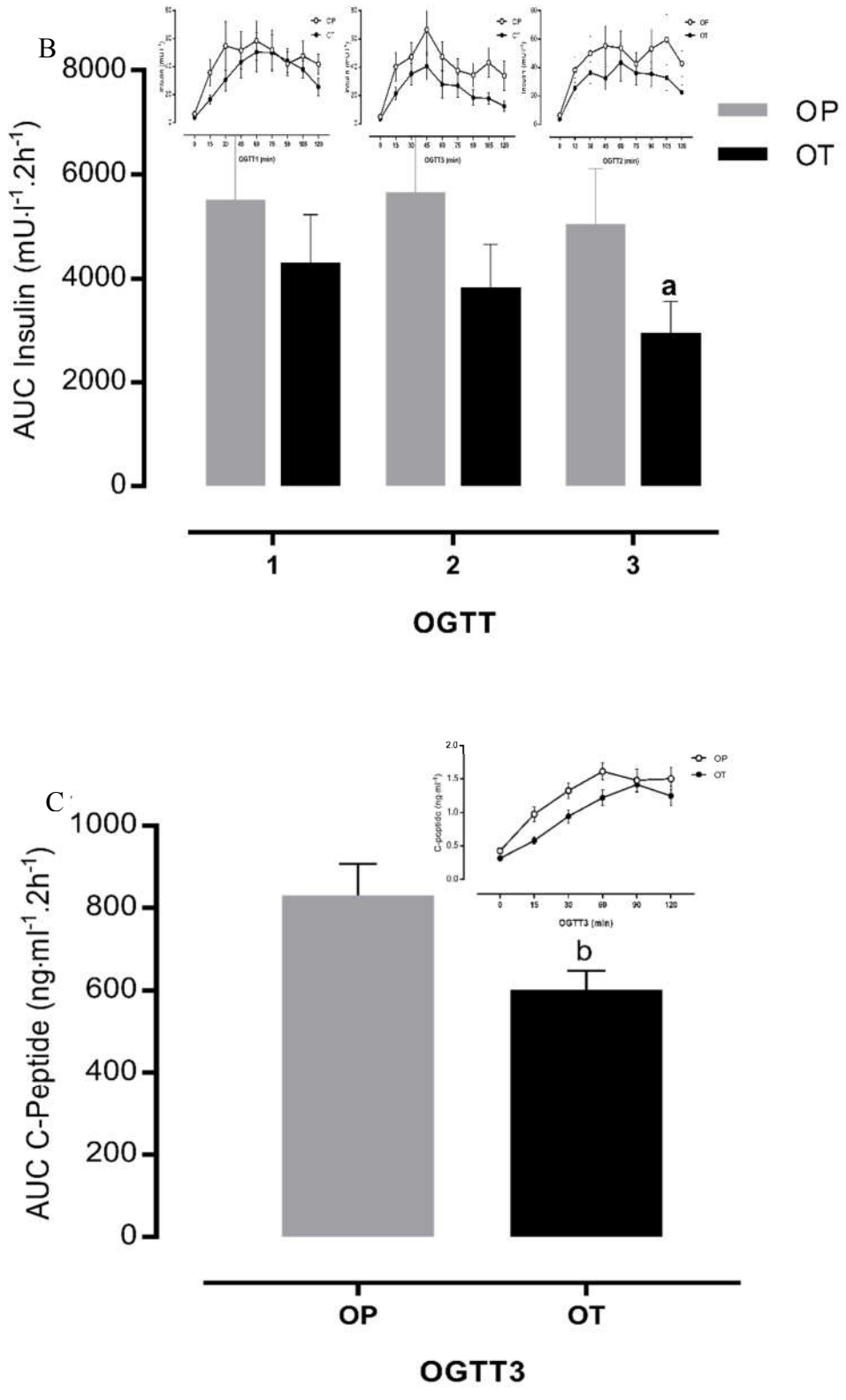


Figure 5-1 changes in insulin, glucose and C-peptide (A-F). Values are means ± SEM. a Significantly different from baseline from repeated measure ANOVA, P<0.05; b significantly different between two groups from independent t-test, P<0.05. AUC: area under curve.

5.4.3 Insulin resistance and sensitivity

Over the RET, HOMA-β and HOMA-IR did not change significantly ($P<0.05$). However, GIR-Index and Matsuda increased significantly in YP ($P<0.05$) and Cederholm index increased significantly in testosterone group ($P<0.05$, Table 5-1).

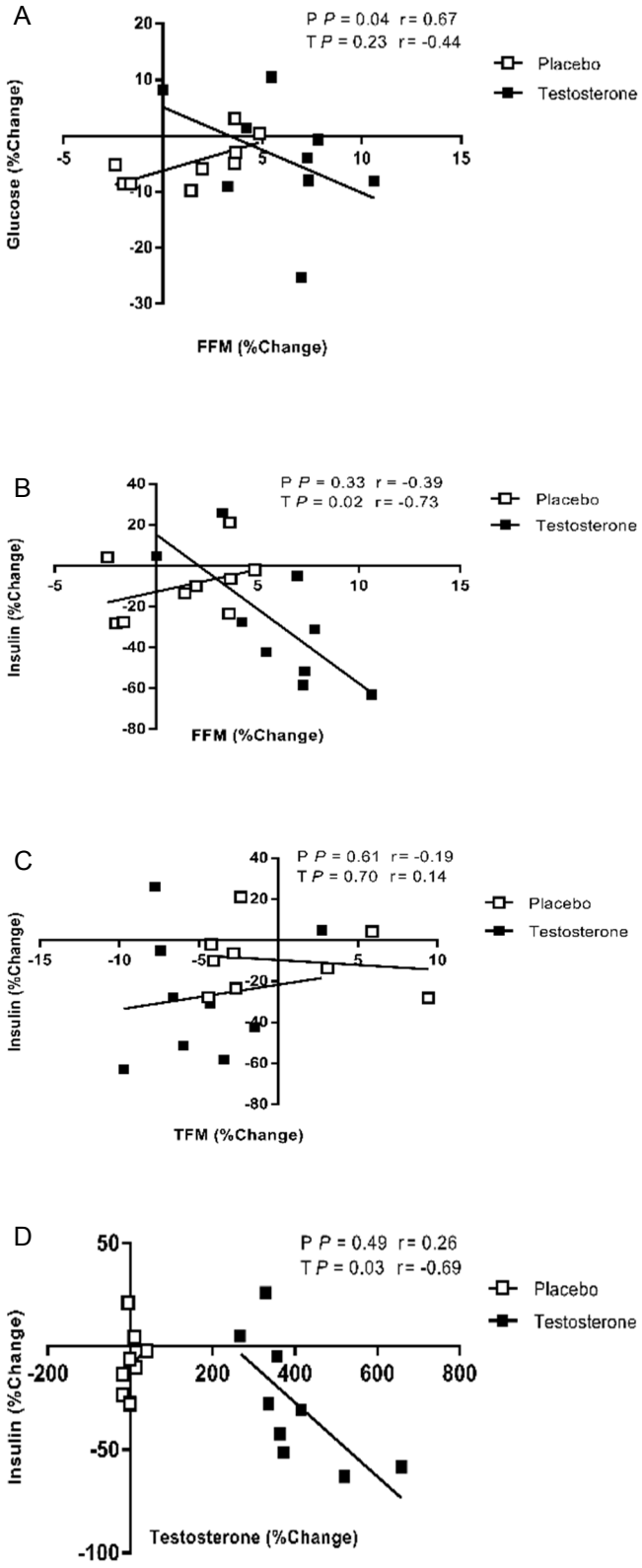
Table 5-1 Glycaemic control-related calculations.

Index	Group	OGTT1	OGTT2	OGTT3
HOMA-β	P(n=9)	----------	----------	36.7±5.1
	T(n=9)	----------	----------	27.1±4.8
HOMA-IR	P(n=9)	1.33±0.2	1.37±0.2	1.03±0.2
	T(n=9)	0.89±0.1	0.88±0.2	0.78±0.1
GIR-Index	P(n=9)	----------	----------	2.4±0.6
	T(n=9)	----------	----------	2.7±0.3
Matsuda	P(n=9)	8.4±2.4	8.1±2.1	10.1±2.6
	T(n=9)	12.2±2.8	13.4±2.5	15.3±2.6
Cederholm	P(n=9)	49.1±3.7	49.8±3.7	57.1±5.1
	T(n=9)	57.3±5.1	60.1±3.5	68.6±5.3[a]

[a] significantly different from OGTT1, [b] significantly different from OGTT2, [c] significantly different based on fold changes, [d] significantly different between groups ($P<0.05$) from two-way ANOVA. HOMA-IR: homeostasis model assessment for insulin resistance; HOMA-B: homeostasis model assessment for beta cell function; GIR-index: Glucose infusion rate index.

5.4.4 Correlations and ratios

Increase in FFM was correlated with increase in glucose levels in placebo group with lower testosterone levels (Figure 5-2 A); however, increase in FFM was negatively correlated with increase in insulin levels in testosterone group with higher testosterone levels (Figure 5-2 B). Moreover, insulin levels was negatively correlated with testosterone levels in testosterone group (Figure 5-2 D). Nevertheless, there was no correlation between insulin and TFM (Figure 5-2 C). In addition, in line with moderately further decrease in glucose levels per unit of FFM in testosterone group (Figure 5-2 F), there was a trend in decreased insulin levels per unit of FFM and per unit of glucose in testosterone group during 6-wks RET. (Figure 5-2 G and H)


A
P P = 0.04 r= 0.67
T P = 0.23 r= -0.44
Placebo
Testosterone
Glucose (%Change)
FFM (%Change)
B
P P = 0.33 r= -0.39
T P = 0.02 r= -0.73
Placebo
Testosterone
Insulin (%Change)
FFM (%Change)
C
P P = 0.61 r= -0.19
T P = 0.70 r= 0.14
Placebo
Testosterone
Insulin (%Change)
TFM (%Change)
D
P P = 0.49 r= 0.26
T P = 0.03 r= -0.69
Placebo
Testosterone
Insulin (%Change)
Testosterone (%Change)

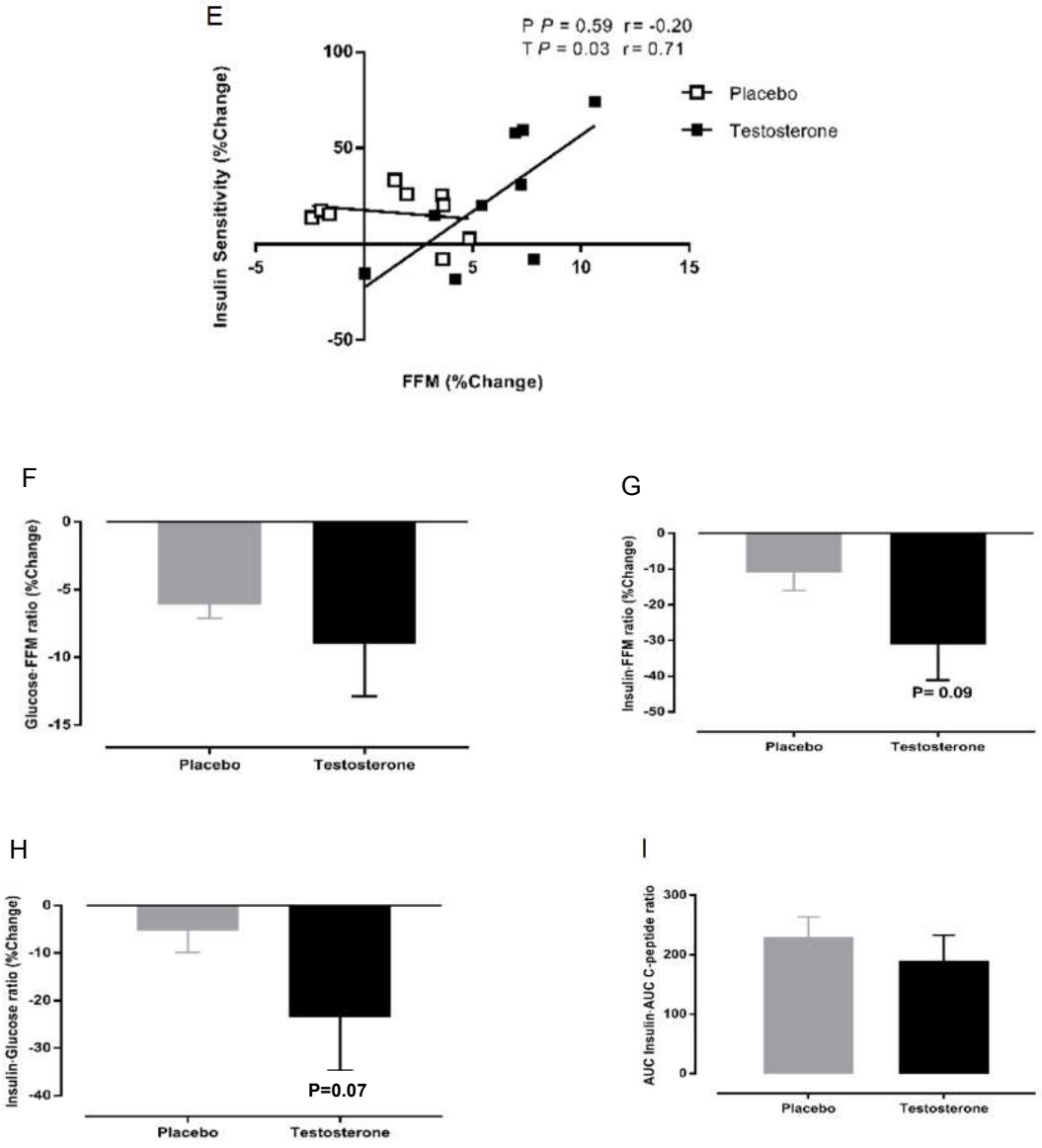


Figure 5-2 Correlations (A-E) and ratios (F-I). Values are means ± SEM. a Significantly different from YP, P<0.05; d significantly different from OT, P<0.05. AUC: area under curve.

5.5 **Discussion**

There is a great need within the scientific field to identifying the role of testosterone and other interventions that alter glucose metabolism with ageing. Herein, we elucidated that just 6-wks whole-body RET coupled with provision of exogenous testosterone in older males had significant effects; i.e. testosterone group showed an attenuated AUC insulin (vs. baseline).

It is reported that long term, 16-wks (Shaibi et al. 2006; Ibañez et al. 2005), 20-wks (Smutok et al. 1993) and 3-month (Eriksson et al. 1997), RET augmented insulin sensitivity similar to those observed following endurance training. However, Zachwieja et al. (1996) (Zachwieja et al. 1996a) showed glycaemic control did not change after 16-wks RET in older men. Indeed, increase in FFM enhanced the available glucose storage area, thereby facilitating the clearance of glucose from the circulation and reducing the amount of insulin required to maintain a normal glucose tolerance (Ibañez et al. 2005). Thus, augmented FFM is associated with improving glycaemic control after RET in men (~8% decrease in glucose levels) (Eriksson et al. 1997); albeit without altering the intrinsic capacity of the muscle to respond to insulin (Poehlman et al. 2000a; Poehlman et al. 2000b; Miller, Sherman, and Ivy 1984). In the present study, we showed the groups had augmented FFM gain (i.e. testosterone), attenuated insulin levels and augmented insulin sensitivity

after 6-wks RET. However, in line with previous studies (Takala, Nuutila, Knuuti, Luotolahti, and Yki-Jarvinen 1999; Takala, Nuutila, Knuuti, Luotolahti, and Yki-Järvinen 1999), RET did not change insulin-stimulated glucose uptake per kilogram of FFM after 6-wks RET, and as increased glycaemic control represents a true long-term adaptation to RET (Ebeling et al. 1993); thus, it seems failure to show increase in insulin sensitivity in placebo group was probably due to short-term (6-wks) RET (Takala, Nuutila, Knuuti, Luotolahti, and Yki-Järvinen 1999). Nevertheless, 12-wks combined RET and aerobic training had no effect on glycaemic control (i.e. glucose and insulin levels) in men (Galvão et al. 2009). Further, 16-wks (Zachwieja et al. 1996b) RET did not improve glycaemic control (involving C-peptide levels) in older men, but 12-wks (Iglay et al. 2007) and 4-months (Hansen et al. 2012) RET decreased fasting C-Peptide levels but not estimated insulin sensitivity and insulin levels in older and middle aged healthy men, respectively. Furthermore, 16-wks heavy RET increased FFM but did not change glucose, insulin, and C-peptide levels in healthy older men (Zachwieja et al. 1996b). Therefore, given to a positive correlation between FFM gain and glucose levels in placebo group, and negative correlation between testosterone and insulin in testosterone group, the positive effects of RET on glycaemic control in testosterone group cannot be ascribed to a mere increase in FFM (Andersen et al. 2003) and likely via factors independent of changes in FFM (Shaibi et al. 2006).

While the relationship of hypogonadism with the development of the metabolic syndrome is not well-defined (Laaksonen et al. 2004), low testosterone levels *per se* is associated with metabolic syndrome (i.e. increased insulin resistance (Grossmann 2014a; Wang et al. 2016), decreased insulin sensitivity (Keating, O'Malley, and Smith 2006) and type 2 diabetes), probably via decrease in FFM and increase in TFM (Wang et al. 2016). Indeed, to support our result showed testosterone provision in testosterone group during RET was associated with attenuated insulin levels, it is postulated that higher testosterone levels and FFM were associated with up-regulated glucose metabolism in healthy men (Hou et al. 2015; Pitteloud, Mootha, Dwyer, Hardin, Lee, Eriksson, Tripathy, Yialamas, Groop, Elahi, et al. 2005). Indeed, testosterone therapy combats glycaemic control deficiency during ageing/illnesses (Grossmann, Gianatti, and Zajac 2010); e.g. provision of exogenous testosterone (200 mg, every 2-wks for 3-monts (Kapoor et al. 2006) or oral 160 mg/day for 6-months) (Emmelot-Vonk et al. 2008) has been shown to reduce insulin resistance and improves glycaemic control in hypogonadal men with type 2 diabetes and healthy older men, respectively. Conversely, testosterone therapy alone for 40-wks (1000 mg injection, every ~2 month) did not improve insulin resistance and glycaemic control in hypogonadal men with type 2 diabetes, notwithstanding increased in FFM and decrease in TFM (Gianatti et al. 2014); indicating the greater benefits of testosterone therapy adjuvant to RET in our study. In our study, we assessed C-peptide levels to measure endogenous insulin secretion, GIR and β-cell function (Jones and

Hattersley 2013). Both C-peptide and insulin are secreted in equimolar amounts from the beta cell as the reliable surrogate for assessing pancreatic β-cells function and GIR (Chailurkit et al. 2007; Ohkura et al. 2013). However, C-peptide is only minimally extracted or metabolized by the liver with longer half-life than insulin (20–30 min vs. 3–5 min) (Jones and Hattersley 2013). In our study, older group with exogenous testosterone provision had lower levels of C-peptide after 6-wks RET, showing decreasing secretion of insulin with higher peripheral insulin sensitivity and GIR. Therefore, testosterone therapy adjuvant to 6-wks RET improved glycaemic control; and is an effective strategy to combat andropause-related metabolic disorders.

In conclusion, testosterone therapy enhanced insulin sensitivity (supported by reduced c-peptide) in older, coupled with RET, showing testosterone is a permissive mediator of RET-induced improvements in insulin sensitivity; and testosterone therapy adjuvant to pre/re-habilitation RET may be of value to overcome the burgeoning trend of worsening glycaemic control with aging

Chapter 6 Overview

6.1 **Overview of the study results and general conclusion**

In this study, we postulated that short-term testosterone suppression blunts adaptations/responses to resistance exercise/training in younger men. In accordance, we showed shorter-term testosterone suppression led to attenuated increases in whole-body muscle growth and glycaemic control, muscle function, MRFs, muscle anabolic signalling, and protein synthesis in adaptive responses during RET. Thus, our data suggest that endogenous testosterone in healthy younger men can be considered as of paramount importance for the muscular adaptation to RET. We established that the younger placebo group developed greater strength gains which also is strongly correlated with testosterone levels. In order to assess the mechanisms underlying the ergogenic effects of normal endogenous testosterone levels on muscle adaptation during RET in younger men, we quantified cumulative rates of myofibrillar protein synthesis over the 6-wks of RET. In doing so, we demonstrated that, in contrast to Zoladex group, placebo group with normal testosterone levels augmented MPS during RET while concomitantly increased muscle protein breakdown (MPB) and crucially, with net balance calculations illustrating MPS>MPB. In addition, in other phase of our study, there is a great deal of interest in identifying the interventions that improve muscle health, combat muscle wasting and also corresponding mechanisms. Herein, we showed that just 6-wks whole-body RET coupled with the exogenous provision of testosterone had significant positive effects on muscle mass, performance, and also peripheral glycaemic control via

inducing net protein accretion through anabolic pathways in older men - thus offsetting age-related muscle loss in adaptive responses to RET. Further, we showed the groups had augmented FFM gain (i.e. testosterone) represented attenuated insulin levels and augmented insulin sensitivity, as well as mitochondrial function/biogenesis after 6-wks RET, indicating the greater energetic adaptation of testosterone therapy adjuvant to RET in older men in our study.

Generally, as the interpretation of our results, long-term testosterone therapy can be an effective therapeutic strategy, especially for patients unable to do RET as the anabolic stimuli, but its application has been limited because of severe side effects. Our study, specifically, targeted healthy men with normal testosterone levels and subjected them to a gain exercise program (RET), with additional randomization to testosterone or placebo in older men and to testosterone suppression or placebo in younger men for ~6 weeks. The principle findings of this study are in general agreement with the literature reporting the role of testosterone on body composition and constitutional symptoms. This study also showed that beneficial impacts of RET are blunted after testosterone withdrawal in younger men, suggesting that higher testosterone levels will likely be required to gain muscle mass and peripheral glycaemic control and these adaptations were associated with increase in muscle strength. On the other hand, lower levels of testosterone did not elicit significant increases in muscle mass or decreases in fat mass in older and younger men in our study, suggesting that the role of testosterone in adaptation (i.e. muscle

plasticity and growth regulation) to short-term RET (i.e. 6-wks) is permissive in older and younger men. This central component role indicated that higher testosterone levels increased the trainability in individuals and that it is associated with high-responders in younger and older men. This demonstrates the potential of testosterone reversing effects on ageing-induced muscle wasting, and anabolic resistance.

In our study, not only we showed short-term testosterone provision is beneficial coupled with RET, but also we investigated the cellular mechanism behind the treatment which helps to enhance our understanding of the androgenic regulation of muscle mass growth and the molecular factors modulated by androgens may help in the identification of effective muscle-specific therapeutic targets to combat the effects of ageing-induced muscle wasting (i.e. sarcopenia). In the first phase of this study, we extensively investigated the mechanistic role of endogenous testosterone in muscle mass growth, function and glycaemic control during RET in healthy young men. Further, our results revealed the likely complex mechanism by which testosterone regulates muscle mass growth through higher expression of AR leads to an increase in protein turnover (with greater increases in MPS), enhancing translational efficiency and capacity, and inducing pro-myogenic and testosterone metabolism gene regulation. In the second phase of our study, higher testosterone levels in older men resulted from testosterone provision led to muscle growth and glycaemic control which mechanistically followed the same pattern as the eugonadal younger group. Finally, to summarize

whole study, given moderate increase in whole body FFM in the younger and older groups with low testosterone levels (i.e. 1.3% increase in older placebo group and 0.07% increase in younger Zoladex group), we should that testosterone has the permissive role in adaptation to RET in young and older men. Accordingly, administration of testosterone coupled to RET is an effective short-term (6-wks) intervention to overcome age-related blunted in the responsiveness of older muscle to RET. As far as the high intensity, albeit short-term, RET adjuvant to testosterone administration were both well-tolerated without any life-threating side-effects or injuries, the strategy could be useful as both pre- and rehabilitation interventions in other clinical-related procedures in older men. However, older men receiving testosterone administration should be carefully monitored because of its potential risks.

6.2 Future research directions

Depart from the physiological and biological impacts of testosterone on regulation of muscle mass in men, testosterone is secreted by the ovaries and the adrenal glands in women (Kalantaridou and Calis 2006). Similar to what we showed in our young participants; premenopausal oophorectomy results in ~5% decrease in testosterone levels, and similar to what we showed in our older participants; women in their 60s have half the testosterone levels of women in their 20s (Kalantaridou and Calis 2006; Dillon et al. 2010). Like men, androgens are important in increase in muscle mass and strength in women (Notelovitz 2002). In accordance, it is reported that transdermal testosterone therapy (150 mg/d) is associated with augmentation in muscle mass and strength in women (Miller et al. 1998; Dolan et al. 2004). This is why despite estrogen and progestin hormone therapy, 67% of young women with premature ovarian failure have the bone density and muscle mass that have been associated with an increased risk for hip fracture (Anasti et al. 1998). It is reported that concurrent estrogen-testosterone therapy increased muscle mass and decreased fat mass in postmenopausal women; and testosterone provision augmented the reduction in core fat mass achieved with estrogen therapy alone (Davis et al. 1995). This is a beneficial impact of testosterone therapy on muscle protein synthesis in women (Dillon et al. 2010). Further, despite the gender differences in endogenous testosterone levels, it is demonstrated age related declines in myofibrillar FSR in both men and women; with higher FSR in women across the

lifespan when compared to men (Henderson et al. 2009). This study showed 12-months testosterone or DHEA therapy did not have any beneficial effects on muscle mass growth in men and women. However, the previous studies have not completely supported the use of testosterone therapy to increase or maintain the muscle mass in women and results are highly contentious. Therefore, further studies are needed to investigate the effects of testosterone therapy adjuvant to RET on muscle quality/programming in premenopausal and/or postmenopausal women.

However, we recognized that there were a number of limitations to our study. Given the results of our study, it seems further studies are needed to fully investigate the testosterone effects on catabolic aspects of muscle (e.g. cortisol levels) growth during short term RET. In addition, regarding the supra-physiological levels of testosterone during first days after each testosterone provision in our study, it seems further studies are needed to investigate the effects of physiological vs. supra-physiological doses of testosterone on pre-requisites to efficacy. Further, we showed that 250 mg testosterone provision bi-weekly coupled to 6-wks RET is an effective strategy to combat the ageing-induced muscle loss, but we still need to know whether this is an effective treatment and precautionary measure in clinical population suffering from muscle atrophy (i.e. sarcopenia, cancer, cachexia, diabetes). In addition, this study showed the beneficial effects of short-term testosterone therapy coupled to RET on muscle gain; but the findings of this current study need to be confirmed and extended in

larger longer term RET after decreasing testosterone doses to avoid any possible high doses-induced side-effects.

References

Abate, Nicola, Steven M Haffner, Abhimanyu Garg, Ronald M Peshock, and Scott M Grundy. 2002. 'Sex steroid hormones, upper body obesity, and insulin resistance', *The Journal of Clinical Endocrinology & Metabolism*, 87: 4522-27.

Abmayr, Susan M, and Grace K Pavlath. 2012. 'Myoblast fusion: lessons from flies and mice', *Development*, 139: 641-56.

Adams, Duncan D. 2008. 'Autoimmune destruction of pericytes as the cause of diabetic retinopathy', *Clinical ophthalmology (Auckland, NZ)*, 2: 295.

Ahtiainen, Juha P., Kai Nyman, Ilpo Huhtaniemi, Tapani Parviainen, Mika Helste, Antti Rannikko, William J. Kraemer, and Keijo Häkkinen. 2015. 'Effects of resistance training on testosterone metabolism in younger and older men', *Experimental gerontology*, 69: 148-58.

Alberti, Kurt George Matthew Mayer, and Paul Z Zimmet. 1998. 'Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation', *Diabetic Medicine*, 15: 539-53.

Anasti, James N, Sophia N Kalantaridou, Lorene M Kimzey, Rubi A Defensor, and Lawrence M Nelson. 1998. 'Bone loss in young women with karyotypically normal spontaneous premature ovarian failure', *Obstetrics & Gynecology*, 91: 12-15.

Andersen, J. L., P. Schjerling, L. L. Andersen, and F. Dela. 2003. 'Resistance training and insulin action in humans: effects of de-training', *The Journal of physiology*, 551: 1049-58.

Andersen, Jesper L., and Per Aagaard. 2000. 'Myosin heavy chain IIX overshoot in human skeletal muscle', *Muscle & nerve*, 23: 1095-104.

Arny A. Ferrando, Melinda Sheffield-Moore Catherine W. Yeckel Charles Gilkison Jie Jiang Alison Achacosa Steven A. Lieberman Kevin Tipton Robert R. Wolfe, and Urban Randall J. 2002. 'Testosterone administration to older men improves muscle function: molecular and physiological mechanisms', *Am J Physiol Endocrinol Metab*, 282: 601–7.

Atherton, PJ, and K Smith. 2012. 'Muscle protein synthesis in response to nutrition and exercise', *The Journal of physiology*, 590: 1049-57.

Atkinson, R. A., U. Srinivas-Shankar, S. A. Roberts, M. J. Connolly, J. E. Adams, J. A. Oldham, F. C. Wu, O. R. Seynnes, C. E. Stewart, C. N. Maganaris, and M. V. Narici. 2010. 'Effects of testosterone on skeletal muscle architecture in intermediate-frail and frail elderly men', *J Gerontol A Biol Sci Med Sci*, 65: 1215-9.

Atlantis, Evan, Sean A Martin, Matthew T Haren, Anne W Taylor, and Gary A Wittert. 2009. 'Inverse associations between muscle mass, strength, and the metabolic syndrome', *Metabolism*, 58: 1013-22.

Aversa, Antonio, Roberto Bruzziches, Davide Francomano, Giuseppe Rosano, Andrea M Isidori, Andrea Lenzi, and Giovanni Spera. 2010. 'Effects of testosterone undecanoate on cardiovascular risk factors and atherosclerosis in middle-aged men with late-onset hypogonadism and metabolic syndrome: results from a 24-month, randomized, double-blind, placebo-controlled study', *The journal of sexual medicine*, 7: 3495-503.

Baar, Keith, and Karyn Esser. 1999. 'Phosphorylation of p70S6kcorrelates with increased skeletal muscle mass following resistance exercise', *American Journal of Physiology-Cell Physiology*, 276: C120-C27.

Baar, Keith, Gustavo Nader, and Sue Bodine. 2006. 'Resistance exercise, muscle loading/unloading and the control of muscle mass', *Essays in biochemistry*, 42: 61.

Balakrishnan, Vaidyanatha S, Madhumathi Rao, Vandana Menon, Patricia L Gordon, Monika Pilichowska, Francisco Castaneda, and Carmen Castaneda-Sceppa. 2010. 'Resistance training increases muscle mitochondrial biogenesis in patients with chronic kidney disease', *Clinical Journal of the American Society of Nephrology*: 996-1002.

Balkau, Beverley, Xavier Jouven, Pierre Ducimetière, and Eveline Eschwège. 1999. 'Diabetes as a risk factor for sudden death', *The Lancet*, 354: 1968-69.

Bandeen-Roche, Karen, Qian-Li Xue, Luigi Ferrucci, Jeremy Walston, Jack M Guralnik, Paulo Chaves, Scott L Zeger, and Linda P Fried. 2006. 'Phenotype of frailty: characterization in the women's health and aging studies', *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 61: 262-66.

Barrett-Connor, Elizabeth. 1992. 'Lower endogenous androgen levels and dyslipidemia in men with non-insulin-dependent diabetes mellitus', *Annals of Internal Medicine*, 117: 807-11.

Basaria, Shehzad, Andrea D Coviello, Thomas G Travison, Thomas W Storer, Wildon R Farwell, Alan M Jette, Richard Eder, Sharon Tennstedt, Jagadish Ulloor, and Anqi Zhang. 2010. 'Adverse events associated with testosterone administration', *New England Journal of Medicine*, 363: 109-22.

Bass, Joseph J, Daniel J Wilkinson, Debbie Rankin, Bethan E Phillips, Nathaniel J Szewczyk, Kenneth Smith, and Philip J Atherton. 2017. 'An overview of technical considerations for Western blotting applications to physiological research', *Scandinavian journal of medicine & science in sports*, 27: 4-25.

Basualto-Alarcón, Carla, Gonzalo Jorquera, Francisco Altamirano, Enrique Jaimovich, and Manuel Estrada. 2013. 'Testosterone signals through mTOR and androgen receptor to induce muscle hypertrophy', *Medicine and science in sports and exercise*, 45: 1712-20.

Baumgartner, Richard N. 2000. 'Body composition in healthy aging', *Annals of the New York Academy of Sciences*, 904: 437-48.

Baumgartner, Richard N, Debra L Waters, Dympna Gallagher, John E Morley, and Philip J Garry. 1999. 'Predictors of skeletal muscle mass in elderly men and women', *Mechanisms of ageing and development*, 107: 123-36.

Behre, Hermann M, and Susan Nieschlag. 2012. *Testosterone* (Springer).

Bellew, JW. 2002. 'The effect of strength training on control of force in older men and women', *Aging clinical and experimental research*, 14: 35-41.

Bennett, Nigel C, Robert A Gardiner, John D Hooper, David W Johnson, and Glenda C Gobe. 2010. 'Molecular cell biology of androgen receptor signalling', *The international journal of biochemistry & cell biology*, 42: 813-27.

Berkes, Charlotte A, and Stephen J Tapscott. 2005. "MyoD and the transcriptional control of myogenesis." In *Seminars in cell & developmental biology*, 585-95. Elsevier.

Bhasin, S, L Woodhouse, and TW Storer. 2001. 'Proof of the effect of testosterone on skeletal muscle', *Journal of endocrinology*, 170: 27-38.

Bhasin, S., T. W. Storer, N. Berman, C. Callegari, B. Clevenger, J. Phillips, T. J. Bunnell, R. Tricker, A. Shirazi, and R. Casaburi. 1996a. 'The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men', *N Engl J Med*, 335: 1-7.

Bhasin, Shalender, Thomas W Storer, Nancy Berman, Carlos Callegari, Brenda Clevenger, Jeffrey Phillips, Thomas J Bunnell, Ray Tricker, Aida Shirazi, and Richard Casaburi. 1996b. 'The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men', *New England Journal of Medicine*, 335: 1-7.

Bhasin, Shalender, Wayne E Taylor, Rajan Singh, Jorge Artaza, Indrani Sinha-Hikim, Ravi Jasuja, Helen Choi, and Nestor F Gonzalez-Cadavid. 2003. 'The mechanisms of androgen effects on body composition: mesenchymal pluripotent cell as the target of androgen action', *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 58: 1103-10.

Bhasin, Shalender, Linda Woodhouse, Richard Casaburi, Atam B Singh, Dimple Bhasin, Nancy Berman, Xianghong Chen, Kevin E Yarasheski, Lynne Magliano, and Connie Dzekov. 2001a. 'Testosterone dose-response relationships in healthy young men', *American Journal of Physiology-Endocrinology And Metabolism*, 281: 1172-81.

Bhasin, Shalender, Linda Woodhouse, Richard Casaburi, Atam B Singh, Ricky Phong Mac, Martin Lee, Kevin E Yarasheski, Indrani Sinha-Hikim, Connie Dzekov, and Jeanne Dzekov. 2005. 'Older men are as responsive as young men to the anabolic effects of graded doses of testosterone on the skeletal muscle', *The Journal of Clinical Endocrinology & Metabolism*, 90: 678-88.

Biolo, Gianni, Sergio P Maggi, Bradley D Williams, Kevin D Tipton, and Robert R Wolfe. 1995. 'Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans', *American Journal of Physiology-Endocrinology And Metabolism*, 268: E514-E20.

Bird, JW, JULIA H Carter, RICHARD E Triemer, RM Brooks, and AM Spanier. 1980. "Proteinases in cardiac and skeletal muscle." In *Federation proceedings*, 20-25.

Birkeland, Kare I, Kristian F Hanssen, Peter A Torjesen, and Stein Vaaler. 1993. 'Level of sex hormone-binding globulin is positively correlated with insulin sensitivity in men with type 2 diabetes', *The Journal of Clinical Endocrinology & Metabolism*, 76: 275-78.

Bodine, Sue C. 2006. 'mTOR signaling and the molecular adaptation to resistance exercise', *Medicine and science in sports and exercise*, 38: 1950-57.

Bodine, Sue C, Trevor N Stitt, Michael Gonzalez, William O Kline, Gretchen L Stover, Roy Bauerlein, Elizabeth Zlotchenko, Angus Scrimgeour, John C Lawrence, and David J Glass. 2001. 'Akt/mTOR pathway is a crucial

regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo', *Nature cell biology*, 3: 1014.

Bolster, Douglas R, Stephen J Crozier, Scot R Kimball, and Leonard S Jefferson. 2002. 'AMP-activated protein kinase suppresses protein synthesis in rat skeletal muscle through down-regulated mammalian target of rapamycin (mTOR) signaling', *Journal of Biological Chemistry*, 277: 23977-80.

Booth, Frank W, and P Darrell Neufer. 2005. 'Exercise Controls Gene Expression: The activity level of skeletal muscle modulates a range of genes that produce dramatic molecular changes—and keep us healthy', *American Scientist*, 93: 28-35.

Borgenvik, Marcus, William Apró, and Eva Blomstrand. 2011. 'Intake of branched-chain amino acids influences the levels of MAFbx mRNA and MuRF-1 total protein in resting and exercising human muscle', *American Journal of Physiology-Endocrinology And Metabolism*, 302: E510-E21.

Borst, Stephen E, and Joshua F Yarrow. 2015. 'Injection of testosterone may be safer and more effective than transdermal administration for combating loss of muscle and bone in older men', *American Journal of Physiology-Endocrinology And Metabolism*, 308: 1035-42.

Breier, BH, and PD Gluckman. 1991. 'The regulation of postnatal growth: nutritional influences on endocrine pathways and function of the somatotrophic axis', *Livestock Production Science*, 27: 77-94.

Brodsky, Irwin G, P Balagopal, and K Sreekumaran Nair. 1996. 'Effects of testosterone replacement on muscle mass and muscle protein synthesis in hypogonadal men--a clinical research center study', *The Journal of Clinical Endocrinology & Metabolism*, 81: 3469-75.

Brook, Matthew S, Daniel J Wilkinson, William K Mitchell, Jonathan N Lund, Bethan E Phillips, Nathaniel J Szewczyk, Paul L Greenhaff, Kenneth Smith, and Philip J Atherton. 2016. 'Synchronous deficits in cumulative muscle protein synthesis and ribosomal biogenesis underlie age-related anabolic resistance to exercise in humans', *The Journal of physiology*, 594: 7399-417.

Brook, Matthew S, Daniel J Wilkinson, Kenneth Smith, and Philip J Atherton. 2016. 'The metabolic and temporal basis of muscle hypertrophy in response to resistance exercise', *European journal of sport science*, 16: 633-44.

Brook, Matthew S., Daniel J. Wilkinson, William K. Mitchell, Jonathan N. Lund, Nathaniel J. Szewczyk, Paul L. Greenhaff, Ken Smith, and Philip J. Atherton. 2015. 'Skeletal muscle hypertrophy adaptations predominate in the early stages of resistance exercise training, matching deuterium oxide-derived measures of muscle protein synthesis and mechanistic target of rapamycin complex 1 signaling', *The FASEB Journal*, 29: 4485-96.

Brook, MS, DJ Wilkinson, BE Phillips, J Perez-Schindler, A Philp, K Smith, and PJ Atherton. 2016. 'Skeletal muscle homeostasis and plasticity in youth and ageing: impact of nutrition and exercise', *Acta Physiologica*, 216: 15-41.

Burd, Nicholas A, Benjamin T Wall, and Luc JC van Loon. 2012. 'The curious case of anabolic resistance: old wives' tales or new fables?', *Journal of Applied Physiology*, 112: 1233-35.

Burd, Nicholas A, Daniel WD West, Daniel R Moore, Philip J Atherton, Aaron W Staples, Todd Prior, Jason E Tang, Michael J Rennie, Steven K Baker, and Stuart M Phillips. 2011. 'Enhanced amino acid sensitivity of myofibrillar protein synthesis persists for up to 24 h after resistance exercise in young men', *The Journal of Nutrition*, 141: 568-73.

Burd, Nicholas A, Daniel WD West, Aaron W Staples, Philip J Atherton, Jeff M Baker, Daniel R Moore, Andrew M Holwerda, Gianni Parise, Michael J Rennie, and Steven K Baker. 2010. 'Low-load high volume resistance exercise stimulates muscle protein synthesis more than high-load low volume resistance exercise in young men', *PloS one*, 5: e12033.

Burton, L. A., and D. Sumukadas. 2010a. 'Optimal management of sarcopenia', *Clin Interv Aging*, 5: 217-28.

Burton, Louise A, and Deepa Sumukadas. 2010b. 'Optimal management of sarcopenia', *Clinical interventions in aging*, 5: 217.

Cartee, Gregory D. 2015. 'Mechanisms for greater insulin-stimulated glucose uptake in normal and insulin-resistant skeletal muscle after acute exercise', *American Journal of Physiology-Endocrinology And Metabolism*, 309: E949-E59.

Casaburi, Richard, Shalender Bhasin, Louis Cosentino, Janos Porszasz, Attila Somfay, Michael I Lewis, Mario Fournier, and Thomas W Storer. 2004. 'Effects of testosterone and resistance training in men with chronic obstructive pulmonary disease', *American journal of respiratory and critical care medicine*, 170: 870-8.

Caserotti, Paolo, P Aagaard, J Buttrup Larsen, and L Puggaard. 2008. 'Explosive heavy-resistance training in old and very old adults: changes in rapid muscle force, strength and power', *Scandinavian journal of medicine & science in sports*, 18: 773-82.

Castoria, Gabriella, Maria Lombardi, Maria Vittoria Barone, Antonio Bilancio, Marina Di Domenico, Daniela Bottero, Flavia Vitale, Antimo Migliaccio, and Ferdinando Auricchio. 2003. 'Androgen-stimulated DNA synthesis and cytoskeletal changes in fibroblasts by a nontranscriptional receptor action', *J Cell Biol*, 161: 547-56.

Cederholm, Jan, and Lars Wibell. 1990. 'Insulin release and peripheral sensitivity at the oral glucose tolerance test', *Diabetes research and clinical practice*, 10: 167-75.

Chai, Ruth Jinfen, Jana Vukovic, Sarah Dunlop, Miranda D Grounds, and Thea Shavlakadze. 2011. 'Striking denervation of neuromuscular junctions without lumbar motoneuron loss in geriatric mouse muscle', *PloS one*, 6: e28090.

Chaillou, Thomas. 2017. 'Impaired ribosome biogenesis could contribute to anabolic resistance to strength exercise in the elderly', *The Journal of physiology*, 595: 1447-8.

Chailurkit, La-or, Wallaya Jongjaroenprasert, Suwannee Chanprasertyothin, and Boonsong Ongphiphadhanakul. 2007. 'Insulin and C-peptide levels, pancreatic beta cell function, and insulin resistance across glucose

tolerance status in Thais', *Journal of clinical laboratory analysis*, 21: 85-90.

Chal, Jérome, and Olivier Pourquié. 2017. 'Making muscle: skeletal myogenesis in vivo and in vitro', *Development*, 144: 2104-22.

Chandel, Anil, Sandeep Dhindsa, Shehzad Topiwala, Ajay Chaudhuri, and Paresh Dandona. 2008. 'Testosterone Concentration in Young Patients With Diabetes', *DIABETES CARE*, 31: 2013-17.

Chang, J. H., K. H. Lin, C. H. Shih, Y. J. Chang, H. C. Chi, and S. L. Chen. 2006. 'Myogenic basic helix-loop-helix proteins regulate the expression of peroxisomal proliferator activated receptor-gamma coactivator-1alpha', *Endocrinology*, 147: 3093-106.

Charge, Sophie BP, and Michael A Rudnicki. 2004. 'Cellular and molecular regulation of muscle regeneration', *Physiological reviews*, 84: 209-38.

Chiang, J. L., M. S. Kirkman, L. M. Laffel, and A. L. Peters. 2014. 'Type 1 diabetes through the life span: a position statement of the American Diabetes Association', *DIABETES CARE*, 37: 2034-54.

Chondrogianni, Niki, and Efstathios S Gonos. 2007. 'Overexpression of hUMP1/POMP proteasome accessory protein enhances proteasome-mediated antioxidant defence', *Experimental gerontology*, 42: 899-903.

Christ-Roberts, Christine Y, Thongchai Pratipanawatr, Wilailak Pratipanawatr, Rachele Berria, Renata Belfort, Sangeeta Kashyap, and Lawrence J Mandarino. 2004. 'Exercise training increases glycogen synthase activity and GLUT4 expression but not insulin signaling in overweight nondiabetic and type 2 diabetic subjects', *Metabolism*, 53: 1233-42.

Churchward-Venne, Tyler A, Nicholas A Burd, and Stuart M Phillips. 2012. 'Nutritional regulation of muscle protein synthesis with resistance exercise: strategies to enhance anabolism', *Nutrition & Metabolism*, 9: 40.

Clague, J. E., F. C. Wu, and M. A. Horan. 1999. 'Difficulties in measuring the effect of testosterone replacement therapy on muscle function in older men', *Int J Androl*, 22: 261-5.

Cleasby, Mark E, Pauline M Jamieson, and Philip J Atherton. 2016. 'Insulin resistance and sarcopenia: mechanistic links between common co-morbidities', *Journal of Endocrinology*, 229: R67-R81.

Coffey, Vernon G, and John A Hawley. 2007. 'The molecular bases of training adaptation', *Sports Medicine*, 37: 737-63.

Coffey, Vernon G, Anthony Shield, Benedict J Canny, Kate A Carey, David Cameron-Smith, and John A Hawley. 2006. 'Interaction of contractile activity and training history on mRNA abundance in skeletal muscle from trained athletes', *American Journal of Physiology-Endocrinology And Metabolism*, 290: E849-E55.

Coffey, Vernon G, Zhihui Zhong, Anthony Shield, Benedict J Canny, Alexander V Chibalin, Juleen R Zierath, and John A Hawley. 2006. 'Early signaling responses to divergent exercise stimuli in skeletal muscle from well-trained humans', *The FASEB Journal*, 20: 190-92.

Corona, Giovanni, Matteo Monami, Giulia Rastrelli, Antonio Aversa, A Sforza, A Lenzi, G Forti, E Mannucci, and M Maggi. 2011. 'Type 2 diabetes mellitus and testosterone: A meta-analysis study', *International journal of andrology*, 34: 528-40.

Costello, LC, and Renty B Franklin. 1993. 'Testosterone regulates pyruvate dehydrogenase activity of prostate mitochondria', *Hormone and metabolic research*, 25: 268-70.

Coyle, EF, JM Hagberg, BF Hurley, WH Martin, AA Ehsani, and JO Holloszy. 1983. 'Carbohydrate feeding during prolonged strenuous exercise can delay fatigue', *Journal of Applied Physiology*, 55: 230-35.

Croymans, Daniel M, Ergit Paparisto, Mary M Lee, Nina Brandt, Brian K Le, Derek Lohan, Cathy C Lee, and Christian K Roberts. 2013. 'Resistance training improves indices of muscle insulin sensitivity and β-cell function in overweight/obese, sedentary young men', *Journal of Applied Physiology*, 115: 1245-53.

Cruz-Jentoft, Alfonso J, Jean Pierre Baeyens, Jürgen M Bauer, Yves Boirie, Tommy Cederholm, Francesco Landi, Finbarr C Martin, Jean-Pierre Michel, Yves Rolland, and Stéphane M Schneider. 2010. 'Sarcopenia: European consensus on definition and diagnosisReport of the European Working Group on Sarcopenia in Older PeopleA. J. Cruz-Gentoft et al', *Age and ageing*, 39: 412-23.

Cunningham, J. T., J. T. Rodgers, D. H. Arlow, F. Vazquez, V. K. Mootha, and P. Puigserver. 2007. 'mTOR controls mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex', *Nature*, 450: 736-40.

Cuthbertson, Daniel J, John Babraj, Kenneth Smith, Emilie Wilkes, Mark J Fedele, Karyn Esser, and Michael Rennie. 2006. 'Anabolic signaling and protein synthesis in human skeletal muscle after dynamic shortening or lengthening exercise', *American Journal of Physiology-Endocrinology And Metabolism*, 290: E731-E38.

Dandona, Paresh, and Sandeep Dhindsa. 2011. 'Update: hypogonadotropic hypogonadism in type 2 diabetes and obesity', *The Journal of Clinical Endocrinology & Metabolism*, 96: 2643-51.

Datta, Sandeep Robert, Henryk Dudek, Xu Tao, Shane Masters, Haian Fu, Yukiko Gotoh, and Michael E Greenberg. 1997. 'Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery', *Cell*, 91: 231-41.

Davis, Susan R, Philip McCloud, Boyd JG Strauss, and Henry Burger. 1995. 'Testosterone enhances estradiol's effects on postmenopausal bone density and sexuality', *Maturitas*, 21: 227-36.

Dayanidhi, Sudarshan, and Richard L Lieber. 2014. 'Skeletal muscle satellite cells: mediators of muscle growth during development and implications for developmental disorders', *Muscle & nerve*, 50: 723-32.

Dennis, Michael D, Leonard S Jefferson, and Scot R Kimball. 2012. 'Role of p70S6K1-mediated phosphorylation of eIF4B and PDCD4 proteins in the regulation of protein synthesis', *Journal of Biological Chemistry*, 287: 42890-99.

Desa, UN. 2014. 'World urbanization prospects, the 2011 revision', *Population Division, Department of Economic and Social Affairs, United Nations Secretariat*.

Deschenes, M. R. 2004a. 'Effects of aging on muscle fibre type and size', *Sports Med*, 34: 809-24.

Deschenes, Michael R. 2004b. 'Effects of aging on muscle fibre type and size', *Sports Medicine*, 34: 809-24.

Deschenes, Michael R, Jonathan Covault, William J Kraemer, and Carl M Maresh. 1994. 'The Neuromuscular Junction', *Sports Medicine*, 17: 358-72.

Dhindsa, Sandeep, Husam Ghanim, Manav Batra, and Paresh Dandona. 2018. 'Hypogonadotropic Hypogonadism in Men With Diabesity', *DIABETES CARE*, 41: 1516-25.

Dhindsa, Sandeep, Husam Ghanim, Manav Batra, Nitesh D Kuhadiya, Sanaa Abuaysheh, Sartaj Sandhu, Kelly Green, Antoine Makdissi, Jeanne Hejna, and Ajay Chaudhuri. 2016. 'Insulin resistance and inflammation in hypogonadotropic hypogonadism and their reduction after testosterone replacement in men with type 2 diabetes', *DIABETES CARE*, 39: 82-91.

Diehl, J Alan, Mangeng Cheng, Martine F Roussel, and Charles J Sherr. 1998. 'Glycogen synthase kinase-3β regulates cyclin D1 proteolysis and subcellular localization', *Genes & development*, 12: 3499-511.

Dietrichson, P, J Coakley, PE Smith, RD Griffiths, TR Helliwell, and RH Edwards. 1987. 'Conchotome and needle percutaneous biopsy of skeletal muscle', *Journal of Neurology, Neurosurgery & Psychiatry*, 50: 1461-7.

Dillon, E Lichar, William J Durham, Randall J Urban, and Melinda Sheffield-Moore. 2010. 'Hormone treatment and muscle anabolism during aging: androgens', *Clinical Nutrition*, 29: 697-700.

Dohoney, Paula, Joseph A. Chromiak, Derek Lemire, Ben R. Abadie, and Christopher Kovacs. 2002. 'Prediction of one repetition maximum (1-RM) strength from a 4-6 RM and a 7-10 RM submaximal strength test in healthy young adult males', *J Exerc Physiol*, 5: 54-9.

Dolan, Sara, Stephanie Wilkie, Negar Aliabadi, Meghan P Sullivan, Nesli Basgoz, Benjamin Davis, and Steven Grinspoon. 2004. 'Effects of testosterone administration in human immunodeficiency virus–infected women with low weight: a randomized placebo-controlled study', *Archives of Internal Medicine*, 164: 897-904.

Dreyer, Hans C, Micah J Drummond, Bart Pennings, Satoshi Fujita, Erin L Glynn, David L Chinkes, Shaheen Dhanani, Elena Volpi, and Blake B Rasmussen. 2008. 'Leucine-enriched essential amino acid and carbohydrate ingestion following resistance exercise enhances mTOR signaling and protein synthesis in human muscle', *American Journal of Physiology-Endocrinology And Metabolism*, 294: E392-E400.

Dreyer, Hans C, Satoshi Fujita, Jerson G Cadenas, David L Chinkes, Elena Volpi, and Blake B Rasmussen. 2006. 'Resistance exercise increases AMPK activity and reduces 4E-BP1 phosphorylation and protein synthesis in human skeletal muscle', *The Journal of physiology*, 576: 613-24.

Drummond, Micah J, Hans C Dreyer, Bart Pennings, Christopher S Fry, Shaheen Dhanani, Edgar L Dillon, Melinda Sheffield-Moore, Elena Volpi, and Blake B Rasmussen. 2008. 'Skeletal muscle protein anabolic response to resistance exercise and essential amino acids is delayed with aging', *Journal of Applied Physiology*, 104: 1452-61.

Drummond, Micah J, Christopher S Fry, Erin L Glynn, Hans C Dreyer, Shaheen Dhanani, Kyle L Timmerman, Elena Volpi, and Blake B Rasmussen. 2009. 'Rapamycin administration in humans blocks the contraction-induced increase in skeletal muscle protein synthesis', *The Journal of physiology*, 587: 1535-46.

Dubois, V., M. Laurent, S. Boonen, D. Vanderschueren, and F. Claessens. 2012. 'Androgens and skeletal muscle: cellular and molecular action mechanisms underlying the anabolic actions', *Cell Mol Life Sci*, 69: 1651-67.

Ebeling, Pertti, Raymond Bourey, Laszlo Koranyi, Juha A Tuominen, Leif C Groop, Jan Henriksson, Mike Mueckler, A Sovijärvi, and Veikko A Koivisto. 1993. 'Mechanism of enhanced insulin sensitivity in athletes. Increased blood flow, muscle glucose transport protein (GLUT-4) concentration, and glycogen synthase activity', *The Journal of clinical investigation*, 92: 1623-31.

Eliasson, Jorgen, Thibault Elfegoun, Johnny Nilsson, Rickard Kohnke, Björn Ekblom, and Eva Blomstrand. 2006. 'Maximal lengthening contractions increase p70 S6 kinase phosphorylation in human skeletal muscle in the absence of nutritional supply', *American Journal of Physiology-Endocrinology And Metabolism*, 291: E1197-E205.

Emery, Alan EH. 2002. 'Muscular dystrophy into the new millennium', *Neuromuscular Disorders*, 12: 343-49.

Emmelot-Vonk, Marielle H, Harald JJ Verhaar, Hamid R Nakhai Pour, André Aleman, Tycho MTW Lock, JLH Ruud Bosch, Diederick E Grobbee, and Yvonne T van der Schouw. 2008. 'Effect of testosterone supplementation on functional mobility, cognition, and other parameters in older men: a randomized controlled trial', *Jama*, 299: 39-52.

Eriksson, J., S. Taimela, K. Eriksson, S. Parviainen, J. Peltonen, and U. Kujala. 1997. 'Resistance training in the treatment of non-insulin-dependent diabetes mellitus', *Int J Sports Med*, 18: 242-6.

Evans, William J, and Jan Lexell. 1995. 'Human aging, muscle mass, and fiber type composition', *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 50: 11-16.

Evans, William J, John E Morley, Josep Argilés, Connie Bales, Vickie Baracos, Denis Guttridge, Aminah Jatoi, Kamyar Kalantar-Zadeh, Herbert Lochs, and Giovanni Mantovani. 2008. 'Cachexia: a new definition', *Clinical Nutrition*, 27: 793-99.

Fagan, Julie M, Lloyd Waxman, and Alfred L Goldberg. 1987. 'Skeletal muscle and liver contain a soluble ATP+ ubiquitin-dependent proteolytic system', *Biochemical Journal*, 243: 335-43.

Fan, WuQiang, Toshihiko Yanase, Masatoshi Nomura, Taijiro Okabe, Kiminobu Goto, Takashi Sato, Hirotaka Kawano, Shigeaki Kato, and Hajime Nawata. 2005. 'Androgen receptor null male mice develop late-onset obesity caused by decreased energy expenditure and lipolytic activity but show normal insulin sensitivity with high adiponectin secretion', *Diabetes*, 54: 1000-8.

Fanzani, Alessandro, Viviane M Conraads, Fabio Penna, and Wim Martinet. 2012. 'Molecular and cellular mechanisms of skeletal muscle atrophy: an update', *Journal of cachexia, sarcopenia and muscle*, 3: 163-79.

Favier, François B, Henri Benoit, and Damien Freyssenet. 2008. 'Cellular and molecular events controlling skeletal muscle mass in response to altered use', *Pflügers Archiv-European Journal of Physiology*, 456: 587-600.

Feldman, Henry A, Christopher Longcope, Carol A Derby, Catherine B Johannes, Andre B Araujo, Andrea D Coviello, William J Bremner, and John B McKinlay. 2002. 'Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts male aging study', *The Journal of Clinical Endocrinology & Metabolism*, 87: 589-98.

Feldstein, Adrianne C, Gregory A Nichols, David H Smith, Victor J Stevens, Keith Bachman, A Gabriela Rosales, and Nancy Perrin. 2008. 'Weight change in diabetes and glycemic and blood pressure control', *DIABETES CARE*, 31: 1960-65.

Ferguson-Stegall, Lisa, Erin McCleave, Zhenping Ding, Phillip G Doerner III, Yang Liu, Bei Wang, Marin Healy, Maximilian Kleinert, Benjamin Dessard, and David G Lassiter. 2011. 'Aerobic exercise training adaptations are increased by postexercise carbohydrate-protein supplementation', *Journal of nutrition and metabolism*, 2011.

Fernández-Balsells, M Mercè, Mohammad Hassan Murad, Melanie Lane, Juliana F Lampropulos, Felipe Albuquerque, Rebecca J Mullan, Neera Agrwal, Mohamed B Elamin, Juan F Gallegos-Orozco, and Amy T Wang. 2010. 'Adverse effects of testosterone therapy in adult men: a systematic review and meta-analysis', *The Journal of Clinical Endocrinology & Metabolism*, 95: 2560-75.

Ferrando, Arny A, Melinda Sheffield-Moore, Catherine W Yeckel, Charles Gilkison, Jie Jiang, Alison Achacosa, Steven A Lieberman, Kevin Tipton, Robert R Wolfe, and Randall J Urban. 2002a. 'Testosterone administration to older men improves muscle function: molecular and physiological mechanisms', *American Journal of Physiology-Endocrinology And Metabolism*.282: E601-E07.

Ferrando, Arny A, Kevin D Tipton, David Doyle, Stuart M Phillips, Joaquin Cortiella, and Robert R Wolfe. 1998a. 'Testosterone injection stimulates net protein synthesis but not tissue amino acid transport', *American Journal of Physiology-Endocrinology And Metabolism*, 275: E864-E71.

Figueiredo, Vandre Casagrande, and John J McCarthy. 2018. 'Regulation of ribosome biogenesis in skeletal muscle hypertrophy', *Physiology*, 34: 30-42.

Fiorotto, Marta L, Robert J Schwartz, and M Craig Delaughter. 2003. 'Persistent IGF-I overexpression in skeletal muscle transiently enhances DNA accretion and growth', *The FASEB Journal*, 17: 59-60.

Flück, M, and H Hoppeler. 2003. 'Molecular basis of skeletal muscle plasticity-from gene to form and function.' in, *Reviews of physiology, biochemistry and pharmacology* (Springer).

Folland, Jonathan P, and Alun G Williams. 2007. 'Morphological and neurological contributions to increased strength', *Sports Medicine*, 37: 145-68.

Franchi, Martino V., Daniel J. Wilkinson, Jonathan I. Quinlan, William K. Mitchell, Jonathan N. Lund, John P. Williams, Neil D. Reeves, Kenneth Smith, Philip J. Atherton, and Marco V. Narici. 2015. 'Early structural

remodeling and deuterium oxide-derived protein metabolic responses to eccentric and concentric loading in human skeletal muscle', *Physiological Reports*, 3: 1-11.

Frontera, Walter R, and Julien Ochala. 2015. 'Skeletal muscle: a brief review of structure and function', *Calcified tissue international*, 96: 183-95.

Fry, Christopher S, Micah J Drummond, Erin L Glynn, Jared M Dickinson, David M Gundermann, Kyle L Timmerman, Dillon K Walker, Shaheen Dhanani, Elena Volpi, and Blake B Rasmussen. 2011. 'Aging impairs contraction-induced human skeletal muscle mTORC1 signaling and protein synthesis', *Skeletal muscle*, 1: 11.

Fry, Christopher S, Micah J Drummond, Erin L Glynn, Jared M Dickinson, David M Gundermann, Kyle L Timmerman, Dillon K Walker, Elena Volpi, and Blake B Rasmussen. 2012. 'Skeletal muscle autophagy and protein breakdown following resistance exercise are similar in younger and older adults', *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences*, 68: 599-607.

Galavazi, Gerbrand, and John A Szirmai. 1971. 'Cytomorphometry of skeletal muscle: The influence of age and testosterone on the rat M. levator ani', *Zeitschrift für Zellforschung und Mikroskopische Anatomie*, 121: 507-30.

Galvão, Daniel A., Dennis R. Taaffe, Nigel Spry, David Joseph, and Robert U. Newton. 2009. 'Combined Resistance and Aerobic Exercise Program Reverses Muscle Loss in Men Undergoing Androgen Suppression Therapy for Prostate Cancer Without Bone Metastases: A Randomized Controlled Trial', *Journal of Clinical Oncology*, 28: 340-47.

Gianatti, Emily J, Philippe Dupuis, Rudolf Hoermann, Boyd J Strauss, John M Wentworth, Jeffrey D Zajac, and Mathis Grossmann. 2014. 'Effect of testosterone treatment on glucose metabolism in men with type 2 diabetes: a randomized controlled trial', *DIABETES CARE*, 37: 2098-107.

Glass, David J. 2003. 'Molecular mechanisms modulating muscle mass', *Trends in molecular medicine*, 9: 344-50.

Glintborg, Dorte, LL Christensen, Thue Kvorning, Rasmus Larsen, K Brixen, DM Hougaard, Bjørn Richelsen, JM Bruun, and M Andersen. 2013. 'Strength training and testosterone treatment have opposing effects on migration inhibitor factor levels in ageing men', *Mediators of inflammation*: 1-7.

Glynn, Erin L, Christopher S Fry, Micah J Drummond, Hans C Dreyer, Shaheen Dhanani, Elena Volpi, and Blake B Rasmussen. 2010. 'Muscle protein breakdown has a minor role in the protein anabolic response to essential amino acid and carbohydrate intake following resistance exercise', *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 299: R533-R40.

Goldspink, David F. 2005. 'Ageing and activity: their effects on the functional reserve capacities of the heart and vascular smooth and skeletal muscles', *Ergonomics*, 48: 1334-51.

Goldspink, Geoffrey, and Stephen DR Harridge. 2004. 'Growth factors and muscle ageing', *Experimental gerontology*, 39: 1433-38.

Graff, Jeremy R, Bruce W Konicek, Ann M McNulty, Zejing Wang, Keith Houck, Sheryl Allen, Jonathan D Paul, Ahed Hbaiu, Robin G Goode, and George E Sandusky. 2000. 'Increased AKT activity contributes to prostate cancer progression by dramatically accelerating prostate tumor growth and diminishing p27Kip1 expression', *Journal of Biological Chemistry*, 275: 24500-05.

Gray, Anna, Henry A Feldman, John B Mckinlay, and Christopher Longcope. 1991. 'Age, disease, and changing sex hormone levels in middle-aged men: results of the Massachusetts Male Aging Study', *The Journal of Clinical Endocrinology & Metabolism*, 73: 1016-25.

Griggs, Robert C, William Kingston, Ralph F Jozefowicz, Barbara E Herr, Gilbert Forbes, and David Halliday. 1989. 'Effect of testosterone on muscle mass and muscle protein synthesis', *Journal of Applied Physiology*, 66: 498-503.

Groennebaek, Thomas, and Kristian Vissing. 2017. 'Impact of resistance training on skeletal muscle mitochondrial biogenesis, content, and function', *Frontiers in physiology*, 8: 1-7.

Grossmann, Mathis. 2014a. 'Testosterone and glucose metabolism in men: current concepts and controversies', *Journal of Endocrinology*, 220: R37-R55.

Grossmann, Mathis, Emily J Gianatti, and Jeffrey D Zajac. 2010. 'Testosterone and type 2 diabetes', *Current Opinion in Endocrinology, Diabetes and Obesity*, 17: 247-56.

Groth, Kristian A, Anne Skakkebæk, Christian Høst, Claus Højbjerg Gravholt, and Anders Bojesen. 2013. 'Klinefelter syndrome—a clinical update', *The Journal of Clinical Endocrinology & Metabolism*, 98: 20-30.

Gruenewald, David A, and Alvin M Matsumoto. 2003. 'Testosterone supplementation therapy for older men: potential benefits and risks', *Journal of the American Geriatrics Society*, 51: 101-15.

Guo, W., S. Wong, M. Li, W. Liang, M. Liesa, C. Serra, R. Jasuja, A. Bartke, J. L. Kirkland, O. Shirihai, and S. Bhasin. 2012. 'Testosterone plus low-intensity physical training in late life improves functional performance, skeletal muscle mitochondrial biogenesis, and mitochondrial quality control in male mice', *PloS one*, 7: 1-12.

Gupta, Vandana, Shalender Bhasin, Wen Guo, Rajan Singh, Rika Miki, Pratibha Chauhan, Karen Choong, Tamara Tchkonia, Nathan K Lebrasseur, and John N Flanagan. 2008. 'Effects of dihydrotestosterone on differentiation and proliferation of human mesenchymal stem cells and preadipocytes', *Molecular and cellular endocrinology*, 296: 32-40.

Guralnik, Jack M, Luigi Ferrucci, Eleanor M Simonsick, Marcel E Salive, and Robert B Wallace. 1995. 'Lower-extremity function in persons over the age of 70 years as a predictor of subsequent disability', *New England Journal of Medicine*, 332: 556-62.

Gwinn, Dana M, David B Shackelford, Daniel F Egan, Maria M Mihaylova, Annabelle Mery, Debbie S Vasquez, Benjamin E Turk, and Reuben J Shaw. 2008. 'AMPK phosphorylation of raptor mediates a metabolic checkpoint', *Molecular cell*, 30: 214-26.

Hackett, Geoffrey, Nigel Cole, Mithun Bhartia, David Kennedy, Jessie Raju, Peter Wilkinson, and BLAST Study Group. 2014. 'Testosterone

replacement therapy improves metabolic parameters in hypogonadal men with type 2 diabetes but not in men with coexisting depression: the BLAST study', *The journal of sexual medicine*, 11: 840-56.

Hackett, GI. 2016. 'Testosterone replacement therapy and mortality in older men', *Drug safety*, 39: 117-30.

Haffner, Steven M, Pauli Karhapää, Leena Mykkänen, and Markku Laakso. 1994. 'Insulin Resistance, Body Fat Distribution, and Sex Hormones in Men', *Diabetes*, 43: 212-19.

Hagenfeldt, Lars, and J Wahren. 1971. 'Human forearm muscle metabolism during exercise VI. Substrate utilization in prolonged fasting', *Scandinavian Journal of Clinical and Laboratory Investigation*, 27: 299-306.

Haidar, Ahmad, Aksam Yassin, Farid Saad, and Ridwan Shabsigh. 2007. 'Effects of androgen deprivation on glycaemic control and on cardiovascular biochemical risk factors in men with advanced prostate cancer with diabetes', *The Aging Male*, 10: 189-96.

Haizlip, KM, BC Harrison, and LA Leinwand. 2015. 'Sex-based differences in skeletal muscle kinetics and fiber-type composition', *Physiology*, 30: 30-39.

Hakkinen, K, and A Pakarinen. 1993. 'Acute hormonal responses to two different fatiguing heavy-resistance protocols in male athletes', *Journal of Applied Physiology*, 74: 882-87.

Häkkinen, K, and A Pakarinen. 1994. 'Serum hormones and strength development during strength training in middle-aged and elderly males and females', *Acta Physiologica Scandinavica*, 150: 211-19.

Häkkinen, Keijo, and Arto Pakarinen. 1993. 'Muscle strength and serum testosterone, cortisol and SHBG concentrations in middle-aged and elderly men and women', *Acta Physiologica Scandinavica*, 148: 199-207.

Hakkinen, Keijo, Arto Pakarinen, William J Kraemer, Robert U Newton, and Markku Alen. 2000. 'Basal concentrations and acute responses of serum hormones and strength development during heavy resistance training in middle-aged and elderly men and women', *Journals of Gerontology-Biological Sciences and Medical Sciences*, 55: 95-105.

Hall, Katharine E, Matthew W McDonald, Kenneth N Grisé, Oscar A Campos, Earl G Noble, and CW James Melling. 2013. 'The role of resistance and aerobic exercise training on insulin sensitivity measures in STZ-induced Type 1 diabetic rodents', *Metabolism*, 62: 1485-94.

Hameed, M, RW Orrell, M Cobbold, G Goldspink, and SDR Harridge. 2003. 'Expression of IGF-I splice variants in young and old human skeletal muscle after high resistance exercise', *The Journal of physiology*, 547: 247-54.

Hammes, Annette, Thomas K Andreassen, Robert Spoelgen, Jens Raila, Norbert Hubner, Herbert Schulz, Jochen Metzger, Florian J Schweigert, Peter B Luppa, and Anders Nykjaer. 2005. 'Role of endocytosis in cellular uptake of sex steroids', *Cell*, 122: 751-62.

Hansen, Elisabeth, Bodil J Landstad, Kjell T Gundersen, Peter A Torjesen, and Sven Svebak. 2012. 'Insulin sensitivity after maximal and endurance

resistance training', *The Journal of Strength & Conditioning Research*, 26: 327-34.

Harber, Matthew P, Justin D Crane, Jared M Dickinson, Bozena Jemiolo, Ulrika Raue, Todd A Trappe, and Scott W Trappe. 2009. 'Protein synthesis and the expression of growth-related genes are altered by running in human vastus lateralis and soleus muscles', *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 296: R708-R14.

Haren, MT, AM Siddiqui, HJ Armbrecht, RT Kevorkian, MJ Kim, MJ Haas, A Mazza, Vijaya B Kumar, M Green, and WA Banks. 2011. 'Testosterone modulates gene expression pathways regulating nutrient accumulation, glucose metabolism and protein turnover in mouse skeletal muscle', *International journal of andrology*, 34: 55-68.

Harman, S Mitchell, E Jeffrey Metter, Jordan D Tobin, Jay Pearson, and Marc R Blackman. 2001. 'Longitudinal effects of aging on serum total and free testosterone levels in healthy men', *The Journal of Clinical Endocrinology & Metabolism*, 86: 724-31.

Haun, Cody T, Christopher G Vann, Brandon M Roberts, Andrew D Vigotsky, Brad J Schoenfeld, and Michael D Roberts. 2019. 'A critical evaluation of the biological construct skeletal muscle hypertrophy: size matters but so does the measurement', *Frontiers in physiology*, 10: 247.

Hawkins, Vivian N, Karen Foster-Schubert, Jessica Chubak, Bess Sorensen, Cornelia M Ulrich, Frank Z Stancyzk, Stephen Plymate, Janet Stanford, Emily White, and John D Potter. 2008. 'Effect of exercise on serum sex hormones in men: a 12-month randomized clinical trial', *Medicine and science in sports and exercise*, 40: 223-33.

Hawley, John A, Louise M Burke, Stuart M Phillips, and Lawrence L Spriet. 2010. 'Nutritional modulation of training-induced skeletal muscle adaptations', *Journal of Applied Physiology*, 110: 834-45.

Hawley, John A, Kevin D Tipton, and Mindy L Millard-Stafford. 2006. 'Promoting training adaptations through nutritional interventions', *Journal of sports sciences*, 24: 709-21.

Hawley, Simon A, David A Pan, Kirsty J Mustard, Louise Ross, Jenny Bain, Arthur M Edelman, Bruno G Frenguelli, and D Grahame Hardie. 2005. 'Calmodulin-dependent protein kinase kinase-β is an alternative upstream kinase for AMP-activated protein kinase', *Cell metabolism*, 2: 9-19.

Hayes, Valerie Y, Randall J Urban, Jie Jiang, Taylor J Marcell, Kevin Helgeson, and Nelly Mauras. 2001. 'Recombinant human growth hormone and recombinant human insulin-like growth factor I diminish the catabolic effects of hypogonadism in man: metabolic and molecular effects', *The Journal of Clinical Endocrinology & Metabolism*, 86: 2211-19.

He, Zhiheng, Darren M Opland, Kerrie J Way, Kohjiro Ueki, Natalya Bodyak, Peter M Kang, Seigo Izumo, Rohit N Kulkarni, Bo Wang, and Ronglih Liao. 2006. 'Regulation of vascular endothelial growth factor expression and vascularization in the myocardium by insulin receptor and PI3K/Akt pathways in insulin resistance and ischemia', *Arteriosclerosis, thrombosis, and vascular biology*, 26: 787-93.

Henderson, Gregory C, Ketan Dhatariya, G Charles Ford, Katherine A Klaus, Rita Basu, Robert A Rizza, Michael D Jensen, Sundeep Khosla, Peter O'Brien, and K Sreekumaran Nair. 2009. 'Higher muscle protein synthesis in women than men across the lifespan, and failure of androgen administration to amend age-related decrements', *The FASEB Journal*, 23: 631-41.

Hirata, Yu, Kazuhiro Nomura, Yoko Senga, Yuko Okada, Kenta Kobayashi, Shiki Okamoto, Yasuhiko Minokoshi, Michihiro Imamura, Shin'ichi Takeda, and Tetsuya Hosooka. 2019. 'Hyperglycemia induces skeletal muscle atrophy via a WWP1/KLF15 axis', *JCI insight*, 4.

Holloszy, John O, and HT Narahara. 1967a. 'Enhanced permeability to sugar associated with muscle contraction: studies of the role of Ca++', *The Journal of general physiology*, 50: 551-62.

Holmäng, A, and P Björntorp. 1992. 'The effects of testosterone on insulin sensitivity in male rats', *Acta Physiologica Scandinavica*, 146: 505-10.

Hoppeler, Hans, and Martin Flück. 2002. 'Normal mammalian skeletal muscle and its phenotypic plasticity', *Journal of Experimental Biology*, 205: 2143-52.

Hornberger, Troy A, and Shu Chien. 2006. 'Mechanical stimuli and nutrients regulate rapamycin-sensitive signaling through distinct mechanisms in skeletal muscle', *Journal of cellular biochemistry*, 97: 1207-16.

Host, Helen H, Polly A Hansen, Lorraine A Nolte, May M Chen, and John O Holloszy. 1998. 'Rapid reversal of adaptive increases in muscle GLUT-4 and glucose transport capacity after training cessation', *Journal of Applied Physiology*, 84: 798-802.

Hotamisligil, Gökhan S. 2006. 'Inflammation and metabolic disorders', *Nature*, 444: 860.

Hou, WW, MA Tse, TH Lam, GM Leung, and CM Schooling. 2015. 'Adolescent testosterone, muscle mass and glucose metabolism: evidence from the 'Children of 1997'birth cohort in Hong Kong', *Diabetic Medicine*, 32: 505-12.

Hourde, C, C Jagerschmidt, P Clément-Lacroix, A Vignaud, Patrick Ammann, GS Butler-Browne, and A Ferry. 2009. 'Androgen replacement therapy improves function in male rat muscles independently of hypertrophy and activation of the Akt/mTOR pathway', *Acta Physiologica*, 195: 471-82.

Hughes, I. A. 1986. 'CHAPTER 6 - The Gonads.' in I. A. Hughes (ed.), *Handbook of Endocrine Investigations in Children* (Butterworth-Heinemann).

Hulmi, Juha J., Christopher M. Lockwood, and Jeffrey R. Stout. 2010. 'Effect of protein/essential amino acids and resistance training on skeletal muscle hypertrophy: A case for whey protein', *Nutrition & Metabolism*, 7: 51.

Ibañez, Javier, Mikel Izquierdo, Iñaki Argüelles, Luis Forga, José L Larrión, Marisol García-Unciti, Fernando Idoate, and Esteban M Gorostiaga. 2005. 'Twice-weekly progressive resistance training decreases abdominal fat and improves insulin sensitivity in older men with type 2 diabetes', *DIABETES CARE*, 28: 662-67.

Iglay, Heidi B, John P Thyfault, John W Apolzan, and Wayne W Campbell. 2007. 'Resistance training and dietary protein: effects on glucose

tolerance and contents of skeletal muscle insulin signaling proteins in older persons', *The American journal of clinical nutrition*, 85: 1005-13.

Inoki, Ken, Tianqing Zhu, and Kun-Liang Guan. 2003. 'TSC2 mediates cellular energy response to control cell growth and survival', *Cell*, 115: 577-90.

Isidori, Andrea M, Elisa Giannetta, Emanuela A Greco, Daniele Gianfrilli, Vincenzo Bonifacio, Aldo Isidori, Andrea Lenzi, and Andrea Fabbri. 2005. 'Effects of testosterone on body composition, bone metabolism and serum lipid profile in middle-aged men: A meta-analysis', *Clinical endocrinology*, 63: 280-93.

Jarow, Jonathan P, Haolin Chen, WILLIAM ROSNER, STEPHANIE TRENTACOSTE, and Barry R Zirkin. 2001. 'Assessment of the androgen environment within the human testis: minimally invasive method to obtain intratesticular fluid', *Journal of andrology*, 22: 640-45.

Jeon, Sang-Min. 2016. 'Regulation and function of AMPK in physiology and diseases', *Experimental & molecular medicine*, 48: e245.

Jiang, Mei, Yunsheng Ma, Congcong Chen, Xuping Fu, Shu Yang, Xia Li, Guohua Yu, Yumin Mao, Yi Xie, and Yao Li. 2009. 'Androgen-responsive gene database: integrated knowledge on androgen-responsive genes', *Molecular endocrinology*, 23: 1927-33.

Jones, AG, and AT Hattersley. 2013. 'The clinical utility of C-peptide measurement in the care of patients with diabetes', *Diabetic Medicine*, 30: 803-17.

Jones, T Hugh, Stefan Arver, Hermann M Behre, Jacques Buvat, Eric Meuleman, Ignacio Moncada, Antonio Martin Morales, Maurizio Volterrani, Ann Yellowlees, and Julian D Howell. 2011. 'Testosterone replacement in hypogonadal men with type 2 diabetes and/or metabolic syndrome (the TIMES2 study)', *DIABETES CARE*, 34: 828-37.

Joubert, Yolaine, and Christine Tobin. 1995. 'Testosterone treatment results in quiescent satellite cells being activated and recruited into cell cycle in rat levator ani muscle', *Developmental biology*, 169: 286-94.

Kadi, F. 2008. 'Cellular and molecular mechanisms responsible for the action of testosterone on human skeletal muscle. A basis for illegal performance enhancement', *Br J Pharmacol*, 154: 522-8.

Kadi, F., A. Eriksson, S. Holmner, and L. E. Thornell. 1999. 'Effects of anabolic steroids on the muscle cells of strength-trained athletes', *Med Sci Sports Exerc*, 31: 1528-34.

Kadi, F., G. Hägg, R. Håkansson, S. Holmner, G. S. Butler-Browne, and L.-E. Thornell. 1998. 'Structural changes in male trapezius muscle with work-related myalgia', *Acta Neuropathologica*, 95: 352-60.

Kalantaridou, Sophia N, and Karim A Calis. 2006. "Testosterone therapy in premenopausal women." In *Seminars in reproductive medicine*, 106-14. Copyright© 2006 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New ….

Kalinchenko, Svetlana Y, Yuliya A Tishova, George J Mskhalaya, Louis JG Gooren, Erik J Giltay, and Farid Saad. 2010. 'Effects of testosterone supplementation on markers of the metabolic syndrome and inflammation in hypogonadal men with the metabolic syndrome: the double-blinded placebo-controlled Moscow study', *Clinical endocrinology*, 73: 602-12.

Kalyani, Rita Rastogi, Mark Corriere, and Luigi Ferrucci. 2014. 'Age-related and disease-related muscle loss: the effect of diabetes, obesity, and other diseases', *The lancet Diabetes & endocrinology*, 2: 819-29.

Kapoor, D., E. Goodwin, K. S. Channer, and T. H. Jones. 2006. 'Testosterone replacement therapy improves insulin resistance, glycaemic control, visceral adiposity and hypercholesterolaemia in hypogonadal men with type 2 diabetes', *Eur J Endocrinol*, 154: 899-906.

Karamizadeh, Zohreh, Mahmoodreza Tabebordbar, Forough Saki, Hamdollah Karamifar, and Gholamhossein Amirhakimi. 2013. 'The side effects of gonadotropin releasing hormone analog (diphereline) in treatment of idiopathic central precocious puberty', *Acta Medica Iranica*: 41-46.

Katznelson, L, DI Rosenthal, MS Rosol, EJ Anderson, DL Hayden, DA Schoenfeld, and A Klibanski. 1998. 'Using quantitative CT to assess adipose distribution in adult men with acquired hypogonadism', *AJR. American journal of roentgenology*, 170: 423-27.

Katznelson, LAURENCE, JOEL S Finkelstein, DAVID A Schoenfeld, DANIEL I Rosenthal, ELLEN J Anderson, and ANNE Klibanski. 1996. 'Increase in bone density and lean body mass during testosterone administration in men with acquired hypogonadism', *The Journal of Clinical Endocrinology & Metabolism*, 81: 4358-65.

Keating, Nancy L., A. James O'Malley, and Matthew R. Smith. 2006. 'Diabetes and Cardiovascular Disease During Androgen Deprivation Therapy for Prostate Cancer', *Journal of Clinical Oncology*, 24: 4448-56.

Kelly, Daniel M, and T Hugh Jones. 2013. 'Testosterone: a metabolic hormone in health and disease', 217: R25.

Kemp, CM, WT Oliver, TL Wheeler, AH Chishti, and Mohammad Koohmaraie. 2013. 'The effects of Capn1 gene inactivation on skeletal muscle growth, development, and atrophy, and the compensatory role of other proteolytic systems', *Journal of Animal Science*, 91: 3155-67.

Kenny, Anne M, Alison Kleppinger, Kristen Annis, Margaret Rathier, Bruce Browner, James O Judge, and Daniel McGee. 2010. 'Effects of transdermal testosterone on bone and muscle in older men with low bioavailable testosterone levels, low bone mass, and physical frailty', *Journal of the American Geriatrics Society*, 58: 1134-43.

Khaw, Kay-Tee, and Elizabeth Barrett-Connor. 1992. 'Lower endogenous androgens predict central adiposity in men', *Ann Epidemiol*, 2: 675-82.

Kim, Hee-jae, Byunghun So, Mijung Choi, Dongheon Kang, and Wook Song. 2015. 'Resistance exercise training increases the expression of irisin concomitant with improvement of muscle function in aging mice and humans', *Experimental gerontology*, 70: 11-17.

Kim, Hyo-Gun, Bin Guo, and Gustavo A Nader. 2019. 'Regulation of Ribosome Biogenesis During Skeletal Muscle Hypertrophy', *Exercise and sport sciences reviews*, 47: 91-97.

Kim, Paul L, Robert S Staron, and Stuart M Phillips. 2005. 'Fasted-state skeletal muscle protein synthesis after resistance exercise is altered with training', *The Journal of physiology*, 568: 283-90.

Kimball, Scot R, Rick L Horetsky, and Leonard S Jefferson. 1998. 'Implication of eIF2B rather than eIF4E in the regulation of global protein synthesis

by amino acids in L6 myoblasts', *Journal of Biological Chemistry*, 273: 30945-53.

Kimball, Scot R, and Leonard S Jefferson. 2010. 'Control of translation initiation through integration of signals generated by hormones, nutrients, and exercise', *Journal of Biological Chemistry*, 285: 29027-32.

Kintzel, Polly E, Sandra L Chase, Lisa M Schultz, and Timothy J O'Rourke. 2008. 'Increased risk of metabolic syndrome, diabetes mellitus, and cardiovascular disease in men receiving androgen deprivation therapy for prostate cancer', *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, 28: 1511-22.

Koenig, Harold, Alfred Goldstone, and Cy Lu. 1980. 'Androgens regulate mitochondrial cytochrome c oxidase and lysosomal hydrolases in mouse skeletal muscle', *Biochemical Journal*, 192: 349-53.

Kon, Michihiro, Nao Ohiwa, Akiko Honda, Takeo Matsubayashi, Tatsuaki Ikeda, Takayuki Akimoto, Yasuhiro Suzuki, Yuichi Hirano, and Aaron P Russell. 2014. 'Effects of systemic hypoxia on human muscular adaptations to resistance exercise training', *Physiological Reports*, 2.

Koopman, René, Bart Pennings, Antoine HG Zorenc, and Luc JC Van Loon. 2007. 'Protein ingestion further augments S6K1 phosphorylation in skeletal muscle following resistance type exercise in males', *The Journal of Nutrition*, 137: 1880-86.

Kostek, Matthew C, Yi-Wen Chen, Daniel J Cuthbertson, Rongye Shi, Mark J Fedele, Karyn A Esser, and Michael J Rennie. 2007. 'Gene expression responses over 24 h to lengthening and shortening contractions in human muscle: major changes in CSRP3, MUSTN1, SIX1, and FBXO32', *Physiological genomics*, 31: 42-52.

Kovacheva, E. L., A. P. Hikim, R. Shen, I. Sinha, and I. Sinha-Hikim. 2010. 'Testosterone supplementation reverses sarcopenia in aging through regulation of myostatin, c-Jun NH2-terminal kinase, Notch, and Akt signaling pathways', *Endocrinology*, 151: 628-38.

Koziris, L Perry, William J Kraemer, Scott E Gordon, Thomas Incledon, and Howard G Knuttgen. 2000. 'Effect of acute postexercise ethanol intoxication on the neuroendocrine response to resistance exercise', *Journal of Applied Physiology*, 88: 165-72.

Kraemer, William J, Keijo Häkkinen, Robert U Newton, Matthew McCormick, Bradley C Nindl, Jeff S Volek, Lincoln A Gotshalk, Steven J Fleck, Wayne W Campbell, and Scott E Gordon. 1998. 'Acute hormonal responses to heavy resistance exercise in younger and older men', *European journal of applied physiology and occupational physiology*, 77: 206-11.

Kraemer, William J, Keijo Häkkinen, Robert U Newton, Bradley C Nindl, Jeff S Volek, Matthew McCormick, Lincoln A Gotshalk, Scott E Gordon, Steven J Fleck, and Wayne W Campbell. 1999. 'Effects of heavy-resistance training on hormonal response patterns in younger vs. older men', *Journal of Applied Physiology*, 87: 982-92.

Kraemer, William J., Louis Marchitelli, Scott E. Gordon, Everett Harman, Joseph E. Dziados, Robert Mello, Peter Frykman, Dini McCurry, and Steven J. Fleck. 1990. 'Hormonal and growth factor responses to heavy

resistance exercise protocols', *Journal of Applied Physiology*, 69: 1442-50.

Krakstad, Camilla, and Martha Chekenya. 2010. 'Survival signalling and apoptosis resistance in glioblastomas: opportunities for targeted therapeutics', *Molecular cancer*, 9: 135.

Kumar, Vinod, Philip J Atherton, Anna Selby, Debbie Rankin, John Williams, Kenneth Smith, Natalie Hiscock, and Michael J Rennie. 2012. 'Muscle protein synthetic responses to exercise: effects of age, volume, and intensity', *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences*, 67: 1170-77.

Kumar, Vinod, Anna Selby, Debbie Rankin, Rekha Patel, Philip Atherton, Wulf Hildebrandt, John Williams, Kenneth Smith, Olivier Seynnes, and Natalie Hiscock. 2009. 'Age-related differences in the dose–response relationship of muscle protein synthesis to resistance exercise in young and old men', *The Journal of physiology*, 587: 211-17.

Kvorning, Thue, Marianne Andersen, Kim Brixen, and Klavs Madsen. 2006a. 'Suppression of endogenous testosterone production attenuates the response to strength training: a randomized, placebo-controlled, and blinded intervention study', *American Journal of Physiology-Endocrinology And Metabolism*, 291: E1325-E32.

Kvorning, Thue, Marianne Andersen, Kim Brixen, Peter Schjerling, Charlotte Suetta, and Klavs Madsen. 2007a. 'Suppression of testosterone does not blunt mRNA expression of myoD, myogenin, IGF, myostatin or androgen receptor post strength training in humans', *The Journal of physiology*, 578: 579-93.

Laaksonen, David E., Leo Niskanen, Kari Punnonen, Kristiina Nyyssönen, Tomi-Pekka Tuomainen, Veli-Pekka Valkonen, Riitta Salonen, and Jukka T. Salonen. 2004. 'Testosterone and Sex Hormone–Binding Globulin Predict the Metabolic Syndrome and Diabetes in Middle-Aged Men', *DIABETES CARE*, 27: 1036-41.

Lakens, Daniël. 2013. 'Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs', *Frontiers in psychology*, 4: 1-12.

Lassar, Andrew, and Andrea Münsterberg. 1994. 'Wiring diagrams: regulatory circuits and the control of skeletal myogenesis', *Current opinion in cell biology*, 6: 432-42.

Lawrence, RD. 1926. 'The effect of exercise on insulin action in diabetes', *British medical journal*, 1: 648.

Lee, JungHoon, DoHoun Kim, and ChangKeun Kim. 2017. 'Resistance Training for Glycemic Control, Muscular Strength, and Lean Body Mass in Old Type 2 Diabetic Patients: A Meta-Analysis', *Diabetes therapy : research, treatment and education of diabetes and related disorders*, 8: 459-73.

Lee, R. C., Z. Wang, M. Heo, R. Ross, I. Janssen, and S. B. Heymsfield. 2000. 'Total-body skeletal muscle mass: development and cross-validation of anthropometric prediction models', *Am J Clin Nutr*, 72: 796-803.

Lei, ZM, S Mishra, W Zou, B Xu, M Foltz, X Li, and Ch V Rao. 2001. 'Targeted disruption of luteinizing hormone/human chorionic gonadotropin receptor gene', *Molecular endocrinology*, 15: 184-200.

Leiter, Jeff RS, Jason Peeler, and Judy E Anderson. 2011. 'Exercise-induced muscle growth is muscle-specific and age-dependent', *Muscle & nerve*, 43: 828-38.

Lemmer, Jeff T, DIANE E HURLBUT, GREG F MARTEL, BRIAN L TRACY, FRED M EY IV, E Jeffrey Metter, JAMES L FOZARD, JEROME L FLEG, and BEN F HURLEY. 2000. 'Age and gender responses to strength training and detraining', *Medicine & Science in Sports & Exercise*, 32: 1505-12.

Leney, Sophie E, and Jeremy M Tavare. 2009. 'The molecular basis of insulin-stimulated glucose uptake: signalling, trafficking and potential drug targets', *The Journal of endocrinology*, 203: 1-18.

Lewis, Michael I, Gail D Horvitz, David R Clemmons, and Mario Fournier. 2002. 'Role of IGF-1 and IGF binding proteins within diaphragm muscle in modulating the effects of nandrolone', *American Journal of Physiology-Endocrinology And Metabolism*.

Liu, Peter Y, Ronald S Swerdloff, and Johannes D Veldhuis. 2004. 'The rationale, efficacy and safety of androgen therapy in older men: future research and current practice recommendations', *The Journal of Clinical Endocrinology & Metabolism*, 89: 4789-96.

Lobley, GE, Alexmary Connell, Eric Milne, Vivien Buchan, Alexander Graham Calder, Susan E Anderson, and Hazel Vint. 1990. 'Muscle protein synthesis in response to testosterone administration in wether lambs', *British journal of Nutrition*, 64: 691-704.

Loo, Simone Y, Laurent Azoulay, Rui Nie, Sophie Dell'Aniello, Oriana Hoi Yun Yu, and Christel Renoux. 2019. 'Cardiovascular and cerebrovascular safety of testosterone replacement therapy among aging men with low testosterone levels: a cohort study', *The American Journal of Medicine*.

Losel, Ralf M, Elisabeth Falkenstein, Martin Feuring, Armin Schultz, Hanns-Christian Tillmann, Karin Rossol-Haseroth, and Martin Wehling. 2003. 'Nongenomic steroid action: controversies, questions, and answers', *Physiological reviews*, 83: 965-1016.

Luetjens, C Marc, and Gerhard F Weinbauer. 2012. 'Testosterone: biosynthesis, transport, metabolism and (non genomic) actions', *Testosterone: action, deficiency, substitution. Nieschlag E, Behre HM (eds.). Cambridge University Press, Cambridge*, 2: 15-33.

MacDonald, AJ, AC Small, CA Greig, H Husi, JA Ross, NA Stephens, KCH Fearon, and T Preston. 2013. 'A novel oral tracer procedure for measurement of habitual myofibrillar protein synthesis', *Rapid Communications in Mass Spectrometry*, 27: 1769-77.

Manfredi, Thomas G, Michael Monteiro, Linda S Lamont, Maria Fiatarone Singh, Mona Foldvari, Sebrina White, Arthur Cosmas, and Maria L Urso. 2013. 'Post-Menopausal Effects of Resistance Training on Muscle Damage and Mitochondria', *Journal of strength and conditioning research/National Strength & Conditioning Association*, 27: 1-13.

Martel, Gregory F, Stephen M Roth, Frederick M Ivey, Jeffrey T Lemmer, Brian L Tracy, Diane E Hurlbut, E Jeffrey Metter, Ben F Hurley, and Marc A Rogers. 2006. 'Age and sex affect human muscle fibre

adaptations to heavy-resistance strength training', *Experimental physiology*, 91: 457-64.
Martino, Fabiana, Ana R Perestrelo, Vladimír Vinarský, Stefania Pagliari, and Giancarlo Forte. 2018. 'Cellular mechanotransduction: from tension to function', *Frontiers in physiology*, 9.
Mateescu, RG, and ML Thonney. 2005. 'Effect of testosterone on insulin-like growth factor-I, androgen receptor, and myostatin gene expression in splenius and semitendinosus muscles in sheep', *Journal of Animal Science*, 83: 803-09.
Matsuda, M., and R. A. DeFronzo. 1999. 'Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp', *DIABETES CARE*, 22: 1462-70.
Matthews, D. R., J. P. Hosker, A. S. Rudenski, B. A. Naylor, D. F. Treacher, and R. C. Turner. 1985. 'Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man', *Diabetologia*, 28: 412-9.
Mauras, Nelly, Valerie Hayes, Susan Welch, Annie Rini, Kevin Helgeson, Maryanne Dokler, Johannes D Veldhuis, and Randall J Urban. 1998. 'Testosterone deficiency in young men: marked alterations in whole body protein kinetics, strength, and adiposity', *The Journal of Clinical Endocrinology & Metabolism*, 83: 1886-92.
Mayhew, David L, Jeong-su Kim, James M Cross, Arny A Ferrando, and Marcas M Bamman. 2009. 'Translational signaling responses preceding resistance training-mediated myofiber hypertrophy in young and old humans', *Journal of Applied Physiology*, 107: 1655-62.
McCarthy, John J, Jyothi Mula, Mitsunori Miyazaki, Rod Erfani, Kelcye Garrison, Amreen B Farooqui, Ratchakrit Srikuea, Benjamin A Lawson, Barry Grimes, and Charles Keller. 2011. 'Effective fiber hypertrophy in satellite cell-depleted skeletal muscle', *Development*, 138: 3657-66.
McGee, Sean L, David Sparling, Ann-Louise Olson, and Mark Hargreaves. 2006. 'Exercise increases MEF2-and GEF DNA-binding activity in human skeletal muscle', *The FASEB Journal*, 20: 348-49.
Melov, Simon, Mark A Tarnopolsky, Kenneth Beckman, Krysta Felkey, and Alan Hubbard. 2007a. 'Resistance exercise reverses aging in human skeletal muscle', *PloS one*, 2: 1-9.
Mendler, Luca, Zsuzsanna Baka, Andrea Kovács-Simon, and László Dux. 2007. 'Androgens negatively regulate myostatin expression in an androgen-dependent skeletal muscle', *Biochemical and biophysical research communications*, 361: 237-42.
Messi, María Laura, and Osvaldo Delbono. 2003. 'Target-derived trophic effect on skeletal muscle innervation in senescent mice', *Journal of Neuroscience*, 23: 1351-59.
Meyuhas, Oded. 2000. 'Synthesis of the translational apparatus is regulated at the translational level', *European Journal of Biochemistry*, 267: 6321-30.
Miller, Karen, Colleen Corcoran, Catharina Armstrong, Kim Caramelli, Ellen Anderson, Deborah Cotton, Nesli Basgoz, Lisa Hirschhorn, Ruth Tuomala, and David Schoenfeld. 1998. 'Transdermal testosterone administration in women with acquired immunodeficiency syndrome

wasting: a pilot study', *The Journal of Clinical Endocrinology & Metabolism*, 83: 2717-25.
Miller, W. J., W. M. Sherman, and J. L. Ivy. 1984. 'Effect of strength training on glucose tolerance and post-glucose insulin response', *Med Sci Sports Exerc*, 16: 539-43.
Mitch, W. E., and A. L. Goldberg. 1996a. 'Mechanisms of muscle wasting. The role of the ubiquitin-proteasome pathway', *N Engl J Med*, 335: 1897-905.
Mitch, William E, and Alfred L Goldberg. 1996b. 'Mechanisms of muscle wasting—the role of the ubiquitin–proteasome pathway', *New England Journal of Medicine*, 335: 1897-905.
Mitchell, W Kyle, John Williams, Philip Atherton, Mike Larvin, John Lund, and Marco Narici. 2012. 'Sarcopenia, dynapenia, and the impact of advancing age on human skeletal muscle size and strength; a quantitative review', *Frontiers in physiology*, 3: 1-18.
Mitchell, William Kyle, Philip James Atherton, John Williams, Michael Larvin, Jonathan Norman Lund, and Marco Narici. 2012. 'Sarcopenia, dynapenia, and the impact of advancing age on human skeletal muscle size and strength; a quantitative review', *Frontiers in physiology*, 3: 260.
Molina, Patricia E, and McGraw-Hill Education. 2006. *Endocrine physiology* (Lange Medical Books/McGraw-Hill).
Moore, Daniel R, Tyler A Churchward-Venne, Oliver Witard, Leigh Breen, Nicholas A Burd, Kevin D Tipton, and Stuart M Phillips. 2014. 'Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men', *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences*, 70: 57-62.
Moore, Daniel R, Stuart M Phillips, John A Babraj, Kenneth Smith, and Michael J Rennie. 2005. 'Myofibrillar and collagen protein synthesis in human skeletal muscle in young men after maximal shortening and lengthening contractions', *American Journal of Physiology-Endocrinology And Metabolism*, 288: E1153-E59.
Moore, Daniel R, Meghann J Robinson, Jessica L Fry, Jason E Tang, Elisa I Glover, Sarah B Wilkinson, Todd Prior, Mark A Tarnopolsky, and Stuart M Phillips. 2008. 'Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men', *The American journal of clinical nutrition*, 89: 161-68.
Morgan, JB, TL Wheeler, M Koohmaraie, JD Crouse, and JW Savell. 1993. 'Effect of castration on myofibrillar protein turnover, endogenous proteinase activities, and muscle growth in bovine skeletal muscle', *Journal of Animal Science*, 71: 408-14.
Morley, John E, Fran E Kaiser, Horace M Perry III, Ping Patrick, Patricia MK Morley, Patricia M Stauber, Bruno Vellas, Richard N Baumgartner, and Phillip J Garry. 1997. 'Longitudinal changes in testosterone, luteinizing hormone, and follicle-stimulating hormone in healthy older men', *Metabolism*, 46: 410-13.
Mosaferi Ziaaldini, Mohammad, Seyyed Reza Attarzadeh Hosseini, and Mehrdad Fathei. 2017. 'Mitochondrial adaptations in aged skeletal muscle: effect of exercise training', *Physiological Research*, 66: 1-14.

Moss, FP, and CP Leblond. 1971. 'Satellite cells as the source of nuclei in muscles of growing rats', *The Anatomical Record*, 170: 421-35.
Mounier, Rémi, Louise Lantier, Jocelyne Leclerc, Athanassia Sotiropoulos, Marc Foretz, and Benoit Viollet. 2011. 'Antagonistic control of muscle cell size by AMPK and mTORC1', *Cell Cycle*, 10: 2640-46.
Mul, Joram D, Kristin I Stanford, Michael F Hirshman, and Laurie J Goodyear. 2015. 'Exercise and regulation of carbohydrate metabolism.' in, *Progress in molecular biology and translational science* (Elsevier).
Murphy, Robyn M. 2010. 'Calpains, skeletal muscle function and exercise', *Clinical and Experimental Pharmacology and Physiology*, 37: 385-91.
Murton, AJ, D Constantin, and PL Greenhaff. 2008. 'The involvement of the ubiquitin proteasome system in human skeletal muscle remodelling and atrophy', *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, 1782: 730-43.
Nader, Gustavo A, Ferdinand von Walden, Chang Liu, Jessica Lindvall, Laurie Gutmann, Emidio Edward Pistilli, and Paul M Gordon. 2014. 'Resistance exercise training modulates acute gene expression during human skeletal muscle hypertrophy', *Journal of Applied Physiology*, 116: 693-702.
Narici, Marco V, and Nicola Maffulli. 2010. 'Sarcopenia: characteristics, mechanisms and functional significance', *British Medical Bulletin*, 95: 139-59.
Narici, MV, and MD De Boer. 2011. 'Disuse of the musculo-skeletal system in space and on earth', *European journal of applied physiology*, 111: 403-20.
Naya, Francisco J, and Eric Olson. 1999. 'MEF2: a transcriptional target for signaling pathways controlling skeletal muscle growth and differentiation', *Current opinion in cell biology*, 11: 683-88.
Nieschlag, Eberhard, and Hermann M Behre. 2004. 'Clinical uses of testosterone in hypogonadism and other conditions', *Testosterone: action, deficiency, substitution*, 14: 292-308.
Noorkoiv, M., K. Nosaka, and A. J. Blazevich. 2010. 'Assessment of quadriceps muscle cross-sectional area by ultrasound extended-field-of-view imaging', *Eur J Appl Physiol*, 109: 631-9.
Notelovitz, Morris. 2002. 'Androgen effects on bone and muscle', *Fertility and sterility*, 77: 34-41.
Nygaard, E. 1981. 'Skeletal muscle fibre characteristics in young women', *Acta Physiologica Scandinavica*, 112: 299-304.
O'Neil, Dominic, Heike Glowatz, and Martin Schlumpberger. 2013. 'Ribosomal RNA depletion for efficient use of RNA-seq capacity', *Current protocols in molecular biology*, 103: 4.19. 1-4.19. 8.
O'Neill, Brian T, Kevin Y Lee, Katherine Klaus, Samir Softic, Megan T Krumpoch, Joachim Fentz, Kristin I Stanford, Matthew M Robinson, Weikang Cai, and Andre Kleinridders. 2016. 'Insulin and IGF-1 receptors regulate FoxO-mediated signaling in muscle proteostasis', *The Journal of clinical investigation*, 126: 3433-46.
Ohkura, Tsuyoshi, Hideki Shiochi, Youhei Fujioka, Keisuke Sumi, Naoya Yamamoto, Kazuhiko Matsuzawa, Shoichiro Izawa, Hiroshi Kinoshita, Hiroko Ohkura, and Masahiko Kato. 2013. '20/(fasting C-peptide×

fasting plasma glucose) is a simple and effective index of insulin resistance in patients with type 2 diabetes mellitus: a preliminary report', *Cardiovasc Diabetol*, 12: 21.

Öner, Jale, Hakan Öner, Zeliha Sahin, Ramazan Demir, and İsmail Üstünel. 2008. 'Melatonin is as effective as testosterone in the prevention of soleus muscle atrophy induced by castration in rats', *The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology: Advances in Integrative Anatomy and Evolutionary Biology*, 291: 448-55.

Orwoll, Eric, Lori C Lambert, Lynn M Marshall, Janet Blank, Elizabeth Barrett-Connor, Jane Cauley, Kris Ensrud, and Steven R Cummings. 2006. 'Endogenous testosterone levels, physical performance, and fall risk in older men', *Archives of Internal Medicine*, 166: 2124-31.

Osterberg, E. Charles, Aaron M. Bernie, and Ranjith Ramasamy. 2014. 'Risks of testosterone replacement therapy in men', *Indian journal of urology : IJU : journal of the Urological Society of India*, 30: 2-7.

Ottenbacher, Kenneth J, Margaret E Ottenbacher, Allison J Ottenbacher, Ana Alfaro Acha, and Glenn V Ostir. 2006. 'Androgen treatment and muscle strength in elderly men: a meta-analysis', *Journal of the American Geriatrics Society*, 54: 1666-73.

Palazzolo, Isabella, Alessandra Gliozzi, Paola Rusmini, Daniela Sau, Valeria Crippa, Francesca Simonini, Elisa Onesto, Elena Bolzoni, and Angelo Poletti. 2008. 'The role of the polyglutamine tract in androgen receptor', *The Journal of steroid biochemistry and molecular biology*, 108: 245-53.

Pallafacchina, G, B Blaauw, and S Schiaffino. 2013. 'Role of satellite cells in muscle growth and maintenance of muscle mass', *Nutrition, Metabolism and Cardiovascular Diseases*, 23: S12-S18.

Park, Seok Won, Bret H Goodpaster, Elsa S Strotmeyer, Lewis H Kuller, Robert Broudeau, Candace Kammerer, Nathalie De Rekeneire, Tamara B Harris, Ann V Schwartz, and Frances A Tylavsky. 2007. 'Accelerated loss of skeletal muscle strength in older adults with type 2 diabetes: the health, aging, and body composition study', *DIABETES CARE*, 30: 1507-12.

Parkington, Jascha D, Nathan K LeBrasseur, Adam P Siebert, and Roger A Fielding. 2004. 'Contraction-mediated mTOR, p70S6k, and ERK1/2 phosphorylation in aged skeletal muscle', *Journal of Applied Physiology*, 97: 243-48.

Pasiakos, Stefan M, Lisa M Vislocky, John W Carbone, Nicholas Altieri, Karen Konopelski, Hedley C Freake, Jeffrey M Anderson, Arny A Ferrando, Robert R Wolfe, and Nancy R Rodriguez. 2010. 'Acute energy deprivation affects skeletal muscle protein synthesis and associated intracellular signaling proteins in physically active adults', *The Journal of Nutrition*, 140: 745-51.

Perry, Christopher GR, James Lally, Graham P Holloway, George JF Heigenhauser, Arend Bonen, and Lawrence L Spriet. 2010. 'Repeated transient mRNA bursts precede increases in transcriptional and mitochondrial proteins during training in human skeletal muscle', *The Journal of physiology*, 588: 4795-810.

Peterson, Mark D, Ananda Sen, and Paul M Gordon. 2011. 'Influence of resistance exercise on lean body mass in aging adults: a meta-analysis', *Medicine and science in sports and exercise*, 43: 249.

Petersson, Stine Juhl, Louise Lehmann Christensen, Jonas Møller Kristensen, Rikke Kruse, Marianne Andersen, and Kurt Højlund. 2014. 'Effect of testosterone on markers of mitochondrial oxidative phosphorylation and lipid metabolism in muscle of aging men with subnormal bioavailable testosterone', *European journal of endocrinology*: 77-88.

Petrella, John K, Jeong-su Kim, James M Cross, David J Kosek, and Marcas M Bamman. 2006. 'Efficacy of myonuclear addition may explain differential myofiber growth among resistance-trained young and older men and women', *American Journal of Physiology-Endocrinology And Metabolism*, 291: E937-E46.

Philippou, A, E Papageorgiou, G Bogdanis, A Halapas, A Sourla, M Maridaki, N Pissimissis, and M Koutsilieris. 2009. 'Expression of IGF-1 isoforms after exercise-induced muscle damage in humans: characterization of the MGF E peptide actions in vitro', *In vivo*, 23: 567-75.

Phillips, Bethan E., John P. Williams, Paul L. Greenhaff, Kenneth Smith, and Philip J. Atherton. 2017. 'Physiological adaptations to resistance exercise as a function of age', *JCI insight*, 2: 1-16.

Phillips, Bethan, John Williams, Philip Atherton, Kenneth Smith, Wulf Hildebrandt, Debbie Rankin, Paul Greenhaff, Ian Macdonald, and Michael J Rennie. 2011. 'Resistance exercise training improves age-related declines in leg vascular conductance and rejuvenates acute leg blood flow responses to feeding and exercise', *Journal of Applied Physiology*, 112: 347-53.

Phillips, Gerald B. 1977. 'Relationship between serum sex hormones and glucose, insulin and lipid abnormalities in men with myocardial infarction', *Proceedings of the National Academy of Sciences*, 74: 1729-33.

Phillips, SM, G Parise, BD Roy, KD Tipton, RR Wolfe, and MA Tarnopolsky. 2002. 'Resistance-training-induced adaptations in skeletal muscle protein turnover in the fed state', *Canadian journal of physiology and pharmacology*, 80: 1045-53.

Phillips, Stuart M. 2007. 'Resistance exercise: good for more than just Grandma and Grandpa's muscles', *Applied Physiology, Nutrition, and Metabolism*, 32: 1198-205.

Phillips, Stuart M, Joseph W Hartman, and Sarah B Wilkinson. 2005. 'Dietary protein to support anabolism with resistance exercise in young men', *Journal of the American College of Nutrition*, 24: 134S-39S.

Phillips, Stuart M, KD Tipton, Arny A Ferrando, and Robert R Wolfe. 1999. 'Resistance training reduces the acute exercise-induced increase in muscle protein turnover', *American Journal of Physiology-Endocrinology And Metabolism*, 276: E118-E24.

Phillips, Stuart M, Kevin D Tipton, ASLE Aarsland, Steven E Wolf, and Robert R Wolfe. 1997. 'Mixed muscle protein synthesis and breakdown after resistance exercise in humans', *American Journal of Physiology-Endocrinology And Metabolism*, 273: E99-E107.

Pigott, Craig R, Halina Mikolajek, Claire E Moore, Stephen J Finn, Curtis W Phippen, Jörn M Werner, and Christopher G Proud. 2012. 'Insights into the regulation of eukaryotic elongation factor 2 kinase and the interplay between its domains', *Biochemical Journal*, 442: 105-18.

Pitteloud, N., V. K. Mootha, A. A. Dwyer, M. Hardin, H. Lee, K. F. Eriksson, D. Tripathy, M. Yialamas, L. Groop, D. Elahi, and F. J. Hayes. 2005. 'Relationship between testosterone levels, insulin sensitivity, and mitochondrial function in men', *DIABETES CARE*, 28: 1636-42.

Pitteloud, Nelly, Vamsi K Mootha, Andrew A Dwyer, Megan Hardin, Hang Lee, Karl-Fredrik Eriksson, Devjit Tripathy, Maria Yialamas, Leif Groop, and Dariush Elahi. 2005a. 'Relationship between testosterone levels, insulin sensitivity, and mitochondrial function in men', *DIABETES CARE*, 28: 1636-42.

Pivarnik, J. M., J. F. Hickson, Jr., and I. Wolinsky. 1989. 'Urinary 3-methylhistidine excretion increases with repeated weight training exercise', *Med Sci Sports Exerc*, 21: 283-7.

Poehlman, E. T., R. V. Dvorak, W. F. DeNino, M. Brochu, and P. A. Ades. 2000a. 'Effects of resistance training and endurance training on insulin sensitivity in nonobese, young women: a controlled randomized trial', *J Clin Endocrinol Metab*, 85: 2463-8.

Poehlman, Eric T, Roman V Dvorak, Walter F DeNino, Martin Brochu, and Philip A Ades. 2000b. 'Effects of resistance training and endurance training on insulin sensitivity in nonobese, young women: a controlled randomized trial', *The Journal of Clinical Endocrinology & Metabolism*, 85: 2463-68.

Porter, C., P. T. Reidy, N. Bhattarai, L. S. Sidossis, and B. B. Rasmussen. 2015a. 'Resistance Exercise Training Alters Mitochondrial Function in Human Skeletal Muscle', *Med Sci Sports Exerc*, 47: 1922-31.

Porter, Craig, Paul T Reidy, Nisha Bhattarai, Labros S Sidossis, and Blake B Rasmussen. 2015b. 'Resistance exercise training alters mitochondrial function in human skeletal muscle', *Medicine and science in sports and exercise*, 47: 1922–31.

Pownall, Mary Elizabeth, Marcus K Gustafsson, and Charles P Emerson Jr. 2002. 'Myogenic regulatory factors and the specification of muscle progenitors in vertebrate embryos', *Annual review of cell and developmental biology*, 18: 747-83.

Quinlan, Jonathan I, Constantinos N Maganaris, Martino V Franchi, Kenneth Smith, Philip J Atherton, Nathaniel J Szewczyk, Paul L Greenhaff, Bethan E Phillips, James I Blackwell, and Catherine Boereboom. 2017. 'Muscle and Tendon Contributions to Reduced Rate of Torque Development in Healthy Older Males', *The Journals of Gerontology: Series A*: 539-45.

Rabiee, A., A. A. Dwyer, L. M. Caronia, F. J. Hayes, M. A. Yialamas, D. K. Andersen, B. Thomas, M. Torriani, and D. Elahi. 2010. 'Impact of acute biochemical castration on insulin sensitivity in healthy adult men', *Endocr Res*, 35: 71-84.

Ragnarsson, Oskar, Morton G Burt, Ken KY Ho, and Gudmundur Johannsson. 2013. 'Effect of short-term GH and testosterone administration on body composition and glucose homoeostasis in men receiving chronic

glucocorticoid therapy', *European journal of endocrinology*, 168: 243-51.

Rahman, Faisal, and Helen C Christian. 2007. 'Non-classical actions of testosterone: an update', *Trends in Endocrinology & Metabolism*, 18: 371-78.

Rasmussen, Blake B, and Stuart M Phillips. 2003. 'Contractile and nutritional regulation of human muscle growth', *Exercise and sport sciences reviews*, 31: 127-31.

Rasmussen, Blake B, Kevin D Tipton, Sharon L Miller, Steven E Wolf, and Robert R Wolfe. 2000. 'An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise', *Journal of Applied Physiology*, 88: 386-92.

Rehfeldt, C, I Fiedler, G Dietl, and K Ender. 2000. 'Myogenesis and postnatal skeletal muscle cell growth as influenced by selection', *Livestock Production Science*, 66: 177-88.

Rehfeldt, Charlotte, Neil C Stickland, Ilse Fiedler, and Jochen Wegner. 1999. 'Environmental and genetic factors as sources of variation in skeletal muscle fibre number', *BAM-PADOVA-*, 9: 235-54.

Rennie, Michael J, and Henning Wackerhage. 2003. 'Connecting the dots for mechanochemical transduction in muscle', *The Journal of physiology*, 553: 1-1.

Rennie, MJ, RHT Edwards, D Halliday, DE Matthews, SL Wolman, and DJ Millward. 1982. 'Muscle protein synthesis measured by stable isotope techniques in man: the effects of feeding and fasting', *Clinical science*, 63: 519-23.

Reynolds, Thomas H, Sue C Bodine, and John C Lawrence. 2002. 'Control of Ser2448 phosphorylation in the mammalian target of rapamycin by insulin and skeletal muscle load', *Journal of Biological Chemistry*, 277: 17657-62.

Rhoden, Ernani Luis, and Abraham Morgentaler. 2004. 'Risks of testosterone-replacement therapy and recommendations for monitoring', *New England Journal of Medicine*, 350: 482-92.

Ribeiro, Manoel Benício Teixeira, Vinicius Guzzoni, Jeffrey M Hord, Gisele Nunes Lopes, Rita de Cássia Marqueti, Rosângela Vieira Andrade, Heloisa Sobreiro Selistre-de-Araujo, and João Luiz Q Durigan. 2017. 'Resistance training regulates gene expression of molecules associated with intramyocellular lipids, glucose signaling and fiber size in old rats', *Scientific reports*, 7: 1-13.

Richter, Erik A, Lawrence P Garetto, Michael N Goodman, and Neil B Ruderman. 1982. 'Muscle glucose metabolism following exercise in the rat: increased sensitivity to insulin', *The Journal of clinical investigation*, 69: 785-93.

Richter, ERIK A, KJ Mikines, Henrik Galbo, and Bente Kiens. 1989. 'Effect of exercise on insulin action in human skeletal muscle', *Journal of Applied Physiology*, 66: 876-85.

Roberts, M. D., M. A. Romero, C. B. Mobley, P. W. Mumford, P. A. Roberson, C. T. Haun, C. G. Vann, S. C. Osburn, H. H. Holmes, R. A. Greer, C. M. Lockwood, H. A. Parry, and A. N. Kavazis. 2018. 'Skeletal muscle mitochondrial volume and myozenin-1 protein differences exist between

high versus low anabolic responders to resistance training', *PeerJ*, 6: 1-25.

Roberts, Michael D, Cody T Haun, Christopher B Mobley, Petey W Mumford, Matthew A Romero, Paul A Roberson, Christopher G Vann, and John J McCarthy. 2018. 'Physiological differences between low versus high skeletal muscle hypertrophic responders to resistance exercise training: current perspectives and future research directions', *Frontiers in physiology*, 9: 1-17.

Rolf, Claus, and Eberhard Nieschlag. 1998. 'Potential adverse effects of long-term testosterone therapy', *Baillière's clinical endocrinology and metabolism*, 12: 521-34.

Rolland, Yves, Charlotte Dupuy, Gabor Abellan van Kan, Sophie Gillette, and Bruno Vellas. 2011. 'Treatment strategies for sarcopenia and frailty', *Medical Clinics*, 95: 427-38.

Rommerts, Focko FG. 1998. 'Testosterone: an overview of biosynthesis, transport, metabolism and nongenomic actions.' in, *Testosterone* (Springer).

Rønnestad, Bent R, Håvard Nygaard, and Truls Raastad. 2011. 'Physiological elevation of endogenous hormones results in superior strength training adaptation', *European journal of applied physiology*, 111: 2249-59.

Rossetti, Michael L, Jennifer L Steiner, and Bradley S Gordon. 2017. 'Androgen-mediated regulation of skeletal muscle protein balance', *Molecular and cellular endocrinology*, 447: 35-44.

Roy, Tracey Ann, Marc R Blackman, S Mitchell Harman, Jordan D Tobin, Matthew Schrager, and E Jeffery Metter. 2002. 'Interrelationships of serum testosterone and free testosterone index with FFM and strength in aging men', *American Journal of Physiology-Endocrinology And Metabolism*, 283: E284-E94.

Rubenstein, Laurence Z, Karen R Josephson, and Alan S Robbins. 1994. 'Falls in the nursing home', *Annals of Internal Medicine*, 121: 442-51.

Ruvinsky, Igor, and Oded Meyuhas. 2006. 'Ribosomal protein S6 phosphorylation: from protein synthesis to cell size', *Trends in biochemical sciences*, 31: 342-48.

Sandri, Marco. 2008. 'Signaling in muscle atrophy and hypertrophy', *Physiology*, 23: 160-70.

Sanes, Joshua R. 2003. 'The basement membrane/basal lamina of skeletal muscle', *Journal of Biological Chemistry*, 278: 12601-04.

Sartorelli, Vittorio, and Marcella Fulco. 2004. 'Molecular and cellular determinants of skeletal muscle atrophy and hypertrophy', *Sci. STKE*, 2004: re11-re11.

Sato, Koji, Motoyuki Iemitsu, Kenji Matsutani, Toshiyuki Kurihara, Takafumi Hamaoka, and Satoshi Fujita. 2014. 'Resistance training restores muscle sex steroid hormone steroidogenesis in older men', *The FASEB Journal*, 28: 1891-97.

Sattler, Fred R. 2013. 'Growth hormone in the aging male', *Best practice & research Clinical endocrinology & metabolism*, 27: 541-55.

Sattler, Fred R, Carmen Castaneda-Sceppa, Ellen F Binder, E Todd Schroeder, Ying Wang, Shalender Bhasin, Miwa Kawakubo, Yolanda Stewart, Kevin E Yarasheski, and Jagadish Ulloor. 2009. 'Testosterone and

growth hormone improve body composition and muscle performance in older men', *The Journal of Clinical Endocrinology & Metabolism*, 94: 1991-2001.

Saunders, Harry L, Bohdan Steciw, and Augusta Kline. 1962. 'Effect of testosterone and insulin on rat skeletal muscle RNA', *Endocrinology*, 71: 314-20.

Sayer, Avan Aihie, Elaine M Dennison, Holly E Syddall, Karen Jameson, Helen J Martin, and Cyrus Cooper. 2008. 'The developmental origins of sarcopenia: using peripheral quantitative computed tomography to assess muscle size in older people', *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 63: 835-40.

Schmittgen, Thomas D, and Kenneth J Livak. 2008. 'Analyzing real-time PCR data by the comparative C T method', *Nature protocols*, 3: 1101-8.

Seidell, Jacob C, Per Björntorp, Lars Sjöström, Henry Kvist, and Rune Sannerstedt. 1990. 'Visceral fat accumulation in men is positively associated with insulin, glucose, and C-peptide levels, but negatively with testosterone levels', *Metabolism*, 39: 897-901.

Serra, Carlo, Shalender Bhasin, Frances Tangherlini, Elisabeth R Barton, Michelle Ganno, Anqi Zhang, Janet Shansky, Herman H Vandenburgh, Thomas G Travison, and Ravi Jasuja. 2011. 'The role of GH and IGF-I in mediating anabolic effects of testosterone on androgen-responsive muscle', *Endocrinology*, 152: 193-206.

Shaibi, Gabriel Q, Martha L Cruz, Geoff DC Ball, Marc J Weigensberg, George J Salem, Noe C Crespo, and Michael I Goran. 2006. 'Effects of resistance training on insulin sensitivity in overweight Latino adolescent males', *Medicine and science in sports and exercise*, 38: 1208.

Sheffield-Moore, M. 2000. 'Androgens and the control of skeletal muscle protein synthesis', *Ann Med*, 32: 181-86.

Sheffield-Moore, Melinda, EL Dillon, KM Randolph, SL Casperson, GR White, K Jennings, J Rathmacher, S Schuette, M Janghorbani, and RJ Urban. 2014. 'Isotopic decay of urinary or plasma 3-methylhistidine as a potential biomarker of pathologic skeletal muscle loss', *Journal of cachexia, sarcopenia and muscle*, 5: 19-25.

Sheffield-Moore, Melinda, and Randall J Urban. 2004. 'An overview of the endocrinology of skeletal muscle', *Trends in Endocrinology & Metabolism*, 15: 110-15.

Sheffield-Moore, Melinda, Randall J Urban, Steven E Wolf, J Jiang, Don H Catlin, David N Herndon, Robert R Wolfe, and Arny A Ferrando. 1999. 'Short-term oxandrolone administration stimulates net muscle protein synthesis in young men', *The Journal of Clinical Endocrinology & Metabolism*, 84: 2705-11.

Sih, Rahmawati, John E Morley, Fran E Kaiser, Horace M Perry III, Ping Patrick, and Candie Ross. 1997. 'Testosterone replacement in older hypogonadal men: a 12-month randomized controlled trial', *The Journal of Clinical Endocrinology & Metabolism*, 82: 1661-67.

Singh, Rajan, Jorge N Artaza, Wayne E Taylor, Nestor F Gonzalez-Cadavid, and Shalender Bhasin. 2003a. 'Androgens stimulate myogenic differentiation and inhibit adipogenesis in C3H 10T1/2 pluripotent cells

through an androgen receptor-mediated pathway', *Endocrinology*, 144: 5081-88.

Singh, Rajan, Shalender Bhasin, Melissa Braga, Jorge N Artaza, Shehla Pervin, Wayne E Taylor, Venkatesh Krishnan, Satyesh K Sinha, Tripathi B Rajavashisth, and Ravi Jasuja. 2008. 'Regulation of myogenic differentiation by androgens: cross talk between androgen receptor/β-catenin and follistatin/transforming growth factor-β signaling pathways', *Endocrinology*, 150: 1259-68.

Sinha-Hikim, I., J. Artaza, L. Woodhouse, N. Gonzalez-Cadavid, A. B. Singh, M. I. Lee, T. W. Storer, R. Casaburi, R. Shen, and S. Bhasin. 2002a. 'Testosterone-induced increase in muscle size in healthy young men is associated with muscle fiber hypertrophy', *Am J Physiol Endocrinol Metab*, 283: 154-64.

Sinha-Hikim, Indrani, Jorge Artaza, Linda Woodhouse, Nestor Gonzalez-Cadavid, Atam B Singh, Martin I Lee, Thomas W Storer, Ricahrd Casaburi, Ruoquing Shen, and Shalender Bhasin. 2002b. 'Testosterone-induced increase in muscle size in healthy, young men is associated with muscle fiber hypertrophy', *American Journal of Physiology-Endocrinology And Metabolism*.

Sinha-Hikim, Indrani, Jorge Artaza, Linda Woodhouse, Nestor Gonzalez-Cadavid, Atam B Singh, Martin I Lee, Thomas W Storer, Richard Casaburi, Ruoquing Shen, and Shalender Bhasin. 2002c. 'Testosterone-induced increase in muscle size in healthy young men is associated with muscle fiber hypertrophy', *American Journal of Physiology-Endocrinology And Metabolism*, 283: E154-E64.

Sinha-Hikim, Indrani, Marcia Cornford, Hilda Gaytan, Martin L Lee, and Shalender Bhasin. 2006a. 'Effects of testosterone supplementation on skeletal muscle fiber hypertrophy and satellite cells in community-dwelling older men', *The Journal of Clinical Endocrinology & Metabolism*, 91: 3024-33.

Sinha-Hikim, Indrani, Stephen M Roth, Martin I Lee, and Shalender Bhasin. 2003. 'Testosterone-induced muscle hypertrophy is associated with an increase in satellite cell number in healthy, young men', *American Journal of Physiology-Endocrinology And Metabolism*, 285: E197-E205.

Sinha-Hikim, Indrani, Wayne E Taylor, Nestor F Gonzalez-Cadavid, Wei Zheng, and Shalender Bhasin. 2004. 'Androgen receptor in human skeletal muscle and cultured muscle satellite cells: up-regulation by androgen treatment', *The Journal of Clinical Endocrinology & Metabolism*, 89: 5245-55.

Sipilä, Sarianna, Marco Narici, Michael Kjaer, Eija Pöllänen, Ross A Atkinson, Mette Hansen, and Vuokko Kovanen. 2013. 'Sex hormones and skeletal muscle weakness', *Biogerontology*, 14: 231-45.

Smith, M. R., H. Lee, and D. M. Nathan. 2006a. 'Insulin sensitivity during combined androgen blockade for prostate cancer', *J Clin Endocrinol Metab*, 91: 1305-8.

Smith, Matthew R., Hang Lee, and David M. Nathan. 2006b. 'Insulin Sensitivity during Combined Androgen Blockade for Prostate Cancer', *The Journal of Clinical Endocrinology & Metabolism*, 91: 1305-08.

Smutok, M. A., C. Reece, P. F. Kokkinos, C. Farmer, P. Dawson, R. Shulman, J. DeVane-Bell, J. Patterson, C. Charabogos, A. P. Goldberg, and et al. 1993. 'Aerobic versus strength training for risk factor intervention in middle-aged men at high risk for coronary heart disease', *Metabolism*, 42: 177-84.

Snijders, Tim, Joshua P. Nederveen, Bryon R. McKay, Sophie Joanisse, Lex B. Verdijk, Luc J. C. van Loon, and Gianni Parise. 2015. 'Satellite cells in human skeletal muscle plasticity', *Frontiers in physiology*, 6.

Snijders, Tim, Lex B Verdijk, Joey SJ Smeets, Bryon R McKay, Joan MG Senden, Fred Hartgens, Gianni Parise, Paul Greenhaff, and Luc JC van Loon. 2014. 'The skeletal muscle satellite cell response to a single bout of resistance-type exercise is delayed with aging in men', *Age (Dordr)*, 36: 9699.

Sriraman, Venkataraman, Moorthy Anbalagan, and A Jagannadha Rao. 2005. 'Hormonal regulation of Leydig cell proliferation and differentiation in rodent testis: a dynamic interplay between gonadotrophins and testicular factors', *Reproductive biomedicine online*, 11: 507-18.

Stanford, Kristin I, and Laurie J Goodyear. 2014. 'Exercise and type 2 diabetes: molecular mechanisms regulating glucose uptake in skeletal muscle', *Advances in physiology education*, 38: 308-14.

Stanworth, Roger D., and T. Hugh Jones. 2008. 'Testosterone for the aging male; current evidence and recommended practice', *Clinical interventions in aging*, 3: 25-44.

Steinberg, Gregory R, and Bruce E Kemp. 2009. 'AMPK in health and disease', *Physiological reviews*, 89: 1025-78.

Storer, Thomas W, Lynne Magliano, Linda Woodhouse, Martin L Lee, Connie Dzekov, Jeanne Dzekov, Richard Casaburi, and Shalender Bhasin. 2003. 'Testosterone dose-dependently increases maximal voluntary strength and leg power, but does not affect fatigability or specific tension', *The Journal of Clinical Endocrinology & Metabolism*, 88: 1478-85.

Sullivan, Dennis H, Paula K Roberson, Larry E Johnson, Osama Bishara, William J Evans, Eugene S Smith, and J Allen Price. 2005. 'Effects of muscle strength training and testosterone in frail elderly males', *Medicine and science in sports and exercise*, 37: 1664-72.

Sundell, Jan. 2011. 'Resistance training is an effective tool against metabolic and frailty syndromes', *Advances in preventive medicine*: 1-7.

Svartberg, J., I. Agledahl, Y. Figenschau, T. Sildnes, K. Waterloo, and R. Jorde. 2008. 'Testosterone treatment in elderly men with subnormal testosterone levels improves body composition and BMD in the hip', *Int J Impot Res*, 20: 378-87.

Swatland, HJ. 1976. "Recent research on postnatal muscle development in swine." In *Proc. 29th Annual Recip. Meat Conf.*

Takala, T. O., P. Nuutila, J. Knuuti, M. Luotolahti, and H. Yki-Jarvinen. 1999. 'Insulin action on heart and skeletal muscle glucose uptake in weight lifters and endurance athletes', *Am J Physiol*, 276: E706-11.

Takala, Teemu O., Pirjo Nuutila, Juhani Knuuti, Matti Luotolahti, and Hannele Yki-Järvinen. 1999. 'Insulin action on heart and skeletal muscle glucose uptake in weight lifters and endurance athletes', *American Journal of Physiology-Endocrinology And Metabolism*, 276: E706-E11.

Tarnow, Lise, Per-Henrik Groop, Samy Hadjadj, Gbenga Kazeem, Francois Cambien, Michel Marre, Carol Forsblom, Hans-Henrik Parving, David Tregouet, and Alexandre Thevard. 2007. 'European rational approach for the genetics of diabetic complications—EURAGEDIC: patient populations and strategy', *Nephrology Dialysis Transplantation*, 23: 161-68.

Taylor, Lockwood G, Steven E Canfield, and Xianglin L Du. 2009. 'Review of major adverse effects of androgen-deprivation therapy in men with prostate cancer', *Cancer*, 115: 2388-99.

Ten Broek, Roel W, Sander Grefte, and Johannes W Von den Hoff. 2010. 'Regulatory factors and cell populations involved in skeletal muscle regeneration', *Journal of cellular physiology*, 224: 7-16.

Terzis, Gerasimos, Konstantinos Spengos, Henrik Mascher, Giorgos Georgiadis, Panagiota Manta, and Eva Blomstrand. 2010. 'The degree of p70 S6k and S6 phosphorylation in human skeletal muscle in response to resistance exercise depends on the training volume', *European journal of applied physiology*, 110: 835-43.

Thomas, David R. 2007. 'Loss of skeletal muscle mass in aging: examining the relationship of starvation, sarcopenia and cachexia', *Clinical Nutrition*, 26: 389-99.

Thompson, LaDora V. 1994. 'Effects of age and training on skeletal muscle physiology and performance', *Physical Therapy*, 74: 71-81.

Thomson, David M, Christopher A Fick, and Scott E Gordon. 2008. 'AMPK activation attenuates S6K1, 4E-BP1, and eEF2 signaling responses to high-frequency electrically stimulated skeletal muscle contractions', *Journal of Applied Physiology*.

Tipton, Kevin D., D. Lee Hamilton, and Iain J. Gallagher. 2018. 'Assessing the Role of Muscle Protein Breakdown in Response to Nutrition and Exercise in Humans', *Sports Med*, 48: 53-64.

Tonini, G, and M Lazzerini. 2000. 'Side effects of GnRH analogue treatment in childhood', *Journal of Pediatric Endocrinology and Metabolism*, 13: 795-804.

Tracy, Brian L, William C Byrnes, and Roger M Enoka. 2004. 'Strength training reduces force fluctuations during anisometric contractions of the quadriceps femoris muscles in old adults', *Journal of Applied Physiology*, 96: 1530-40.

Traish, Abdulmaged M, Bassima Abdallah, and George Yu. 2011. 'Androgen deficiency and mitochondrial dysfunction: implications for fatigue, muscle dysfunction, insulin resistance, diabetes, and cardiovascular disease', *Hormone molecular biology and clinical investigation*, 8: 431-44.

Travison, Thomas G., Hubert W. Vesper, Eric Orwoll, Frederick Wu, Jean Marc Kaufman, Ying Wang, Bruno Lapauw, Tom Fiers, Alvin M. Matsumoto, and Shalender Bhasin. 2017. 'Harmonized Reference Ranges for Circulating Testosterone Levels in Men of Four Cohort Studies in the United States and Europe', *The Journal of Clinical Endocrinology & Metabolism*, 102: 1161-73.

Treebak, Jonas T, Stephan Glund, Atul Deshmukh, Ditte K Klein, Yun Chau Long, Thomas E Jensen, Sebastian B Jørgensen, Benoit Viollet, Leif

Andersson, and Dietbert Neumann. 2006. 'AMPK-mediated AS160 phosphorylation in skeletal muscle is dependent on AMPK catalytic and regulatory subunits', *Diabetes*, 55: 2051-58.

Trounce, Ian A, Yoon L Kim, Albert S Jun, and Douglas C Wallace. 1996. 'Assessment of mitochondrial oxidative phosphorylation in patient muscle biopsies, lymphoblasts, and transmitochondrial cell lines.' in Giuseppe Attardi and Anne Chomyn (eds.), *Mitochondrial Biogenesis and Genetics: Part B* (Elsevier).

Tsai, EC, EJ Boyko, DL Leonetti, and WY Fujimoto. 2000. 'Low serum testosterone level as a predictor of increased visceral fat in Japanese-American men', *International journal of obesity*, 24: 485.

Tsintzas, Kostas, Luke Norton, Kamal Chokkalingam, Nusrat Nizamani, Scott Cooper, Francis Stephens, Rudolf Billeter, and Andrew Bennett. 2013. 'Independent and combined effects of acute physiological hyperglycaemia and hyperinsulinaemia on metabolic gene expression in human skeletal muscle', *Clinical science*, 124: 675-86.

Urban, Randall J, Yvonne H Bodenburg, Charles Gilkison, Judy Foxworth, Andrew R Coggan, Robert R Wolfe, and A Ferrando. 1995a. 'Testosterone administration to elderly men increases skeletal muscle strength and protein synthesis', *American Journal of Physiology-Endocrinology And Metabolism*, 269: 820-26.

Urso, Maria L. 2009. 'Disuse atrophy of human skeletal muscle: cell signaling and potential interventions', *Medicine and science in sports and exercise*, 41: 1860-68.

Usui, Taro, Kazuo Kajita, Toshiko Kajita, Ichiro Mori, Takayuki Hanamoto, Takahide Ikeda, Hideyuki Okada, Koichiro Taguchi, Yoshihiko Kitada, and Hiroyuki Morita. 2014. 'Elevated mitochondrial biogenesis in skeletal muscle is associated with testosterone-induced body weight loss in male mice', *FEBS letters*, 588: 1935-41.

van Breda, Eric, Hans A Keizer, Michaël M Vork, Don AM Surtel, Yvonne F de Jong, Ger J van der Vusse, and Jan FC Glatz. 1992. 'Modulation of fatty-acid-binding protein content of rat heart and skeletal muscle by endurance training and testosterone treatment', *Pflügers Archiv*, 421: 274-79.

van den Beld, Annewieke W, Frank H de Jong, Diederick E Grobbee, Huibert AP Pols, and Steven WJ Lamberts. 2000. 'Measures of bioavailable serum testosterone and estradiol and their relationships with muscle strength, bone density, and body composition in elderly men', *The Journal of Clinical Endocrinology & Metabolism*, 85: 3276-82.

Van der Meer, SFT, RT Jaspers, and H Degens. 2011. 'Is the myonuclear domain size fixed?', *Journal of musculoskeletal & neuronal interactions*, 11: 286-97.

Vega, S Rojas, A Knicker, W Hollmann, W Bloch, and HK Strüder. 2010. 'Effect of resistance exercise on serum levels of growth factors in humans', *Hormone and metabolic research*, 42: 982-86.

Verdijk, Lex B, René Koopman, Gert Schaart, Kenneth Meijer, Hans HCM Savelberg, and Luc JC van Loon. 2007. 'Satellite cell content is specifically reduced in type II skeletal muscle fibers in the elderly',

American Journal of Physiology-Endocrinology And Metabolism, 292: E151-E57.

Vermeulen, Alex, Lieve Verdonck, and Jean M Kaufman. 1999. 'A critical evaluation of simple methods for the estimation of free testosterone in serum', *The Journal of Clinical Endocrinology & Metabolism*, 84: 3666-72.

Vigen, Rebecca, Colin I O'Donnell, Anna E Barón, Gary K Grunwald, Thomas M Maddox, Steven M Bradley, Al Barqawi, Glenn Woning, Margaret E Wierman, and Mary E Plomondon. 2013. 'Association of testosterone therapy with mortality, myocardial infarction, and stroke in men with low testosterone levels', *Jama*, 310: 1829-36.

Vingren, J. L., W. J. Kraemer, N. A. Ratamess, J. M. Anderson, J. S. Volek, and C. M. Maresh. 2010. 'Testosterone physiology in resistance exercise and training: the up-stream regulatory elements', *Sports Med*, 40: 1037-53.

Vingren, Jakob L, William J Kraemer, Disa L Hatfield, Jeffrey M Anderson, Jeff S Volek, Nicholas A Ratamess, Gwendolyn A Thomas, Jen-Yu Ho, Maren S Fragala, and Carl M Maresh. 2008. 'Effect of resistance exercise on muscle steroidogenesis', *Journal of Applied Physiology*, 105: 1754-60.

Vissing, K, SL McGee, J Farup, Tue Kjølhede, MH Vendelbo, and Niels Jessen. 2013. 'Differentiated mTOR but not AMPK signaling after strength vs endurance exercise in training-accustomed individuals', *Scandinavian journal of medicine & science in sports*, 23: 355-66.

Vissing, K., S. McGee, J. Farup, T. Kjolhede, M. Vendelbo, and N. Jessen. 2013. 'Differentiated mTOR but not AMPK signaling after strength vs endurance exercise in training-accustomed individuals', *Scand J Med Sci Sports*, 23: 355-66.

Wallace, Tara M, Jonathan C Levy, and David R Matthews. 2004. 'Use and abuse of HOMA modeling', *DIABETES CARE*, 27: 1487-95.

Walston, Jeremy D. 2012. 'Sarcopenia in older adults', *Current opinion in rheumatology*, 24: 623.

Wang, Huimin, Xiangyu Sun, Lin Zhao, Xiuju Chen, and Jinsheng Zhao. 2016. 'Androgen deprivation therapy is associated with diabetes: Evidence from meta-analysis', *J Diabetes Investig*, 7: 629-36.

Wang, Yu Xin, and Michael A Rudnicki. 2012. 'Satellite cells, the engines of muscle repair', *Nature Reviews Molecular Cell Biology*, 13: 127.

Welle, Stephen, Saara Totterman, and Charles Thornton. 1996. 'Effect of age on muscle hypertrophy induced by resistance training', *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 51: M270-M75.

Wentzensen, Nicolas, and Britton Trabert. 2015. 'Hormone therapy: short-term relief, long-term consequences', *The Lancet*, 385: 1806-08.

West, Daniel WD, Nicholas A Burd, Jason E Tang, Daniel R Moore, Aaron W Staples, Andrew M Holwerda, Steven K Baker, and Stuart M Phillips. 2009. 'Elevations in ostensibly anabolic hormones with resistance exercise enhance neither training-induced muscle hypertrophy nor strength of the elbow flexors', *Journal of Applied Physiology*, 108: 60-67.

West, Daniel WD, Gregory W Kujbida, Daniel R Moore, Philip Atherton, Nicholas A Burd, Jan P Padzik, Michael De Lisio, Jason E Tang, Gianni Parise, and Michael J Rennie. 2009. 'Resistance exercise-induced increases in putative anabolic hormones do not enhance muscle protein synthesis or intracellular signalling in young men', *The Journal of physiology*, 587: 5239-47.

White, James P, Song Gao, Melissa J Puppa, Shuichi Sato, Stephen L Welle, and James A Carson. 2013a. 'Testosterone regulation of Akt/mTORC1/FoxO3a signaling in skeletal muscle', *Molecular and cellular endocrinology*, 365: 174-86.

White, James P., Song Gao, Melissa J. Puppa, Shuichi Sato, Stephen L. Welle, and James A. Carson. 2013b. 'Testosterone regulation of Akt/mTORC1/FoxO3a signaling in skeletal muscle', *Molecular and cellular endocrinology*, 365: 174-86.

White, Robert B, Anne-Sophie Biérinx, Viola F Gnocchi, and Peter S Zammit. 2010. 'Dynamics of muscle fibre growth during postnatal mouse development', *BMC developmental biology*, 10: 21.

Wilborn, Colin D, Lemuel W Taylor, Michael Greenwood, Richard B Kreider, and Darryn S Willoughby. 2009. 'Effects of different intensities of resistance exercise on regulators of myogenesis', *The Journal of Strength & Conditioning Research*, 23: 2179-87.

Wild, Sarah, Gojka Roglic, Anders Green, Richard Sicree, and Hilary King. 2004. 'Global prevalence of diabetes: estimates for the year 2000 and projections for 2030', *DIABETES CARE*, 27: 1047-53.

Wilkinson, Daniel J, Martino V Franchi, Matthew S Brook, Marco V Narici, John P Williams, William K Mitchell, Nathaniel J Szewczyk, Paul L Greenhaff, Philip J Atherton, and Kenneth Smith. 2013. 'A validation of the application of D2O stable isotope tracer techniques for monitoring day-to-day changes in muscle protein subfraction synthesis in humans', *American Journal of Physiology-Endocrinology And Metabolism*, 306: E571-E79.

Wilkinson, Daniel J., Martino V. Franchi, Matthew S. Brook, Marco V. Narici, John P. Williams, William K. Mitchell, Nathaniel J. Szewczyk, Paul L. Greenhaff, Philip J. Atherton, and Kenneth Smith. 2014. 'A validation of the application of D2O stable isotope tracer techniques for monitoring day-to-day changes in muscle protein subfraction synthesis in humans', *American Journal of Physiology - Endocrinology and Metabolism*, 306: 571-79.

Wilkinson, S. B., S. M. Phillips, P. J. Atherton, R. Patel, K. E. Yarasheski, M. A. Tarnopolsky, and M. J. Rennie. 2008a. 'Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle', *J Physiol*, 586: 3701-17.

Wilkinson, S. B., M. A. Tarnopolsky, M. J. Macdonald, J. R. Macdonald, D. Armstrong, and S. M. Phillips. 2007a. 'Consumption of fluid skim milk promotes greater muscle protein accretion after resistance exercise than does consumption of an isonitrogenous and isoenergetic soy-protein beverage', *Am J Clin Nutr*, 85: 1031-40.

Wilkinson, Sarah B, Stuart M Phillips, Philip J Atherton, Rekha Patel, Kevin E Yarasheski, Mark A Tarnopolsky, and Michael J Rennie. 2008b. 'Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle', *The Journal of physiology*, 586: 3701-17.

Wilkinson, Sarah B, Mark A Tarnopolsky, Maureen J MacDonald, Jay R MacDonald, David Armstrong, and Stuart M Phillips. 2007b. 'Consumption of fluid skim milk promotes greater muscle protein accretion after resistance exercise than does consumption of an isonitrogenous and isoenergetic soy-protein beverage', *The American journal of clinical nutrition*, 85: 1031-40.

Wilkinson, Sarah B., Stuart M. Phillips, Philip J. Atherton, Rekha Patel, Kevin E. Yarasheski, Mark A. Tarnopolsky, and Michael J. Rennie. 2008c. 'Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle', *The Journal of physiology*, 586: 3701-17.

Williams, Glenn N, Michael J Higgins, and Michael D Lewek. 2002. 'Aging skeletal muscle: physiologic changes and the effects of training', *Physical Therapy*, 82: 62-68.

Williamson, D, P Gallagher, M Harber, C Hollon, and S Trappe. 2003. 'Mitogen-activated protein kinase (MAPK) pathway activation: effects of age and acute exercise on human skeletal muscle', *The Journal of physiology*, 547: 977-87.

Willoughby, DARRYN S, and Lemuel Taylor. 2004. 'Effects of sequential bouts of resistance exercise on androgen receptor expression', *Medicine and science in sports and exercise*, 36: 1499-506.

Witczak, Carol A, Nobuharu Fujii, Michael F Hirshman, and Laurie J Goodyear. 2007. 'Ca2+/calmodulin-dependent protein kinase kinase-α regulates skeletal muscle glucose uptake independent of AMP-activated protein kinase and Akt activation', *Diabetes*, 56: 1403-09.

Wong, Evelyn, Kathryn Backholer, Emma Gearon, Jessica Harding, Rosanne Freak-Poli, Christopher Stevenson, and Anna Peeters. 2013. 'Diabetes and risk of physical disability in adults: a systematic review and meta-analysis', *The lancet Diabetes & endocrinology*, 1: 106-14.

Wu, Miaozong, Jacqueline Fannin, Kevin M Rice, Bin Wang, and Eric R Blough. 2011. 'Effect of aging on cellular mechanotransduction', *Ageing research reviews*, 10: 1-15.

Wu, Susan C, and Yi Zhang. 2009. 'Minireview: role of protein methylation and demethylation in nuclear hormone signaling', *Molecular endocrinology*, 23: 1323-34.

Wullschleger, Stephan, Robbie Loewith, and Michael N Hall. 2006. 'TOR signaling in growth and metabolism', *Cell*, 124: 471-84.

Xu, Tianshun, Ying Shen, Heather Pink, Jim Triantafillou, Stephen A Stimpson, Philip Turnbull, and Bajin Han. 2004. 'Phosphorylation of p70s6 kinase is implicated in androgen-induced levator ani muscle anabolism in castrated rats', *The Journal of steroid biochemistry and molecular biology*, 92: 447-54.

Yang, Jinzeng. 2014. 'Enhanced skeletal muscle for effective glucose homeostasis.' in, *Progress in molecular biology and translational science* (Elsevier).

Yang, Shiyu, Majed Alnaqeeb, Hamish Simpson, and Geoffrey Goldspink. 1996. 'Cloning and characterization of an IGF-1 isoform expressed in skeletal muscle subjected to stretch', *Journal of Muscle Research & Cell Motility*, 17: 487-95.

Yang, Yifan, Andrew Creer, Bozena Jemiolo, and Scott Trappe. 2005. 'Time course of myogenic and metabolic gene expression in response to acute exercise in human skeletal muscle', *Journal of Applied Physiology*, 98: 1745-52.

Yarasheski, KEVIN E, JEFFREY J Zachwieja, and DENNIS M Bier. 1993. 'Acute effects of resistance exercise on muscle protein synthesis rate in young and elderly men and women', *American Journal of Physiology-Endocrinology And Metabolism*, 265: E210-E14.

Yialamas, M. A., A. A. Dwyer, E. Hanley, H. Lee, N. Pitteloud, and F. J. Hayes. 2007. 'Acute sex steroid withdrawal reduces insulin sensitivity in healthy men with idiopathic hypogonadotropic hypogonadism', *J Clin Endocrinol Metab*, 92: 4254-9.

Zachwieja, J. J., G. Toffolo, C. Cobelli, D. M. Bier, and K. E. Yarasheski. 1996a. 'Resistance exercise and growth hormone administration in older men: effects on insulin sensitivity and secretion during a stable-label intravenous glucose tolerance test', *Metabolism*, 45: 254-60.

Zachwieja, Jeffrey J, Gianna Toffolo, Claudio Cobelli, Dennis M Bier, and Kevin E Yarasheski. 1996b. 'Resistance exercise and growth hormone administration in older men: effects on insulin sensitivity and secretion during a stable-label intravenous glucose tolerance test', *Metabolism*, 45: 254-60.

Zanou, Nadège, and Philippe Gailly. 2013. 'Skeletal muscle hypertrophy and regeneration: interplay between the myogenic regulatory factors (MRFs) and insulin-like growth factors (IGFs) pathways', *Cellular and Molecular Life Sciences*, 70: 4117-30.

Zembroń-Łacny, Agnieszka, Wioletta Dziubek, Łukasz Rogowski, Ewa Skorupka, and Grażyna Dąbrowska. 2014. 'Sarcopenia: monitoring, molecular mechanisms, and physical intervention', *Physiological Research*, 63.

Zhang, Xiao-Jun, David L Chinkes, and Robert R Wolfe. 2002. 'Measurement of muscle protein fractional synthesis and breakdown rates from a pulse tracer injection', *American Journal of Physiology-Endocrinology And Metabolism*, 283: E753-E64.

Zoncu, Roberto, Alejo Efeyan, and David M Sabatini. 2011. 'mTOR: from growth signal integration to cancer, diabetes and ageing', *Nature Reviews Molecular Cell Biology*, 12: 21.

Zurlo, Francesco, Karen Larson, Clifton Bogardus, and Eric Ravussin. 1990. 'Skeletal muscle metabolism is a major determinant of resting energy expenditure', *The Journal of clinical investigation*, 86: 1423-27.

Appendices

Appendix 1

ORIGINAL ARTICLE

Journal of Cachexia, Sarcopenia and Muscle (2019)
Published online in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/jcsm.12472

Testosterone therapy induces molecular programming augmenting physiological adaptations to resistance exercise in older men

Nima Gharahdaghi[1], Supreeth Rudrappa[1], Matthew S. Brook[1], Iskandar Idris[1], Hannah Crossland[1], Claire Hamrock[2], Muhammad Hariz Abdul Aziz[1], Fawzi Kadi[3], Janelle Tarum[3], Paul L. Greenhaff[4], Dumitru Constantin-Teodosiu[4], Jessica Cegielski[1], Bethan E. Phillips[1], Daniel J. Wilkinson[1], Nathaniel J. Szewczyk[1], Kenneth Smith[1] & Philip J. Atherton[1*]

[1]*MRC-ARUK Centre for Musculoskeletal Ageing Research and Nottingham NIHR BRC, School of Medicine, University of Nottingham, Derby, UK,* [2]*Institute of Food and Health, University College Dublin, Belfield, Dublin, Ireland,* [3]*Division of Sports Sciences, School of Health and Medical Sciences, Örebro University, Örebro, Sweden,* [4]*MRC-ARUK Centre for Musculoskeletal Ageing Research, School of Life Sciences, University of Nottingham, Nottingham, Nottingham, UK*

Abstract

Background The andropause is associated with declines in serum testosterone (T), loss of muscle mass (sarcopenia), and frailty. Two major interventions purported to offset sarcopenia are anabolic steroid therapies and resistance exercise training (RET). Nonetheless, the efficacy and physiological and molecular impacts of T therapy adjuvant to short-term RET remain poorly defined.
Methods Eighteen non-hypogonadal healthy older men, 65–75 years, were assigned in a random double-blinded fashion to receive, biweekly, either placebo (P, saline, $n = 9$) or T (Sustanon 250 mg, $n = 9$) injections over 6 week whole-body RET (three sets of 8–10 repetitions at 80% one-repetition maximum). Subjects underwent dual-energy X-ray absorptiometry, ultrasound of vastus lateralis (VL) muscle architecture, and knee extensor isometric muscle force tests; VL muscle biopsies were taken to quantify myogenic/anabolic gene expression, anabolic signalling, muscle protein synthesis (D_2O), and breakdown (extrapolated).
Results Testosterone adjuvant to RET augmented total fat-free mass ($P=0.007$), legs fat-free mass ($P=0.02$), and appendicular fat-free mass ($P=0.001$) gains while decreasing total fat mass ($P=0.02$). Augmentations in VL muscle thickness, fascicle length, and quadriceps cross-section area with RET occured to a greater extent in T ($P < 0.05$). Sum strength ($P=0.0009$) and maximal voluntary contract (e.g. knee extension at 70°) ($P=0.002$) increased significantly more in the T group. Mechanistically, both muscle protein synthesis rates (T: 2.13 ± 0.21%·day^{-1} vs. P: 1.34 ± 0.13%·day^{-1}, $P=0.0009$) and absolute breakdown rates (T: 140.2 ± 15.8 g·day^{-1} vs. P: 90.2 ± 11.7 g·day^{-1}, $P=0.02$) were elevated with T therapy, which led to higher net turnover and protein accretion in the T group (T: 8.3 ± 1.4 g·day^{-1} vs. P: 1.9 ± 1.2 g·day^{-1}, $P=0.004$). Increases in ribosomal biogenesis (RNA:DNA ratio); mRNA expression relating to T metabolism (androgen receptor: 1.4-fold; *Srd5a1*: 1.6-fold; *AKR1C3*: 2.1-fold; and *HSD17β3*: two-fold); insulin-like growth factor (IGF)-1 signalling [*IGF-1Ea* (3.5-fold) and *IGF-1Ec* (three-fold)] and myogenic regulatory factors; and the activity of anabolic signalling (e.g. mTOR, AKT, and RPS6; $P < 0.05$) were all up-regulated with T therapy. Only T up-regulated mitochondrial citrate synthase activity ($P=0.03$) and transcription factor A (1.41 ± 0.2-fold, $P=0.0002$), in addition to peroxisome proliferator-activated receptor-γ co-activator 1-α mRNA (1.19 ± 0.21-fold, $P=0.037$).
Conclusions Administration of T adjuvant to RET enhanced skeletal muscle mass and performance, while up-regulating myogenic gene programming, myocellular translational efficiency and capacity, collectively resulting in higher protein turnover, and net protein accretion. T coupled with RET is an effective short-term intervention to improve muscle mass/function in older non-hypogonadal men.

Keywords Skeletal muscle; Protein turnover; Testosterone; Resistance exercise; Ageing

© 2019 The Authors. Journal of Cachexia, Sarcopenia and Muscle published by John Wiley & Sons Ltd on behalf of the Society on Sarcopenia, Cachexia and Wasting Disorders
This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Appendix 2

Division of Medical Sciences and GEM

University of Nottingham
UK | CHINA | MALAYSIA

Healthy Volunteer Consent Form (HVCF)

Premises covered	MSGEM	This is version	8
Document Created	15.7.2009	Document issued in	June 19
Review Period	Every two years	To be reviewed in	June 21

Project Title:

Please read this form and sign it once the investigator above-named, or their designated representative, has explained fully the aims and procedures of the study to you.

- I voluntarily agree to take part in this study. ☐
- I confirm that I have been given a full explanation of the study and that I have read and understand the information sheet (version number X.X dated XX/XX/XXXX) for the above study and have had the opportunity to ask questions. ☐
- I understand that I am free to withdraw from the study at any time, without having to give a reason for withdrawing. ☐
- I agree to comply with the reasonable instructions of the research team and will notify them immediately of any unexpected unusual symptoms or deterioration of health. ☐
- I give permission to collect, store, analyse and publish information obtained from my participation in this study. I understand my personal details will be kept confidential. ☐
- I understand that data collected in the study may be looked at by responsible individuals from the University of Nottingham and regulatory authorities where it is relevant to my taking part in this study. ☐
- I understand that information about me recorded during the study will be kept in a secure database. If data is transferred to others it will be made anonymous. Data will be kept for 7 years after all results of this study have been published. After this time, it will be disposed of in line with University policy. ☐
- I understand that the tests or procedures are carried out for research only and not for clinical diagnostic purposes. However, if the study investigator should feel it necessary to inform my GP of my participation in the study, or of an adverse event or abnormal test result, I understand I am giving my consent to do so. ☐
- I confirm that I have disclosed relevant medical information before the study. ☐
- I have not been a volunteer in any other research study in the *last three months*, which involved: taking a drug, being paid an inconvenience allowance, having an invasive procedure (e.g. venepuncture >50ml, endoscopy) or exposure to ionising radiation. ☐

- I confirm that I have not been exposed to more than 5 mSv of ionising radiation in the *last 12 months* (to clarify, please discuss with a member of the research team). ☐

- **OPTIONAL** - I agree to my tissue/ blood samples being shared with other researchers including those working outside the University. I understand that I would not gain commercially. ☐

- **OPTIONAL** - Where tissue is removed from my body for the purposes of this study, but not used for this study, and when the ethical approval for this project expires, I agree to the future and continued storage and use of this tissue for future research into functioning of the human body and disease. Such tissue will fall under the University of Nottingham licence of the Human Tissue Act. As specified under the Licence tissue records will be maintained for 25 years. ☐

Personal Data:

- **OPTIONAL** - I agree to my contact details being stored for the purpose of being invited to participate in future research studies. ☐

Volunteer Name:

……
……

Signature: …… **Date:** ……

I confirm that I (investigator) have fully explained the purpose of the study and what is involved to:

……
……

I have given the above named a copy of this form together with the information sheet.

Investigator Name:

……
….

Signature: …… **Date:** ……

PI Signature: …… **Date:** ……

Study Volunteer Number: ……

2 copies: 1 for the participant, 1 for the study

Appendix 3

Healthy Older Volunteer's Information Sheet

Clinical, Metabolic and Molecular Physiology Research Group

Title of Project: The regulation of skeletal muscle protein synthesis by systemic hormones and its influence on ageing and anabolic resistance

Names of Investigators:
Dr Daniel Wilkinson
Dr Phil Atherton
Dr Ken Smith
Dr Bethan Phillips
Dr Iskander Idris,
Dr Haitham Abdullah,
Dr Supreeth Rudrappa

You have been invited to take part in a research study. Before you decide whether to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives if you wish to. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. If you decide to take part you will be given a copy of your consent form and this leaflet.

Background

Muscles represent the largest organ in the body, making up over 50% of total body weight. Most people know that skeletal muscles are important for movement and to support the skeleton, but not everyone is aware of how important muscles are for whole-body health. For example, muscles represent a vast protein store containing amino acids (the building blocks of protein) which can be broken down in times of fasting, infection and disease in order to provide energy to help other vital organs. Hormones can play an important role in the regulation of muscle mass throughout adult life, with reductions in hormones levels due to illness or disease leading to a reduction in muscle mass and strength. It however still remains unclear how these hormones regulate these changes in muscle mass. This knowledge could be key in developing future pharmaceuticals for treatment of muscle wasting disease. Further to this, most people will have noticed that with age people become frail. This is principally due to the wasting of skeletal muscle known as "sarcopenia". Crucially, sarcopenia is more than just a symptom of weakness and poor functional capacity; it exposes people to an increased risk of falls and fractures, impacting quality of life, independence, health status and ultimately lifespan. It is because of these

detrimental effects on health, and the associated health costs that sarcopenia is of grave concern. Interestingly, with ageing there is also an associated reduction in hormone levels and a lower stimulation of hormonal release in response to things like exercise. This association leads to the possibility that reductions in hormone levels with age could contribute to the frailty and sarcopenia associated with normal ageing, and therefore there is a significant clinical need to identify whether this is a significant contributing factors to this muscle loss so that it can be specifically targeted for intervention (i.e., hormonal therapies).

What does the study involve?

Screening Visit

Before you formally enter the study you will be asked to attend our facility (the Medical School, Royal Derby Hospital), without having eaten anything (from 2000h the night before), a screening visit to ensure that you are eligible for participation in this study. During this visit (approximately 60 minutes), we will explain the study in full and answer any questions you may have, then (i) ask you to sign a consent form for the study, (ii) take a blood sample (10-20ml), and (iii) conduct a health assessment - including measuring your height and weight and a trace of your hearts electrical activity (an ECG).

Main Study

Once we have received the results of your blood tests from screening we will contact you to inform you of your results and confirm your inclusion into the study. If you are happy to proceed with the study we will organise a mutually convenient day on which you will attend our facility to begin the study. The study period will last a total of 8 weeks in duration, and over this period you will be asked to attend our exercise suite 3 times a week for supervised exercise training and for collection of samples.

On the initial lab visit a DXA scan and ultrasound will be performed to measure your body composition, your leg muscle mass and muscle architecture (fibre length and thickness). The ultrasound is similar to the scan carried out to image babies during pregnancy and involves some jelly and a probe being placed on your thigh. A leaflet describing the DXA scan in more detail is attached. In brief, this is a whole-body low dose X-ray scan that provides us with information about the tissue under your skin. The radiation exposure from this scan is very low, equivalent to ~3 days background exposure and significantly less than a flight to America. A test called an oral glucose tolerance test (OGTT) will also be performed. This test assesses your body's response to a single glucose drink, and is commonly used by GPs to assess a person's health. This test involves a small 75g glucose drink followed by regular 15 min blood samples over the following two hours, this sampling will be performed via the placement of a small tube in a vein in your arm (the OGTT will be repeated again at the start and end of the exercise training period). Following this your strength will be assessed and you will be familiarised with the exercise protocol to be performed during the study period. Your 1 repetition maximum (1-RM) will then be determined for six muscles (this is the highest amount of weight you can lift once with one leg) so we can determine your workload during the exercise training. Following

completion of the exercise test a muscle biopsy will be collected from the vastus lateralis muscle (i.e., a region in the front of the thigh muscle) after an injection of local anaesthetic to numb the area. After the local anaesthetic a trained clinician will make a small (0.5 cm) cut through the skin and underlying muscle sheath then take the biopsy (a small piece about the size of an orange pip) with a special set of forceps. With the local anaesthetic you may feel pushing during this procedure but no pain. A stitch is placed in the skin afterwards to aid healing. A tight bandage will be placed around the thigh after the biopsy and should remain in place for ~12h to speed healing. You will then provide a saliva sample, and be provided with a drink of 'heavy water' (~250 ml) to consume. This water contains a stable isotope tracer (deuterium in place of hydrogen, but is not radioactive), which is slightly different from normal water, but it is perfectly safe. We will ask you to provide a small sample of saliva 3-4 hours later, (which you can store refrigerated in sealed tubes we will provide). We will also ask you to collect samples of saliva daily around midday and at least an hour after food or drink for the first week. You will be provided with a small volume (~10 ml) of additional heavy water to consume daily for the remainder of the study period. This tracer is what allows us to measure rates of muscle growth and metabolism. In addition to the heavy water, we will provide you with a small drink of labelled creatine and 3-methylhistidine tracers (as with the heavy water, these are not radioactive and are perfectly safe, both creatine and 3-methylhistidine occur naturally in your muscles), we will then ask you to collect urine samples over the next 72h, before returning at 72h for a series of small blood samples. These samples will provide us with a measure of your muscle weight and how quickly your muscle is breaking down, and will be repeated at 3 and 6 weeks into the exercise period to assess for any changes related to the testing.

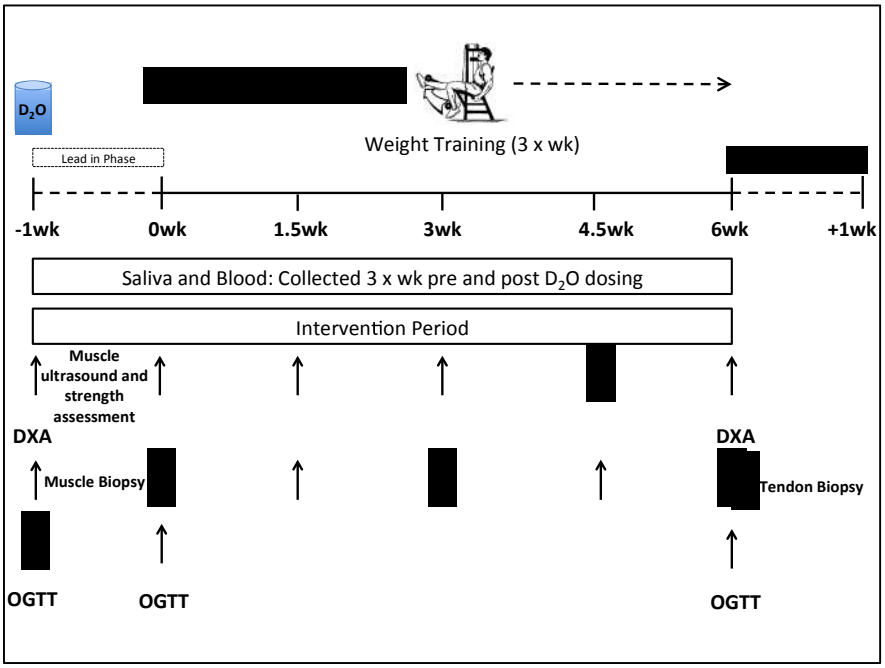
You will then be randomly assigned to one of two testing groups, either: 1) a placebo group, or 2) a testosterone supplemented group (Sustanon 250: 250mg intramuscular injection every 2-3wks). The testosterone intervention is aimed at increasing levels of testosterone in your blood, which are known to be lower in ageing populations and are anticipated to contribute to reduced muscle mass with ageing and in response to exercise training.

Following an initial lead in period of one week, where you will perform no exercise, you will return to the lab 3 x wk for 6-weeks for a whole body progressive supervised resistance exercise training programme. You will be asked to perform 3 sets of 10 repetitions of both upper and lower body exercises at a weight equivalent to 80% of your 1-RM. At the start of the exercise period (designated week 0 on the attached diagram), and every 1.5 weeks up to the end of the exercise period (at 1.5, 3, 4.5 and 6 weeks as highlighted in the diagram, you will be asked to return to the lab for muscle biopsies. As well as using the biopsies to assess changes in metabolism due to the interventions, biopsies will also be used for cell-culture work to explore important mechanisms regulating the numerous roles of muscle.

During the intervention period, you will also have repeat ultrasound measurements, muscle function and strength tests every 1.5 weeks. An additional small patellar tendon biopsy will also be obtained at 6 weeks – this is the tendon that attaches the bottom of your kneecap (patellar) to the top of your shinbone (tibia). This biopsy will be taken using a microneedle technique, guided by ultrasound. This procedure has been conducted regularly within our labs without any reports of discomfort (average discomfort reported during and following the procedure was 2 out of 10; with 1 being no discomfort and 10 being

maximal discomfort). Prior to each muscle biopsy an ultrasound scan of the muscle will be performed (with a repeat DXA scan) to monitor any changes in muscle structure due to the training regime. Following the training regime the intervention will be stopped and you will be monitored for one additional week to ensure hormonal levels have returned to normal. Following this a final muscle biopsy will be obtained. A diagram of the study protocol is provided below.

Why have you been chosen?



Primarily because you have expressed an interest in taking part in the study and you have met with the entry criteria and do not have on-going medical problems. We will be recruiting healthy, recreationally active males aged 65-75y.

Do you have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

What do I have to do?

You are requested to fast on the night before, and avoiding heavy exercise for 48h prior to your visits to the laboratory. We will provide you with a recommended dietary intake for the study period that we would like you to adhere to where possible. This will follow a standard balanced diet according to current

recommended daily allowances for carbohydrates, fats and proteins, and will help us to limit the impact of dietary intake upon the outcomes of study.

What is the drug or procedure that is being tested?

One group of individuals will receive slow release testosterone (the other a placebo), which provides increases in testosterone levels, it is safe to use in healthy humans and similar testosterone products have been previously used in studies within our research group and is regularly used as a treatment in humans. Testosterone (or placebo) will be administered via intramuscular injection every 2-3wks. You will be provided with all necessary information regarding this compound and any potential side effects (see below) and will be continually monitored by our researchers.

What are the side effects of any treatment or procedures received when taking part?

Research studies often involve some risks, not all of which may be currently known. In the light of our experience, muscle and tendon biopsies are well tolerated by all our volunteers. Scarring is hardly perceptible and fades with time. However, the procedure may cause mild pain in some volunteers, but with appropriate local analgesia this is minimal; indeed data from a previous audit of the procedure demonstrated that 83% of our participants experienced less than or the expected discomfort during the muscle biopsy and all respondents said that they would or may be willing to participate in a similar study in the future. Some discomfort (muscle tenderness and stiffness) may be felt for 2-3 days afterwards but mild painkillers will usually effectively abolish this. There is a slight risk of infection at biopsy sites, however all procedures are performed under sterile conditions to minimize any risk of infection. Damage to small nerves of the skin during the biopsy is possible; however these nerves grow back with restoration of normal sensation, without difficulty.

We will provide you with a short, anonymized questionnaire about your experience of the biopsy procedures during this study should you wish to respond.

The risks involved in venous blood sampling are very small. Occasionally, however there may be bruising due to leakage from a blood vessel on withdrawal of the needle, but this is uncommon with good practice. The loss of 60 ml of blood has no ill effects in healthy persons and is much less than if you donate blood.

As with any pharmacological intervention there may be some side effects associated with the administration of the Testosterone, however these are uncommon and are generally only associated with prolonged administration over periods longer than what is being performed in the current study. Those side effects which may be encountered are mild and include; headache, increased hypertension, acne, increased blood count and skin irritation at the gel site (a full list of all potential side effects associated with testosterone are attached with this

information sheet). If you are randomized into a testosterone (vs. placebo) group you will be given the chance to discuss these with any of the clinicians if unsure.

What are the exclusion criteria for this study?

We are recruiting healthy men between the ages of 65 to 75 years, who are of normal weight or slightly overweight. If you have been a subject in any other research study in the last three months which involved: taking a drug, being paid a disturbance allowance or having an invasive procedure (e.g. blood sample >50ml) you would not be eligible to take part.

You would also be unsuitable if you have particular medical conditions or are taking certain medications. If you are interested in this study please discuss these further with the study doctor.

What are the possible disadvantages and risks of taking part?

Healthy volunteers have been chosen for this study; however, it is possible that the routine tests could detect unknown health problems. Should this be the case you will be informed and advised to attend your GP practice for further management. Your GP will be notified. Be advised, however, that future insurance status e.g. for life insurance or private medical insurance, could be affected by these diagnoses.

What if something goes wrong?

In the unlikely event of a complication following the study or that you should wish to voice grievances, in the first instance please contact the clinician involved in the execution of the study or the lead investigator, Dr Phil Atherton (T: 01332 724725; E: philip.atherton@nottingham.ac.uk). If you remain unhappy and wish to complain formally, you should then contact the FMHS Research Ethics Committee Administrator, c/o The University of Nottingham, School of Medicine Education Centre, B Floor, Medical School, Queen's Medical Centre Campus, Nottingham University Hospitals, Nottingham, NG7 2UH. E-mail: louise.sabir@nottingham.ac.uk.

Will my taking part in this study be kept confidential?

We respect your right to privacy and we will take measures to safeguard confidentiality. A single form, on which you are asked to sign to give consent for involvement will carry details of your name and address, but no health related details. This is kept securely in a locked cabinet within the Medical School. Access to this cabinet is restricted to personnel directly involved in the study and to University staff with direct responsibility for ensuring the study is conducted appropriately.

We will follow current ethical and legal practice and all information about you will be handled in confidence. If you join the study, some parts of the data collected about you will be looked at by authorised persons from the University of Nottingham who are organising the research. They may also be looked at by people authorised to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and we will do our best to meet this duty.

All information which is collected about you during the course of the research will be kept strictly confidential, stored in a secure and locked office, and on a password protected database. Any information about you that leaves the University will have your name and address removed (anonymised) and a unique code will be used so that you cannot be recognised from it, with the exception of a letter sent to your GP advising of your participation in this study.

Your personal data (address, telephone number) will be kept for 5 years after the end of the study so that we are able to contact you about the findings of the study and possible follow-up studies. All other data (research data) will be kept securely for 7 years. After this time your data will be disposed of securely. During this time all precautions will be taken by all those involved to maintain your confidentiality and only direct members of the research team will have access to your personal data.

What will happen to any samples I give?

We will use the tendon and biopsy samples to see how your muscle and tendons respond to exercise and/ or the effect of age or hormones. We may also use your muscle samples to generate cell-culture samples so that we can further investigate mechanisms involved in muscle health and disease outside of the human body. We will also seek your consent for any samples remaining after analysis for this study has been completed, to be stored and used in future research. This is optional and you will be asked to give separate consent for this. The samples will be securely stored with a code unique to you at the University of Nottingham under the Universities Human Tissue Research Licence (No. 12265).

Some of these future studies may be carried out by researchers other than the current research team. This may include researchers working for commercial companies. Any samples or data used will be anonymised so that you could not be identified in any way. If you do not agree to this, any remaining samples will be disposed of in accordance with the Human Tissue Authorities code of practice.

Will any genetic tests be done?

In the current study we will collect a blood sample that can be used to obtain information about your genes (RNA and DNA), however this analysis will not give us information on disease prognosis and samples will not be subject to genetic manipulation.

Who is funding the research?

This research is funded by The University of Nottingham.

Who has reviewed the study?

This study has been reviewed and approved by the University of Nottingham Medical School Ethics Committee.

Contact for Further Information

Thank you for taking part in the study. For further information you can contact Mr Nima Gharahdaghi on 01332 724685 or at mzxng@nottingham.ac.uk, or Dr Daniel Wilkinson (Research Fellow) on 01332 724850 or at d.wilkinson@nottingham.ac.uk, both are alternatively contactable at Clinical, Metabolic and Molecular Physiology, University of Nottingham, School of Medicine, Division of Graduate Entry Medicine and Health, Royal Derby Hospital, Uttoxeter Road, Derby, DE22 3DT.

Testosterone Reported Adverse Reactions

(taken from http://www.medicines.org.uk/emc/medicine/22159)

Adverse Drug Reactions terminology used for the classification of incidence: Very common ≥10% - Common ≥1% to <10% - Uncommon ≥0.1% to <1% - Rare ≥0.01% to <0.1% - Very rare <0.01% - Not known (can not be estimated from the available data).
The cumulative safety experience of testosterone is derived from Phase I to Phase III clinical trials and post-marketing experience.
The adverse reactions listed in the table below have been observed in clinical studies with testosterone and/or post-marketing experience.

System Organ Class	Adverse Reaction	Frequency
Nervous disorders	Headache	Common
Vascular disorders	Hypertension worsened	Common
	Hot flushes/flushing	Uncommon
Skin and subcutaneous tissue disorders	Acne	Common
	Pruritus	Uncommon
Reproductive system and breast disorders	Azoospermia	Very rare
General disorders and administration site disorders	Application site reaction (including erythema, rash and pruritus)	Common
	Peripheral oedema	Uncommon
Investigations	PSA increased, haematocrit increased, haemoglobin increased, red blood cell count increased	Common

Uncommonly, gynaecomastia may develop and persist in patients being treated for hypogonadism with testosterone.

Appendix 4

Healthy Younger Volunteer's Information Sheet

Clinical, Metabolic and Molecular Physiology Research Group

Title of Project: The regulation of skeletal muscle protein synthesis by systemic hormones and its influence on ageing and anabolic resistance

Names of Investigators:
Dr Daniel Wilkinson
Dr Phil Atherton
Dr Ken Smith
Dr Bethan Phillips
Dr Iskander Idris,
Dr Haitham Abdullah,
Dr Supreeth Rudrappa

You have been invited to take part in a research study. Before you decide whether to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives if you wish to. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. If you decide to take part you will be given a copy of your consent form and this leaflet.

Background

Muscles represent the largest organ in the body, making up over 50% of total body weight. Most people know that skeletal muscles are important for movement and to support the skeleton, but not everyone is aware of how important muscles are for whole-body health. For example, muscles represent a vast protein store containing amino acids (the building blocks of protein) which can be broken down in times of fasting, infection and disease in order to provide energy to help other vital organs. Hormones can play an important role in the regulation of muscle mass throughout adult life, with reductions in hormones levels due to illness or disease leading to a reduction in muscle mass and strength. It however still remains unclear how these hormones regulate these changes in muscle mass. This knowledge could be key in developing future pharmaceuticals for treatment of muscle wasting disease. Further to this, most people will have noticed that with age people become frail. This is principally due to the wasting of skeletal muscle known as "sarcopenia". Crucially, sarcopenia is more than just a symptom of weakness and poor functional capacity; it exposes people to an increased risk of falls and fractures, impacting quality of life, independence, health status and ultimately lifespan. It is because of these detrimental effects on health, and the associated health costs that sarcopenia is of grave concern. Interestingly, with ageing there is also an associated reduction in hormone levels and a lower stimulation of hormonal release in response to things like exercise. This association leads to the possibility that reductions in hormone levels with age could contribute to the frailty and sarcopenia associated

with normal ageing, and therefore there is a significant clinical need to identify whether this is a significant contributing factors to this muscle loss so that it can be specifically targeted for intervention (i.e., hormonal therapies).

What does the study involve?

Screening Visit

Before you formally enter the study you will be asked to attend our facility (the Medical School, Royal Derby Hospital), without having eaten anything (from 2000h the night before), a screening visit to ensure that you are eligible for participation in this study. During this visit (approximately 60 minutes), we will explain the study in full and answer any questions you may have, then (i) ask you to sign a consent form for the study, (ii) take a blood sample (10-20ml), and (iii) conduct a health assessment - including measuring your height and weight and a trace of your hearts electrical activity (an ECG).

Main Study

Once we have received the results of your blood tests from screening we will contact you to inform you of your results and confirm your inclusion into the study. If you are happy to proceed with the study we will organise a mutually convenient day on which you will attend our facility to begin the study. The study period will last a total of 8 weeks in duration, and over this period you will be asked to attend our exercise suite 3 times a week for supervised exercise training and for collection of samples.

On the initial lab visit a DXA scan and ultrasound will be performed to measure your body composition, your leg muscle mass and muscle architecture (fibre length and thickness). The ultrasound is similar to the scan carried out to image babies during pregnancy and involves some jelly and a probe being placed on your thigh. A leaflet describing the DXA scan in more detail is attached. In brief, this is a whole-body low dose X-ray scan that provides us with information about the tissue under your skin. The radiation exposure from this scan is very low, equivalent to ~3 days background exposure and significantly less than a flight to America. A test called an oral glucose tolerance test (OGTT) will also be performed. This test assesses your body's response to a single glucose drink, and is commonly used by GPs to assess a person's health. This test involves a small 75g glucose drink followed by regular 15 min blood samples over the following two hours, this sampling will be performed via the placement of a small tube in a vein in your arm (the OGTT will be repeated again at the start and end of the exercise training period). Following this, your strength will be assessed and you will be familiarised with the exercise protocol to be performed during the study period. Your 1 repetition maximum (1-RM) will then be determined for six muscles (this is the highest amount of weight you can lift once) so we can determine your workload during the exercise training. Following completion of the exercise test a muscle biopsy will be collected from the vastus lateralis muscle (i.e., a region in the front of the thigh muscle) after an injection of local anaesthetic to numb the area. After the local anaesthetic a trained clinician will make a small (0.5 cm) cut through the skin and underlying muscle sheath then take the biopsy (a small piece about the size of an orange pip) with a special set

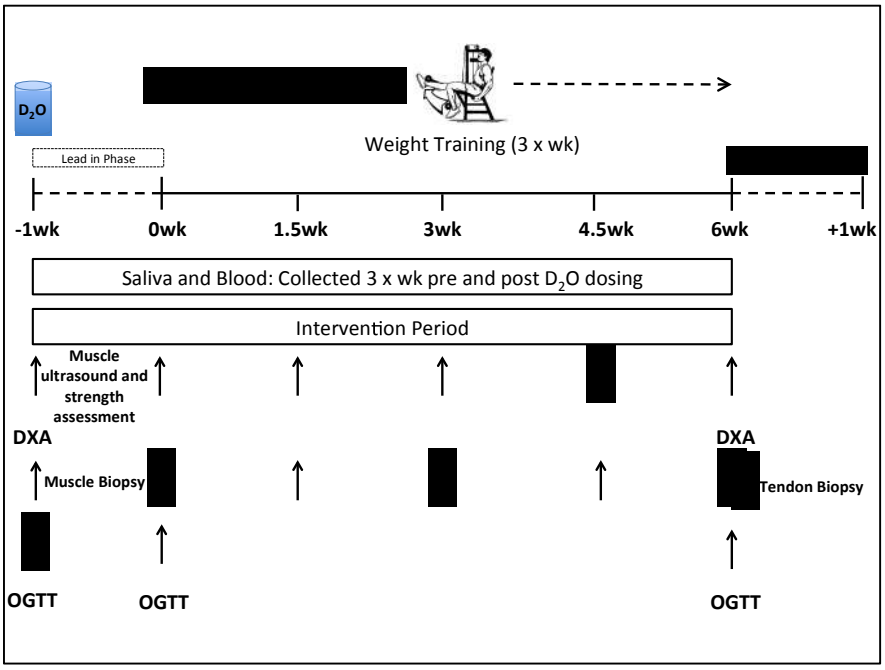
of forceps. With the local anaesthetic you may feel pushing during this procedure but no pain. A stitch is placed in the skin afterwards to aid healing. A tight bandage will be placed around the thigh after the biopsy and should remain in place for ~12h to speed healing. You will then provide a saliva sample, and be provided with a drink of 'heavy water' (~250 ml) to consume. This water contains a stable isotope tracer (deuterium in place of hydrogen, but is not radioactive), which is slightly different from normal water, but it is perfectly safe. We will ask you to provide a small sample of saliva 3-4 hours later, (which you can store refrigerated in sealed tubes we will provide). We will also ask you to collect samples of saliva daily around midday and at least an hour after food or drink for the first week. You will be provided with a small volume (~10 ml) of additional heavy water to consume daily for the remainder of the study period. This tracer is what allows us to measure rates of muscle growth and metabolism. In addition to the heavy water, we will provide you with a small drink of labelled creatine and 3-methylhistidine tracers (as with the heavy water, these are not radioactive and are perfectly safe, both creatine and 3-methylhistidine occur naturally in your muscles), we will then ask you to collect urine samples over the next 72h, before returning at 72h for a series of small blood samples. These samples will provide us with a measure of your muscle weight and how quickly your muscle is breaking down, and will be repeated at 3 and 6 weeks into the exercise period to assess for any changes related to the testing.

You will then be assigned to one of two testing groups, either: 1) a placebo group or 2) a testosterone lowering group (Zoladex: 3.6mg/4weeks). Each of these interventions will be provided via an injection just under your skin. The testosterone lowering intervention is aimed at determining the influence of testosterone levels in your blood on muscle mass gains due to exercise training.

Following an initial lead in period of one week, where you will perform no exercise, you will return to the lab 3 x wk for 6-weeks for a whole body progressive supervised resistance exercise training programme. You will be asked to perform 4 sets of 8 repetitions of both upper and lower body exercises at a weight equivalent to 80% of your 1-RM. At the start of the exercise period (designated week 0 on the attached diagram), and every 1.5 weeks up to the end of the exercise period (at 1.5, 3, 4.5 and 6 weeks as highlighted in the diagram), you will be asked to return to the lab for additional muscle biopsies. As well as using the biopsies to assess changes in metabolism due to the interventions, biopsies will also be used for cell-culture work to explore important mechanisms regulating the numerous roles of muscle.

During the intervention period, you will also have repeat ultrasound measurements, muscle function and strength tests every 1.5 weeks. An additional small patellar tendon biopsy will also be obtained at 6 weeks – this is the tendon that attaches the bottom of your kneecap (patellar) to the top of your shinbone (tibia). This biopsy will be taken using a microneedle technique, guided by ultrasound. This procedure has been conducted regularly within our labs without any reports of discomfort (average discomfort reported during and following the procedure was 2 out of 10; with 1 being no discomfort and 10 being maximal discomfort). Prior to each muscle biopsy an ultrasound scan of the muscle will be performed (with a repeat DXA scan) to monitor any changes in muscle structure due to the training regime. Following the training regime the intervention will be stopped and you will be monitored for one additional week to

ensure hormonal levels have returned to normal. Following this a final muscle



biopsy will be obtained. A diagram of the study protocol is provided below.

Why have you been chosen?

Primarily because you have expressed an interest in taking part in the study and you have met with the entry criteria and do not have on-going medical problems. We will be recruiting healthy, recreationally active males aged 18-30y.

Do you have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

What do I have to do?

You are requested to fast on the night before, and avoiding heavy exercise for 48h prior to your visits to the laboratory. We will provide you with a recommended dietary intake for the study period that we would like you to adhere to where possible. This will follow a standard balanced diet according to current recommended daily allowances for carbohydrates, fats and proteins, and will help us to limit the impact of dietary intake upon the outcomes of study.

What is the drug or procedure that is being tested?

One group of individuals will receive a drug named Zoladex, a gonadotropin releasing hormone analogue. This is a licensed drug that reduces the production of testosterone within the body. It is safe to use in healthy humans and has been previously used in a number of similar studies to assess testosterone

metabolism in humans. This will be administered by a small injection into soft tissue of the abdomen every 4 weeks. You will be provided with all necessary information regarding this drug and any potential side effects (see below) and will be continually monitored by our researchers.

What are the side effects of any treatment or procedures received when taking part?

Research studies often involve some risks, not all of which may be currently known. In the light of our experience, muscle and tendon biopsies are well tolerated by all our volunteers. Scarring is hardly perceptible and fades with time. However, the procedure may cause mild pain in some volunteers, but with appropriate local analgesia this is minimal; indeed data from a previous audit of the procedure demonstrated that 83% of our participants experienced less than or the expected discomfort during the muscle biopsy and all respondents said that they would or may be willing to participate in a similar study in the future. Some discomfort (muscle tenderness and stiffness) may be felt for 2-3 days afterwards but mild painkillers will usually effectively abolish this. There is a slight risk of infection at biopsy sites, however all procedures are performed under sterile conditions to minimize any risk of infection. Damage to small nerves of the skin during the biopsy is possible; however these nerves grow back with restoration of normal sensation, without difficulty.

We will provide you with a short, anonymized questionnaire about your experience of the biopsy procedures during this study should you wish to respond.

The risks involved in venous blood sampling are very small. Occasionally, however there may be bruising due to leakage from a blood vessel on withdrawal of the needle, but this is uncommon with good practice. The loss of 60 ml of blood has no ill effects in healthy persons and is much less than if you donate blood.

As with any pharmacological intervention there may be some side effects associated with Zoladex administration, however these are uncommon and are generally only associated with prolonged administration over periods longer than what is being performed in the current study. Those side effects which may be encountered over the proposed study period are generally mild and include; Acne, hot flushes, erectile dysfunction, altered blood sugar, mood changes and irritation at injection site (a full list of all potential side effects associated with testosterone are attached with this information sheet). If you are randomized into a Zoladex (vs. placebo) group you will be given the chance to discuss these with any of the clinicians if unsure.

What are the exclusion criteria for this study?

We are recruiting healthy men between the ages of 18 to 30 years, who are of normal weight or slightly overweight. If you have been a subject in any other research study in the last three months which involved: taking a drug, being paid a disturbance allowance or having an invasive procedure (e.g. blood sample >50ml) you would not be eligible to take part.

You would also be unsuitable if you have particular medical conditions or are taking certain medications. If you are interested in this study please discuss these further with the study doctor.

What are the possible disadvantages and risks of taking part?

Healthy volunteers have been chosen for this study; however, it is possible that the routine tests could detect unknown health problems. Should this be the case you will be informed and advised to attend your GP practice for further management. Your GP will be notified. Be advised, however, that future insurance status e.g. for life insurance or private medical insurance, could be affected by these diagnoses.

What if something goes wrong?

In the unlikely event of a complication following the study or that you should wish to voice grievances, in the first instance please contact the clinician involved in the execution of the study or the lead investigator, Dr Phil Atherton (T: 01332 724725; E: philip.atherton@nottingham.ac.uk). If you remain unhappy and wish to complain formally, you should then contact the FMHS Research Ethics Committee Administrator, c/o The University of Nottingham, School of Medicine Education Centre, B Floor, Medical School, Queen's Medical Centre Campus, Nottingham University Hospitals, Nottingham, NG7 2UH. E-mail: louise.sabir@nottingham.ac.uk.

Will my taking part in this study be kept confidential?

We respect your right to privacy and we will take measures to safeguard confidentiality. A single form, on which you are asked to sign to give consent for involvement will carry details of your name and address, but no health related details. This is kept securely in a locked cabinet within the Medical School. Access to this cabinet is restricted to personnel directly involved in the study and to University staff with direct responsibility for ensuring the study is conducted appropriately.

We will follow current ethical and legal practice and all information about you will be handled in confidence. If you join the study, some parts of the data collected about you will be looked at by authorised persons from the University of Nottingham who are organising the research. They may also be looked at by people authorised to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and we will do our best to meet this duty.

All information which is collected about you during the course of the research will be kept strictly confidential, stored in a secure and locked office, and on a password protected database. Any information about you that leaves the University will have your name and address removed (anonymised) and a unique code will be used so that you cannot be recognised from it, with the exception of a letter sent to your GP advising of your participation in this study.

Your personal data (address, telephone number) will be kept for 5 years after the end of the study so that we are able to contact you about the findings of the study and possible follow-up studies. All other data (research data) will be kept

securely for 7 years. After this time your data will be disposed of securely. During this time all precautions will be taken by all those involved to maintain your confidentiality and only direct members of the research team will have access to your personal data.

What will happen to any samples I give?

We will use the tendon and biopsy samples to see how your muscle and tendons respond to exercise and/ or the effect of age or hormones. We may also use your muscle samples to generate cell-culture samples so that we can further investigate mechanisms involved in muscle health and disease outside of the human body. We will also seek your consent for any samples remaining after analysis for this study has been completed, to be stored and used in future research. This is optional and you will be asked to give separate consent for this. The samples will be securely stored with a code unique to you at the University of Nottingham under the Universities Human Tissue Research Licence (No. 12265).

Some of these future studies may be carried out by researchers other than the current research team. This may include researchers working for commercial companies. Any samples or data used will be anonymised so that you could not be identified in any way. If you do not agree to this, any remaining samples will be disposed of in accordance with the Human Tissue Authorities code of practice.

Will any genetic tests be done?

In the current study we will collect a blood sample that can be used to obtain information about your genes (RNA and DNA), however this analysis will not give us information on disease prognosis and samples will not be subject to genetic manipulation.

Who is funding the research?

This research is funded by The University of Nottingham.

Who has reviewed the study?

This study has been reviewed and approved by the University of Nottingham Medical School Ethics Committee.

Contact for Further Information

Thank you for taking part in the study. For further information you can contact Mr Nima Gharahdaghi on 01332 724685 or at mzxng@nottingham.ac.uk, or Dr Daniel Wilkinson (Research Fellow) on 01332 724850 or at d.wilkinson@nottingham.ac.uk, both are alternatively contactable at Clinical, Metabolic and Molecular Physiology, University of Nottingham, School of Medicine, Division of Graduate Entry Medicine and Health, Royal Derby Hospital, Uttoxeter Road, Derby, DE22 3DT.

Zoladex reported Undesirable effects (taken from http://www.medicines.org.uk/emc/document.aspx?documentid=7855)

Very common (≥1/10), Common (≥1/100 to <1/10), Uncommon (≥1/1,000 to <1/100), Rare (≥1/10,000 to <1/1,000), Very rare (<1/10,000) and Not known (cannot be estimated from the available data). – These are associated with chronic administration

SOC	Frequency	Males	Females
Neoplasms benign, malignant and unspecified (including cysts and polyps)	Very rare	Pituitary tumour	Pituitary tumour
	Not known	N/A	Degeneration of uterine fibroid
Immune system disorders	Uncommon	Drug hypersensitivity	Drug hypersensitivity
	Rare	Anaphylactic reaction	Anaphylactic reaction
Endocrine disorders	Very rare	Pituitary haemorrhage	Pituitary haemorrhage
Metabolism and nutrition disorders	Common	Glucose tolerance impaireda	N/A
	Uncommon	N/A	Hypercalcaemia
Psychiatric disorders	Very common	Libido decreased	Libido decreased
	Common	Mood changes, depression	Mood changes, depression
	Very rare	Psychotic disorder	Psychotic disorder
Nervous system disorders	Common	Paraesthesia	Paraesthesia
		Spinal cord compression	N/A
		N/A	Headache
Cardiac disorders	Common	Cardiac failure, myocardial infarction	N/A
	Not known	QT prolongation (see sections 4.4 and 4.5)	QT prolongation (see sections 4.4 and 4.5)
Vascular disorders	Very common	Hot flushb	Hot flushb
	Common	Blood pressure abnormal	Blood pressure abnormal
Skin and subcutaneous tissue disorders	Very common	Hyperhidrosisb	Hyperhidrosisb, acnei
	Common	Rashd	Rashd, alopeciag
	Not Known	Alopeciah	(see Common)
Musculoskeletal, connective tissue and bone disorders	Common	Bone paine	N/A
		(see Uncommon)	Arthralgia
	Uncommon	Arthralgia	(see Common)
Renal and urinary disorders	Uncommon	Ureteric obstruction	N/A
Reproductive system and breast disorders	Very common	Erectile dysfunction	N/A
		N/A	Vulvovaginal dryness
		N/A	Breast enlargement
	Common	Gynaecomastia	N/A
	Uncommon	Breast tenderness	N/A
	Rare	N/A	Ovarian cyst
		N/A	Ovarian hyperstimulation syndrome (if concomitantly used with gonadotrophins)
	Not known	N/A	Withdrawal bleeding
General disorders and administration site conditions	Very common	(see Common)	Injection site reaction
	Common	Injection site reaction	(see Very common)
		N/A	Tumour flare, tumour pain (on initiation of treatment)
Investigations	Common	Bone density decreased, weight increased	Bone density decreased, weight increased

Appendix 5

Ethics Approval:

The University of Nottingham

Direct line/e-mail
+44 (0) 115 8232561
Louise.Sabir@nottingham.ac.uk

Faculty of Medicine and Health Sciences

Research Ethics Committee
School of Medicine Education Centre
B Floor, Medical School
Queen's Medical Centre Campus
Nottingham University Hospitals
Nottingham
NG7 2UH

8th April 2016

Dr Dan Wilkinson
Research Fellow
Clinical, Metabolic and Molecular Physiology
MRC-ARUK Centre of Excellence for Musculoskeletal Ageing Research
Division of Medical Sciences and Graduate Entry Medicine
School of Medicine
University of Nottingham
Royal Derby Hospital
DE22 3DT

Dear Dan

Ethics Reference No: G11082015 SoMS MSGEM - **please always quote.**
Study Title: The regulation of skeletal muscle protein synthesis by systemic hormones and its influence on ageing and anabolic resistance.
Chief Researcher/Academic Supervisors: Dr PJ Atherton, Associate Professor Division of Medical Sciences and Graduate Entry Medicine (MSGEM), School of Medicine,Derby.
Other key researchers: Dr DJ Wilkinson, Research Fellow, Dr K Smith Principal Research Fellow, Dr BE Phillips, Assistant Professor, Dr Iskandar Idris, Associate Professor and Consultant Diabetologist, Dr Haitham Abdulla, Clinical Research Fellow, Dr Supreeth Rudrappa, Clinical Research Fellow, MSGEM School of Medicine, Derby
Duration of Study: 01/07/15-30/06/18 36mths
No of Subjects: 40 (18-75 yrs)

Thank you for your letter dated 7th April 2016 notifying the Committee of amendment no 2: 7th April 2016 in summary as follows:

- Older 65-75 year old group change of hormone administration from Testim gel applied daily by the volunteer to the shoulders or upper arms to return to administration of intramuscular injections using a product called Sustanon 250 every 2-3 weeks (total 3 injections over 7 week study period) as there is currently no appropriate placebo for the Testim gel.

- An additional muscle biopsy at the baseline testing period to allow improved characterisation of the molecular mechanisms underlying any changes associated with the intervention. This will increase the total number of biopsies to 6 over the 7 week intervention.

And the following documents were received:

Hormones:
- Notice of Amendment no 2: dated 7th April 2016 outlining changes in detail.
- FMHS Research Ethics Application Form V2, 07/04/2016.
- Study Proposal, V2, 07/04/16
- Revised - Volunteer Information Sheet- Old Version 3. Date 07/04/16
- Revised – Volunteer Information Sheet – Young Version 3 Date 07/04/16

These have been reviewed and are satisfactory and the study amendment no 2 dated 7th April 2016 is approved.

The University of Nottingham

Approval is given on the understanding that the Conditions of Approval set out below are followed.

1. You must follow the protocol agreed and inform the Committee of any changes using a notification of amendment form (please request a form).
2. You must notify the Chair of any serious or unexpected event.
3. This study is approved for the period of active recruitment requested. The Committee also provides a further 5 year approval for any necessary work to be performed on the study which may arise in the process of publication and peer review.
4. An End of Project Progress Report is completed and returned when the study has finished (Please request a form).

Yours sincerely

pp

Professor Ravi Mahajan
Chair, Faculty of Medicine & Health Sciences Research Ethics Committee

Appendix 6

Participants' questionnaire

Questionnaire for Testosterone Study

Dear Participant,

The following questionnaire is developed to examine various parameters thought to be androgen-related. We would greatly appreciate it if you consider the questions and answer them based on the study you have taken part.

1. How would you rate your libido (sex drive)?

1(terrible) 2(poor) 3(average) 4(good) 5(excellent)

Does it mean you experienced an increase in libido (sex drive)?

YES NO

2. How would you rate your energy level?

1(terrible) 2(poor) 3(average) 4(good) 5(excellent)

Does it mean you experienced an increase in amount of energy you have?

YES NO

3. How would you rate your strength/endurance?

1(terrible) 2(poor) 3(average) 4(good) 5(excellent)

Does it mean you experienced an increase in strength and/or endurance?

YES NO

4. How would you rate your enjoyment of life?

1(terrible) 2(poor) 3(average) 4(good) 5(excellent)

Does it mean you noticed an increase in ''enjoyment of life''?

YES NO

5. How would you rate your happiness level?

1(terrible) 2(poor) 3(average) 4(good) 5(excellent)

Does it mean you noticed an increase in your **‘‘**happiness level**’’?**

YES NO

6. How strong are your erections?

(1= extremely weak ; 5= extremely strong)

1 2 3 4 5

Does it mean you experienced an increase in strength of erections?

YES NO

7. How would you rate your work performance over the past 6 weeks?

1(terrible) 2(poor) 3(average) 4(good) 5(excellent)

Does it mean there was **a recent increase in your work performance?**

YES NO

8. How often do you fall asleep after dinner?

1(never) 2(1-2/week) 3(3-4/week) 4(5-6/week)
5(every night)

Does it mean **you experienced change in time when you fall asleep after dinner? YES NO**

If yes, please clarify…

9. How would you rate your sports ability over the past 6 weeks?

1(terrible) 2(poor) 3(average) 4(good)
5(excellent)

Does it mean there was **a recent increase in your sports ability?**

YES NO

Publications, Presentations, Awards and funding:

Publication:

Nima Gharahdaghi, Supreeth Rudrappa, Matthew S. Brook, Iskandar Idris, Hannah Crossland, Claire Hamrock, Mohamed Hariz Abdul-Aziz, Fawzi Kadi, Janelle Tarum, Paul L. Greenhaff, Dumitru Constantin-Teodosiu, Jessica Cegielski, Bethan E. Phillips, Daniel J. Wilkinson, Nathaniel J. Szewczyk, Kenneth Smith & Philip J. Atherton (2019).

Testosterone therapy induces molecular programming augmenting physiological adaptations to resistance exercise in older men. **Journal of Cachexia, Sarcopenia and Muscle (2019).**

Presentations:

55th annual meeting of The European Association for the Study of Diabetes (EASD)- September 2019, Barcelona, Spain.

Physiology- July 2019, Aberdeen, Scotland.

European Conference of Sport Science- July 2019, Prague, Czech.

European Conference of Sport Science- July 2018, Dublin, Ireland.

International Conference on Frailty & Sarcopenia Research– ICFSR- March 2018, Miami, USA.

Third Midlands Academy of Medical Sciences Research Festival- March 2018, Loughborough, UK.

Muscle Physiology and Metabolism- November 2017, London, UK.

Young Life Scientist- November 2017, Derby, UK.

Posters:

55th annual meeting of The European Association for the Study of Diabetes (EASD)- September 2019, Barcelona, Spain.

Physiology- July 2019, Aberdeen, Scotland.

Muscle Physiology and Metabolism- November 2017, London, UK.

International SCWD conference on cachexia, sarcopenia and muscle wasting- December 2017, Rome, Italy

Awards:

European Conference of Sport Science- Young Investigator Award Winner- July 2019, Prague, Czech.

Best Poster prize at international SCWD conference on cachexia, sarcopenia and muscle wasting- December 2017, Rome, Italy

Funding:

Physiological Society travel grant- 2019

Physiological Society travel grant- 2018

School of Medicine Doctoral Programme Committee (DPC) Fund- 2017

PGEC Conference & Educational Fund- 2017

Vice-Chancellor's Scholarship for Research Excellence. (Full Tuition-Fee Scholarship for PhD program). Scholarship Reference: 15698- 2015-2018