

HORMONE-MEDIATED STRATEGIES TO ENHANCE TRAINING AND PERFORMANCE

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by

Christopher Martyn Beaven MSc
Primary Supervisor: Dr Nicholas Gill

“An Engineer? I had grown up amongst engineers, and could remember the engineers of the Twenties very well indeed: their open, shining intellects, their free and gentle humour, their agility and breadth of thought, the ease with which they shifted from one engineering field to another, and for that matter, from technology to social concerns and art. Then, too, they personified good manners and delicacy of taste; well-bred speech that flowed evenly and was free of uncultured words; one of them might play a musical instrument, another dabble in painting; and their faces always bore a spiritual imprint.”

Aleksandr Solzhenitsyn in ‘The Gulag Archipelago’

“It's better to burn out, than to fade away.”

Neil Young, 1979 in ‘My My, Hey Hey (Out Of The Blue)’

Clancy Brown as ‘The Kurgan’, 1986 in ‘The Highlander’

Kurt Cobain, 1994 in his final communication.

“Do not go where the path may lead; go instead where there is no path and leave a trail.”

Ralph Waldo Emerson

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N.B. The list of abbreviations and terms is located at the very back of this thesis for easy reference.

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ATTESTATION OF AUTHORSHIP

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

C. Martyn Beaven

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CO-AUTHORED WORKS

The following is a list of publications and presentations that have arisen from work reported in this thesis:

Published Work

Dose Effect of Caffeine on Testosterone and Cortisol Responses to Resistance Exercise. **C. Martyn Beaven**, Will G. Hopkins, Kier T. Hansen, Matthew R. Wood, John B. Cronin, & Timothy E. Lowe. *International Journal of Sport Nutrition and Exercise Metabolism*, Vol. 18, No. 2, April 2008, pp. 131-141.

(Beaven 75%, Hopkins 10%, Wood 5%, Lowe 5%, Hansen and Cronin 5%)

Ultradian Rhythmicity and Induced Changes in Salivary Testosterone. **C. Martyn Beaven**, John R. Ingram, Nicholas D. Gill, & Will G. Hopkins. *European Journal of Applied Physiology*. In Press, Epub ahead of press 29 May 2010.

(Beaven 85%, Gill 5%, Hopkins 5%, Ingram 5%)

Accepted for Publication

Acute Salivary Hormone Responses to Complex Exercise Bouts. **C. Martyn Beaven**, Nicholas D. Gill, John R. Ingram, & Will G. Hopkins. *Journal of Strength and Conditioning Research*. In Press, accepted 1 September 2009

(Beaven 85%, Hopkins 10%, Gill and Ingram 5%)

Manuscript in Preparation

Lower-body Strength and Power Development during Different Phases of the Circadian Rhythm. **C. Martyn Beaven**, John R. Ingram, & Nicholas D. Gill. *Manuscript in preparation*

(Beaven 90%, Gill 5%, Ingram 5%)

Conference Podium Presentations

Effects of caffeine on salivary testosterone and cortisol responses to responses to resistance exercise. **C. Martyn Beaven**, Will G. Hopkins, Kier T. Hansen, Matthew R. Wood, John B. Cronin, & Timothy E. Lowe. Presented at the *New Zealand Sports Medicine and Science Conference*, November 16-18, 2006, Wellington.

Conference Poster Presentations

Acute salivary hormone responses to complex exercise bouts. **C. Martyn Beaven**, Nicholas D. Gill, Christos K. Argus, John R. Ingram, & Will G. Hopkins. Presented at the *International Conference on Applied Strength and Conditioning*, November 20-22, 2009, Surfers Paradise. (Appendix G).

ABSTRACT

Rugby union is a highly competitive and physically demanding contact sport in which successful outcomes rely, inherently, on strength, power, speed, and endurance. Resistance training is a potent stimulus for skeletal muscle strength and power adaptation, and training stimuli and loads modulate adaptation. Adaptive responses are mediated by cellular and molecular events, and actualised by alterations in gene expression. The steroid hormones testosterone and cortisol play a role in the adaptive process and subsequent training outcomes. This thesis investigates strategies intended to modify salivary hormone responses and affect resistance training outcomes.

A literature review examined the cellular response of skeletal muscle tissue to resistance exercise with attention focussed on how testosterone and cortisol mediate adaptive outcomes. The acute testosterone and cortisol responses to complex training were investigated in Chapter Three. Sixteen rugby players performed four exercise protocols in a cross-over manner: power-power; power-strength; strength-power; or strength-strength. The most noteworthy responses were a small testosterone elevation and a trivial elevation in cortisol following the strength-power protocol, suggesting that this exercise sequence elicited an enhanced anabolic state compared to the other exercise protocols.

An interaction between hormonal circadian rhythms and exercise stimulus was investigated in Chapter Four. Eight rugby players performed identical squat training over four weeks either in the morning or in the afternoon. Clear differences were observed between pre- and post-exercise hormone concentrations, and the ratio of testosterone to cortisol was elevated in the afternoon. Training resulted in similar increases in box squat strength regardless of time performed; however peak power increased to a greater extent

when training was performed in the afternoon. This observation suggested that circadian rhythmicity has the potential to modulate specific adaptations to resistance exercise.

The effect of the ultradian pulsatility of testosterone and cortisol, and their interaction with the hormonal responses to physiological and psychological stimuli, were investigated in Chapter Five. Testosterone and cortisol concentrations of seven males were measured every 10 min between 0800 and 1600 h on three consecutive days. Analysis was consistent with episodic testosterone pulses on non-intervention days. A sprint intervention elicited a small elevation in testosterone and this response correlated with the change in testosterone concentration in the 10 min prior to exercise. Thus, the testosterone response to exercise may be related to the ultradian biorhythm. This interaction could have important implications for adaptation to exercise.

The ability of caffeine to modify the hormonal response to exercise was investigated in Chapter Six. A double-blinded, placebo controlled study with 24 athletes ingesting 0, 200, 400, or 800 mg doses of caffeine, assessed testosterone and cortisol responses to resistance exercise sessions. Exercise elevated testosterone, and caffeine enhanced this response in a dose-dependent manner. However, caffeine also produced an elevation in cortisol, and thus the anabolic effects of the testosterone increase may be counteracted by the catabolic effects of cortisol.

In conclusion, it has been demonstrated that various strategies are capable of modulating the testosterone and cortisol response to exercise stimuli, and thus have implications for subsequent adaptation and performance.

CHAPTER ONE: INTRODUCTION

Rugby is considered the national sport of New Zealand due to its widespread popularity, participation, and media profile. Indeed, rugby is so engrained in the national psychology of New Zealand that it has been suggested that poor performances by the national team (the All Blacks) are associated with negative economic repercussions. The fact that a postgraduate thesis out of the University of Otago (Walter, 2000) investigated this economic phenomenon demonstrates just how pervasive rugby union is in New Zealand culture.

The desire and necessity for successful competitive outcomes in professional sports has resulted in a concerted effort to monitor and understand the components that underpin success. In the rugby codes the size and body mass of the players are recognised as critical components of high level performance. Furthermore, strength and power performance has been shown to differentiate between athletes of different performance levels within contact sports such as American football and rugby league (Fry & Kraemer, 1991; Baker, 2002). Baker went on to emphasise that rugby league players should “strive to increase strength and power to attain NRL professional status in the future” (Baker, 2002). In rugby union, studies have demonstrated that a range of both anthropometric and physical performance variables are able to distinguish between players of differing levels (Quarrie et al., 1995). Training to maximise lean body mass should contribute to rugby performance due to the relationships with strength and power measures described in elite rugby players (Crewther, Lowe, Weatherby, Gill, & Keogh, 2009b).

The aims of resistance training (RT) are to increase strength, power, and muscle mass. As such, resistance exercises have become an integral part of training for athletes participating in a wide range of professional sports. The physiological adaptations achieved as a result of RT are actualised by changes in gene expression which are partially mediated by the endocrine response to the exercise stimulus. Resistance exercise induces a range of

physiological responses including an increase in the anabolic hormone testosterone, as well as the catabolic hormone cortisol (Kraemer & Ratamess, 2005). These steroid hormones have been identified as being involved in the remodeling of skeletal muscle tissue (Storer et al., 2003; Viru & Viru, 2004). Testosterone in particular, as the primary anabolic hormone, has been linked to strength and muscle gains in humans (Bhasin et al., 1996; Bhasin et al., 1997; Ramos, Frontera, Llopart, & Feliciano, 1998; Strawford et al., 1999; Anawalt & Merriam, 2001). Indeed, research has demonstrated the vital importance of the interaction of testosterone with androgen receptors as pharmacologic blockade of testosterone-specific receptors suppresses exercise-induced hypertrophy of skeletal muscle (Inoue, Yamasaki, Fushiki, Okada, & Sugimoto, 1994).

In terms of functional adaptation, Hansen and colleagues (2001) have described a larger relative increase in isometric strength when the anabolic hormonal response was enhanced by additional leg exercises (Hansen, Kvorning, Kjær, & Sjøgaard, 2001). Staron and co-workers (1994) have linked increases in strength and muscle fibre transformation in males to elevated serum testosterone levels (Staron et al., 1994). Testosterone administration has also been reported to positively influence muscle architecture (Blazevich & Giorgi, 2001). In 2008, significant strength gains were demonstrated when exercise protocol variables were prescribed based on individual salivary testosterone responses (Beaven, Cook, & Gill, 2008a). Attenuation of the cortisol response to resistance training via nutritional supplementation has also been associated with enhanced leg strength (Bird, Tarpenning, & Marino, 2006b). These data taken together, demonstrate a link between acute exercise-induced changes in these steroid hormone concentrations and improvements in the functional adaptations associated with resistance training.

It is the intention in this thesis to further investigate practical interventions that have the potential to maximise the functional gains of resistance exercise via hormone manipulation. The three strategies are: modulation of prescribed exercise variables;

understanding natural biorhythms; and supplementation (Figure 1). For individual athletes, it is intended that such strategies will enhance training efficiency and functional adaptation; and that this enhancement will ultimately translate into quantifiable performance gains.

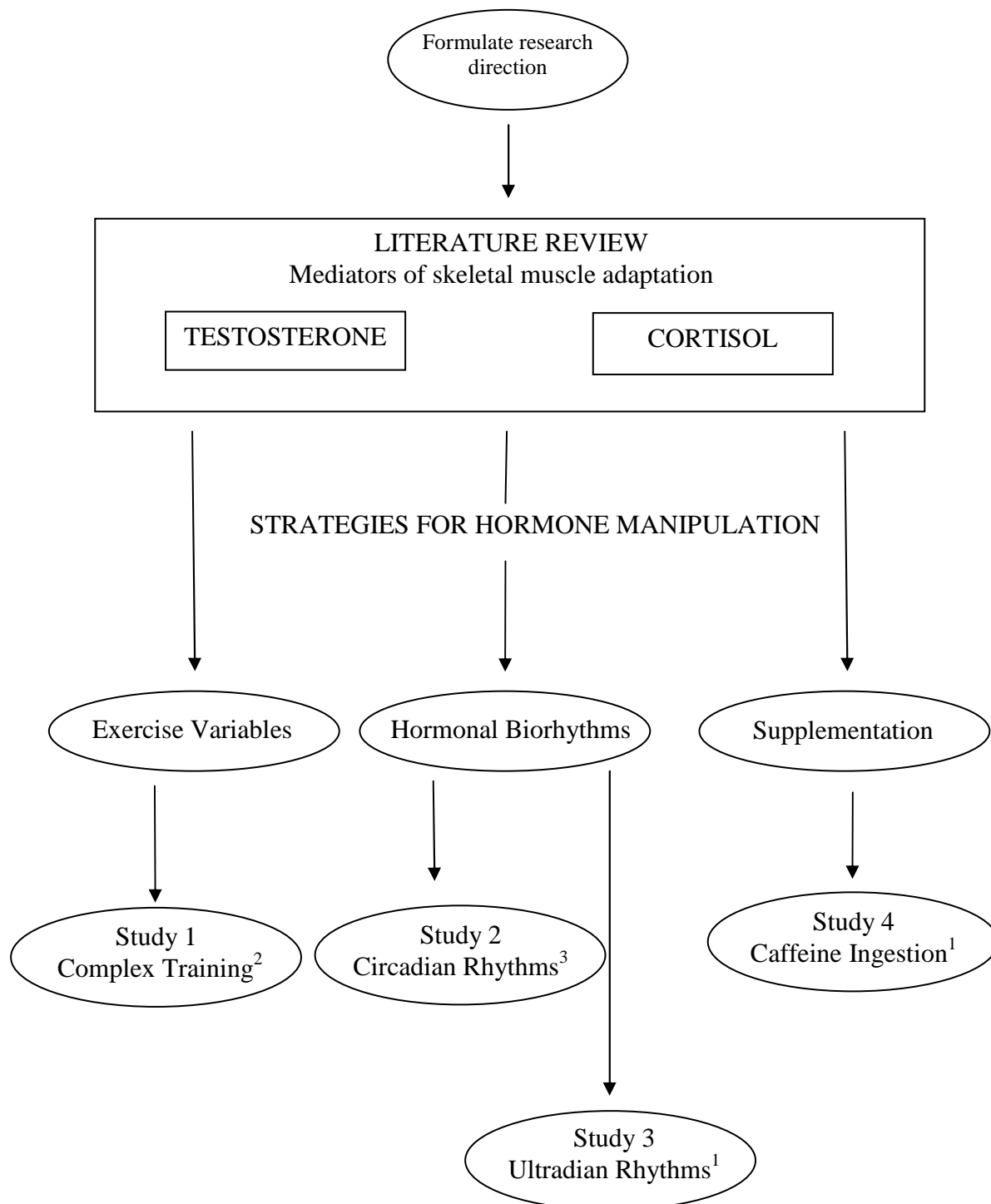


Figure 1. Overview of research project indicating strategies for modulating hormonal response to exercise.

¹ indicates papers that have been published.

² indicates papers that have been accepted for publication.

³ indicates a manuscript that is being prepared for submission.

1.0 Thesis organisation

This thesis consists of seven chapters. Chapter two is a review of the literature. It first examines the molecular response of skeletal muscle tissue to resistance exercise with attention focussed on how testosterone and cortisol may modulate adaptive outcomes. Chapters three, four, five and six are the experimental studies presented in the format of the journals for which they were written, with the exception that each is preceded by a brief explanatory prelude rather than an abstract (instead, the abstracts are included in Appendix F). Consequently, there is some repetition between the review and experimental chapters. The final chapter consists of general conclusions and practical recommendations. The appendices present relevant peripheral material including informed consent forms, ethical approval forms, and subject information sheets.

CHAPTER TWO: LITERATURE REVIEW

2.0 Purpose and Scope

The primary aim of all competitive sporting endeavours is to achieve an optimal level of performance in order to maximise successful outcomes. Preparation and effective adaptation to training are therefore crucial and consume large periods of time both prior to, and within, competitive seasons. Adaptation occurs as a result of the cellular specialisation of skeletal muscle fibres which enables remodeling of the muscle's structural makeup according to alterations in functional demand. Load-dependent and muscle fibre type specific adaptations demonstrate the enormous plasticity of human skeletal muscle and the specificity of adaptive responses.

Mechanical, metabolic, and growth factor stimulation of muscle fibres are integrated by cellular signaling pathways that are capable of modulating the effects of exercise training by influencing protein gene expression. The role steroid hormones play in skeletal muscle tissue adaptation has received much attention and the use, and abuse, of knowledge gained has been applied to enhance athletic performance. It is the intention of this review to discuss the role of the steroid hormones testosterone and cortisol in adaptation, and to highlight the importance of understanding the mechanisms that modulate gene expression to assist in enhancing athletic performance.

2.1 Skeletal Muscle Adaptation

Skeletal muscle has the capacity for significant adaptational plasticity in response to increased, and decreased, activity (Parsons et al., 2004; Bassel-Duby & Olson, 2006; Spangenburg, 2009). This plasticity is readily observable in the dramatic alterations in muscular force, velocity, and endurance in response to imposed demands. Strength training is a potent stimulus for skeletal adaptation and initiates a raft of cellular and molecular processes that are necessary and essential to the adaptive process (Favier, Benoit, & Freyssenet, 2008). The intensity, volume, and type of muscular contractions are important determinants of functional adaptation (Kraemer & Ratamess, 2005; Tidball, 2005). The mechanical, metabolic, and growth factor stimuli of physical activity are integrated by coordinated biochemical pathways. The integration of a variety of signals ultimately alters gene expression and actualises skeletal muscle modifications such as hypertrophy and shifts in muscle fibre phenotype (Hood, Irrcher, Ljubcic, & Joseph, 2006). As a result, understanding the mechanisms by which exercise stimuli mediate specific muscular adaptations is important for strength and conditioning practitioners whose aim is to maximise adaptation and performance.

Muscular hypertrophy occurs when the rate of protein synthesis exceeds the rate of proteolysis, resulting in an increase in the total contractile muscle protein content (Tipton & Wolfe, 2001; Hornberger & Esser, 2004). The first step in eukaryotic protein synthesis is transcription in which RNA polymerase produces a messenger RNA molecule using an unwound DNA sequence as a blueprint (Figure 2). This messenger RNA molecule is then transported through nuclear pores from the cell nucleus to the cytoplasm where ribosomes produce proteins in a process called translation. Protein synthesis via translation proceeds in three steps: initiation; elongation; and termination. Each step in the transcription, transportation, and translation of protein synthesis is subject to various levels of control capable of inhibiting or enhancing gene expression.

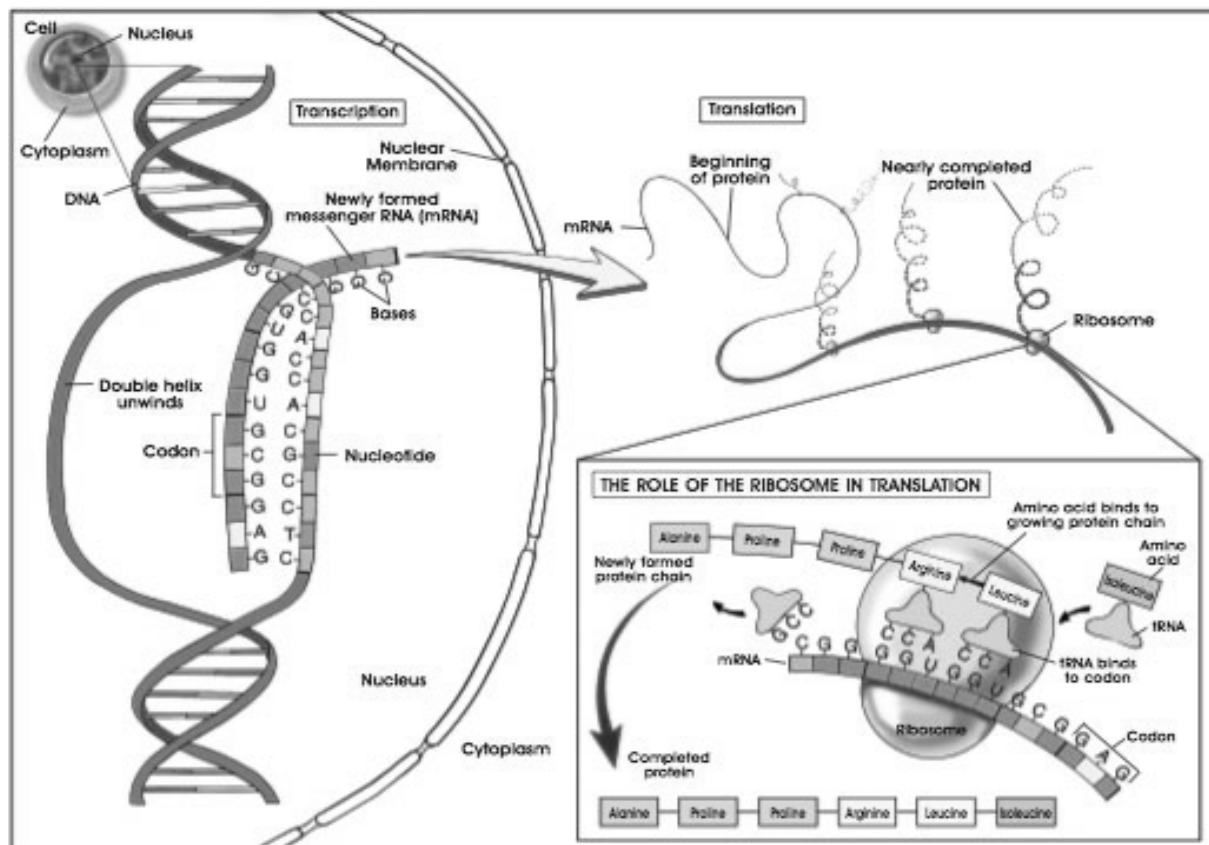


Figure 2. Gene expression.

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2.1.1 Mechanical Stimuli

The potent effects of resistance training on skeletal muscle remodeling indicate the important role of mechanical stimuli. Indeed, exercise protocols designed to maximise muscular hypertrophy are often characterised by relatively high mechanical deformation (i.e. high volume and high load) (McDonagh & Davies, 1984). Importantly, Baar and Esser (1999) demonstrated in a rat model that mechanical loading increased the number of ribosomes associated with messenger RNA and interpreted this finding as an increase in translation initiation (Baar & Esser, 1999). Indeed, mechanical loading is known to activate anabolic cellular signaling pathways (Carson & Wei, 2000; Bodine et al., 2001; Hornberger, Armstrong, Koh, Burkholder, & Esser, 2005; Tidball, 2005; Coffey & Hawley, 2007; Burkholder, 2008; Spangenburg, 2009). Furthermore, it is evident that muscle cells are capable of distinguishing between different mechanical signals and respond by activating distinct and specific signaling pathways (A. Kumar, Chaudhry, Reid, & Boriek, 2002;

Hornberger, et al., 2005). Thus, skeletal muscle can be regarded as a mechanosensitive cell type.

Specifically, mechanical loading of the muscle cell membrane has been shown to increase the phosphorylation of protein kinase B (Akt/PKB) immediately after loading (Nader & Esser, 2001) and this activation is “requisitely involved” in regulating skeletal muscle hypertrophy (Bodine, et al., 2001). Protein kinase B acts by phosphorylating a downstream target, the tuberous sclerosis complex (TSC), which relieves the inhibition on the mammalian target of rapamycin (mTOR) complex (Figure 3) (Ballou & Lin, 2008).

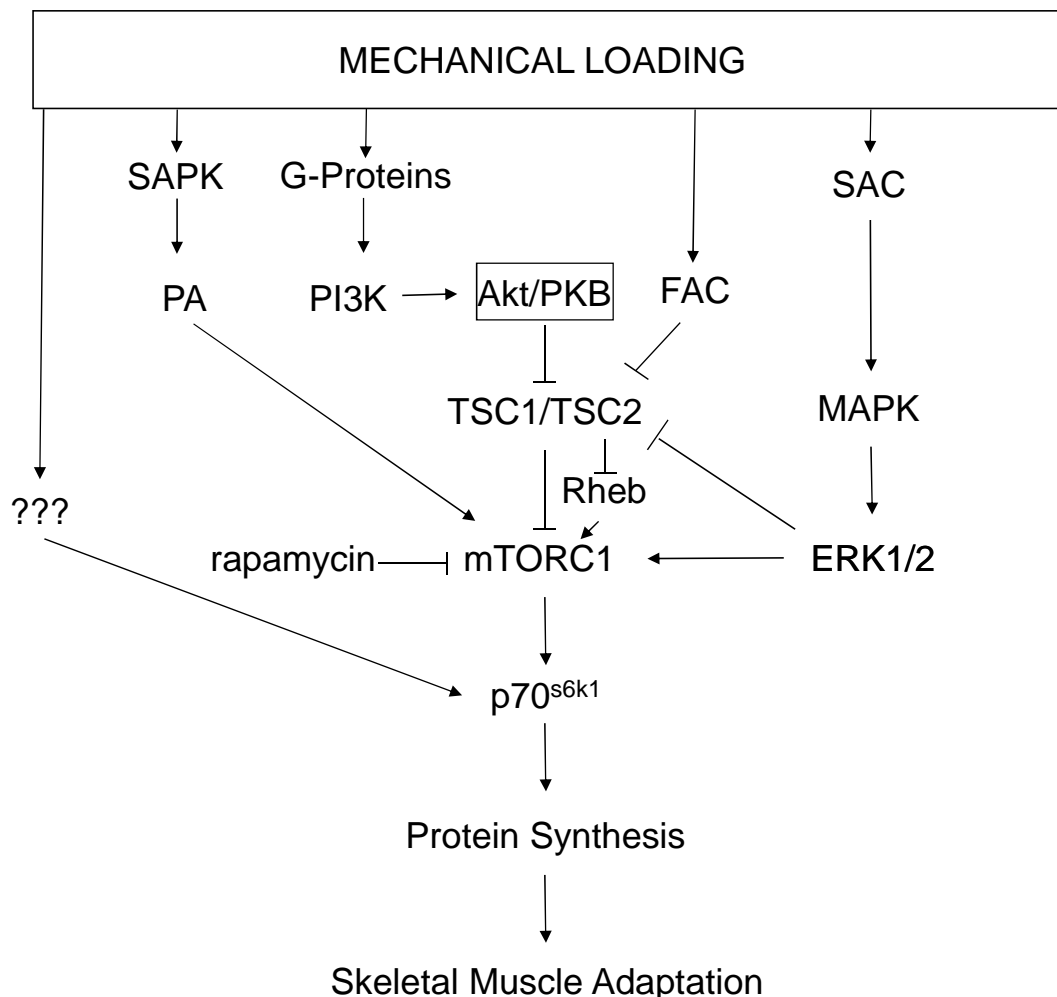


Figure 3. Mechanotransduction of protein synthesis.

SAPK = ~~stress~~-activated protein kinases; G-protein = ~~guanine~~ nucleotide-binding protein; SAC = ~~stretch~~-activated ion channels; PI3-K = ~~phosphoinositide~~ 3-kinase; Akt/PKB = ~~protein~~ kinase B; PA = ~~phosphatidic~~ acid; FAC = ~~focal~~ adhesion complexes; TSC = ~~tuberous~~ sclerosis complex; MAPK = ~~mitogen~~-activated protein kinases; ??? = undefined mechanism; mTORC1 = ~~mammalian~~ target of rapamycin complex 1; ERK = ~~extracellular signal-regulated~~ kinase; p70^{S6k} = ~~ribosomal~~ S6 kinase. Arrows represent an activating step and a blocked line represents an inhibition.

mTOR is a serine/threonine kinase known to form two distinct complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (Deldicque, Theisen, & Francaux, 2005; Proud, 2007). mTORC1 is a complex comprising mTOR, the regulatory associated protein of mTOR (raptor), and a G-protein- β -subunit-like protein; and this complex is susceptible to rapamycin inhibition. mTORC1 is regulated by amino acids and controls several components of translation initiation and ribosome biogenesis (Gulati & Thomas, 2007; MacKenzie, Hamilton, Murray, & Baar, 2007). mTORC2 is a complex comprising of mTOR and the rapamycin-insensitive companion of mTOR, and has been reported to modulate the organisation of the actin cytoskeleton (Deldicque, et al., 2005).

Activation of the mTORC1 complex results in an increase in ribosomal S6 kinase (p70^{S6k}) phosphorylation that subsequently increases protein synthesis by regulating translation (Figure 3) (Baar & Esser, 1999; Bodine, et al., 2001). Indeed, relationships have been reported between p70^{S6k} phosphorylation and increases in muscle mass and strength (Baar & Esser, 1999; Terzis et al., 2008). Interestingly, mTOR phosphorylation has been reported to be preferentially localised in type IIa fibres (Parkington, Siebert, LeBrasseur, & Fielding, 2003). This observation may contribute to the propensity of type II fibres to hypertrophy.

The precise mechanisms by which mechanotransduction of protein synthesis occur remain to be fully elucidated, however a number of researchers have reported distinct pathways. The mechanical loading of the lipid membrane, via heterotrimeric guanine nucleotide-binding protein (G-protein) subunits, has been proposed to activate the Akt/PKB pathway by signaling through a PI3-K (phosphoinositide 3-kinase) dependent pathway (Wymann & Pirola, 1998; Hornberger & Esser, 2004). Interestingly, both uni-axial and multi-axial stretch induced an increase in Akt/PKB phosphorylation but only multi-axial stretch induced p70^{S6k} phosphorylation (Figure 3) (Hornberger, et al., 2005). The

mechanotransduction associated with multi-axial stretch occurred via a novel (and undefined) direct signaling pathway that is independent of the Akt/PKB pathway, but is dependent on the integrity of the actin cytoskeleton. Indeed, pharmacological inhibition of myosin-actin interaction reduces translational activity and gene expression in cultured cells (Soeno, Shimada, & Obinata, 1999).

Focal adhesion complexes are large protein complexes that connect the cytoskeleton of a cell to the extracellular matrix (Spangenburg, 2009). These complexes have been reported to signal mechanical loading of skeletal muscle via phosphorylation of focal adhesion kinase (Gordon, Flück, & Booth, 2001) which directly inactivates the tuberous sclerosis complex (Figure 3) (Gan, Yoo, & Guan, 2006). Another Akt/PKB independent pathway activated by mechanical loading has been identified that involves the synthesis of phosphatidic acid by phospholipase D (Hornberger et al., 2006; O'Neil, Duffy, Frey, & Hornberger, 2009). Interestingly, these studies have reported that phospholipase D is able to bind with α -actinin within the z-band of skeletal muscle where mechanical load is transmitted; and that phospholipase D inhibition is sufficient to prevent stretch-induced activation of the mTOR complex.

Mitogen-activated protein (MAP) kinases have also been reported to be activated in response to mechanical loading and three parallel pathways have been described: extracellular signal-regulated kinase, protein kinase 38, and c-Jun-N-terminal kinases (JNKs) (A. Kumar, et al., 2002). Interestingly, Martineau and Gardiner (2001) reported a quantitative relationship between JNK phosphorylation and peak tension ($r^2 = 0.89$) that was independent of contraction type. However, protein kinase 38 and JNK have been referred to as stress-activated protein kinases and their activation has been reported as being insufficient to induce mTOR activation as inhibition of phospholipase D blocks stretch-induced mTOR activation but not protein kinase 38 or JNK activation (O'Neil, et al., 2009). In contrast, extracellular signal-regulated kinases can inhibit TSC, leading to upregulation of the mTOR pathway

(Figure 3) (Ma, Chen, Erdjument-Bromage, Tempst, & Pandolfi, 2005; Sarbassov, Ali, & Sabatini, 2005). Furthermore, extracellular signal-regulated kinases are important for mTOR activation through phosphorylation of the raptor molecule (Carrière et al., 2008).

The activation of MAP kinases has been reported to be mediated by stretch-activated ion channels (McBride, 2003). Further work has reported a link between the function of these ion channels and activation of the mTOR pathway (Spangenburg & McBride, 2005). Indeed, in an animal model, it has been demonstrated that pharmacological blockade of stretch-activated ion channels “completely abrogated” beneficial adaptations associated with eccentric exercise and attenuated muscle mass gains (Butterfield & Best, 2009).

Interestingly, testosterone has been shown to activate the mTOR pathway through phosphorylation of extracellular signal-regulated kinases (Figure 3) (Altamirano et al., 2009). These authors established that the mTOR activation by testosterone in cardiomyocytes was independent of androgen receptors. Furthermore, they were able to demonstrate that Ca^{2+} release from intracellular stores was “necessary” for the activation of the extracellular signal-regulated kinases and subsequent cardiomyocyte hypertrophy. It is worth noting that researchers have previously demonstrated that testosterone increases intracellular Ca^{2+} release and the phosphorylation of extracellular signal-regulated kinases in skeletal muscle cells (Estrada, Espinosa, Muller, & Jaimovich, 2003) suggesting a conserved mechanism for cellular signaling.

It is apparent that skeletal muscles are mechanosensitive cells capable of distinguishing between distinct deformation types. A number of mechanisms have been identified as important in the mechanotransduction, some of which are independent of the PI3K-Akt/PKB, and even mTOR molecular pathways (Figure 3). Indeed, stressors are incapable of replicating physiological responses when actin-myosin interactions are inhibited (Soeno, et al., 1999). Thus, the importance of an intact and functional actin cytoskeleton has been demonstrated for mechanotransduction of transcriptional activity, gene expression, and

ultimately muscular adaptation. The steroid hormone testosterone is notable for its ability to activate mTORC1 and thus influence adaptive outcomes.

2.1.2 Metabolic Stimuli

The stimuli experienced by skeletal muscle during the repeated contractions associated with resistance exercise are clearly not limited to mechanical loading and deformation. Muscular contractions will, amongst other things: decrease the ATP/AMP ratio, cause localised hypoxia, and induce a rapid inflammatory response which results in prostaglandin release. The metabolic effects of muscular contraction must be integrated and interpreted into molecular signals that mediate cellular processes associated with adaptation.

Protein synthesis is an energy intensive process (Browne & Proud, 2002; Fujita et al., 2007b) and as such, it makes sense from a biological perspective to inhibit protein synthesis during periods when energy levels are depleted. Activation of adenosine monophosphate-activated protein kinase (AMPK) in response to a decrease in the ATP/AMP ratio is known to suppress protein synthesis via TSC phosphorylation and inhibition of the mTOR pathway (Figure 4) (Ballou & Lin, 2008). This activation may explain the lack of muscle hypertrophy seen as a result of endurance-type training, as mTOR function may be inhibited in response to reduced energy stores (Bolster, Crozier, Kimball, & Jefferson, 2002; Hardie & Sakamoto, 2006).

Interestingly, administration of 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), an AMPK agonist, is known to inhibit mTOR (Brugarolas et al., 2004) and increase skeletal muscle glucose transport (Bassel-Duby & Olson, 2006). Furthermore, four weeks of AICAR treatment acted as an exercise mimetic and markedly enhanced endurance performance in rats (44% increase in distance, 23% increase in duration), even when no endurance exercise was performed (Narkar et al., 2008). Assuming that AMPK agonists like AICAR are efficacious in humans, the implications for human health could be tremendous.

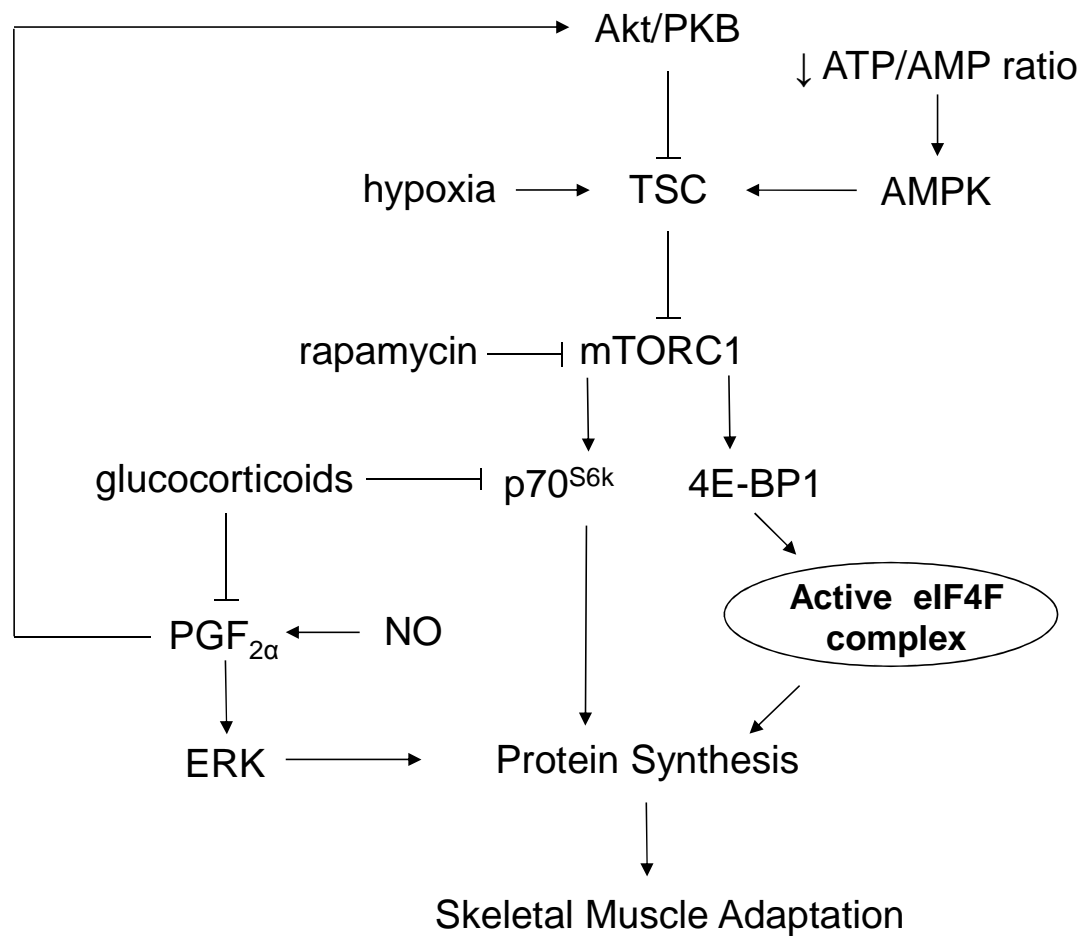


Figure 4. Metabolic factors associated with protein synthesis.

Akt/PKB=protein kinase B; TSC=tuberous sclerosis complex; ATP=adenosine triphosphate; AMP=adenosine monophosphate; AMPK=adenosine monophosphate-activated protein kinase; mTORC1=mammalian target of rapamycin complex 1; p70^{S6k}=ribosomal S6 kinase; 4E-BP1=eukaryotic initiation factor 4E binding protein-1; eIF4F=eukaryotic initiation factor 4F; PGF_{2α}=prostaglandin F_{2α}; NO=Nitric oxide; ERK=extracellular signal-regulated kinases. Arrows represent an activating step and a blocked line represents an inhibition.

Hypoxia associated with repeated contractions has been suggested as an important signal in modulating muscular adaptation (Hoppeler & Vogt, 2001; Flück, 2006). In 2003, it was demonstrated that hypoxia inhibits two of the downstream targets of mTOR: p70^{S6k}; and eukaryotic initiation factor 4E binding protein-1 (4E-BP1) (Arsham, Howell, & Simon, 2003). 4E-BP1 normally sequesters the eukaryotic initiation factor 4E (eIF4E), but hyperphosphorylation of 4E-BP1 by mTOR releases this inhibition of eIF4E and allows it to interact with the eukaryotic initiation factor 4F which initiates translation via an activated eIF4F complex (Figure 4) (Spiering et al., 2008a; Spangenburg, 2009). It has been reported

that mTOR inhibition under hypoxic conditions is dependent on an intact tuberous sclerosis complex and expression of the hypoxia-inducible gene, REDD1 (Brugarolas, et al., 2004).

Interestingly though, training that artificially restricts blood flow (also known as Kaatsu training) has been shown to cause dramatic hypertrophic gains in untrained (Abe et al., 2005b; Abe, Kearns, & Sato, 2006) and trained (Abe et al., 2005a) subjects. The occlusion associated with this novel type of training has been shown to cause localised hypoxia (Kawada, 2005). Such restriction of blood flow has been associated with marked increases in the acute GH response to exercise when compared to a control group (Abe, et al., 2006; Reeves et al., 2006; Fujita et al., 2007a). Increases in basal levels of circulating IGF-1 resulting from two weeks of Kaatsu training have also been reported (Abe, et al., 2005b). Furthermore, blood restriction combined with low-intensity resistance exercise has been shown to increase the phosphorylation of downstream targets of mTOR, promote translation elongation via eukaryotic elongation factor 2, and increase muscle protein synthesis as measured by labeled amino acid incorporation (Fujita, et al., 2007a). These data, taken together, indicate that the increase in growth factor release associated with blood flow restriction is capable of overcoming mTOR inhibition due to hypoxia.

The enzyme cyclooxygenase 2 (COX-2) is known to catalyse the production of prostaglandins such as prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from arachidonic acid (Veliça & Bunce, 2008). The importance of COX-2 pathway for hypertrophy resulting from functional overload has been demonstrated (Soltow et al., 2006). Blocking $PGF_{2\alpha}$ with non-steroidal anti-inflammatory drugs has been reported to blunt protein synthesis after exercise (T. Trappe, Fluckey, White, Lambert, & Evans, 2001; T. A. Trappe et al., 2002). As such, $PGF_{2\alpha}$ has been reported to be an important contributor to post-exercise protein synthesis (T. Trappe, Raue, Williams, Carrithers, & Hickner, 2006; Weinheimer et al., 2007; Veliça & Bunce, 2008). Indeed, $PGF_{2\alpha}$ has been reported to activate $p70^{S6k}$ and induce protein synthesis via both PI3K-Akt/PKB, and extracellular signal-related kinase pathways (Figure

4) (Rao et al., 1999). Interestingly, expression of COX-2 is induced by nitric oxide and this induction is suppressed by glucocorticoids (Honda et al., 2000).

Glucocorticoids are released in response to intense exercise and function to suppress protein synthesis (Virus & Virus, 2004). Indeed, in skeletal muscle cells, glucocorticoids inhibit protein synthetic rates by up to 30% (Shah, Kimball, & Jefferson, 2000c). Further work from this laboratory demonstrated that the negative effects of glucocorticoids on protein expression occur via p70^{S6k} and 4E-BP-1, and independently of the PI3-K pathway (Figure 4) (Shah, Kimball, & Jefferson, 2000d). Glucocorticoid attenuation of growth factor-induced protein translation occurs within two hours of administration and requires a functional glucocorticoid receptor (Shah, et al., 2000d; Shah, Iniguez-Lluhi, Romanelli, Kimball, & Jefferson, 2002). Indeed, glucocorticoids can exhibit both long and short term effects to control protein synthesis via attenuation of translation initiation (short term), and reduction in translational capacity (long term) (Shah, et al., 2002).

Interestingly, suppression of the glucocorticoid response to exercise via nutritional intervention can influence the adaptive process and markedly enhance functional gains (Tarpenning, Wiswell, Hawkins, & Marcell, 2001; Bird, et al., 2006b; Bird, Tarpenning, & Marino, 2006a). Specifically, when weight training was combined with a 6% carbohydrate supplement that effectively blunted acute cortisol response, greater gains ($p < 0.05$) were apparent in both type I (19.1%) and type II (22.5%) muscle fibre area over a 12-wk period compared to a control group (Tarpenning, et al., 2001). These researchers went on to show that a supplement that combined essential amino acids with carbohydrates which also effectively suppressed acute cortisol response, produced even greater gains in muscle cross-sectional area and strength over a 12-wk period than carbohydrate supplementation alone (Bird, et al., 2006b).

Thus, it is apparent that the metabolic effects of exercise are integrated by aspects of the mTOR signaling pathway. Decreases in the ATP/AMP ratio and muscle oxygenation,

and/or increases in glucocorticoids levels, are interpreted as signs of increased energy expenditure. This energy deficit results in the suppression of energy intensive processes, such as protein synthesis. It is apparent that glucocorticoids play an important role in mediating the cellular processes associated with protein synthesis and adaptive outcomes.

2.1.3 Growth Factor Stimuli

The cascade of events that lead to protein synthesis are reliably induced by growth factors such as insulin and insulin-like growth factor 1 (IGF-1) (Hayashi & Proud, 2007; G. R. Adams, 2010; Frystyk, 2010). The release of these factors triggers the Akt/PKB signaling pathway (Hayashi & Proud, 2007; Spiering, et al., 2008a; Wilkinson et al., 2008). The stimulation of the Akt/PKB pathway occurs via PI3K which recruits Akt/PKB to the plasma membrane where it is activated by phosphorylation (Brugarolas, et al., 2004).

As mentioned previously, activation of Akt/PKB leads to the phosphorylation of mTOR which in turn phosphorylates two primary targets: p70^{S6k} and 4E-BP1. Hyperphosphorylation of 4E-BP1 ultimately leads to an enhancement in translational efficiency, protein synthesis, and skeletal muscle adaptation via the formation of an active eIF4F complex (Shah, Anthony, Kimball, & Jefferson, 2000a; Spiering et al., 2008b). Akt/PKB activation also deactivates glycogen synthase kinase 3 (GSK-3), which subsequently enhances protein translation via the eukaryotic elongation factor 2 (Figure 5) (Glass, 2003; Blomstrand, Eliasson, Karlsson, & Köhnke, 2006).

IGF-1 has also been reported to induce an increase in intracellular Ca²⁺ which is associated with activation of the mTOR pathway via extracellular signal-regulated kinases (Espinosa, Estrada, & Jaimovich, 2004). Surprisingly though, it has been reported that a functional IGF-1 receptor is not necessary for overload-induced phosphorylation of p70^{S6k} and muscle growth in mice (Spangenburg, Le Roith, Ward, & Bodine, 2008). Indeed, the short-term effects of human growth hormone, insulin, and IGF-1 have been reported to be “unnecessary” for the realisation of anabolic effects (Rennie, 2007). Insulin does however

appear to play an important role in inhibiting muscle protein breakdown post-exercise (V. Kumar, Atherton, Smith, & Rennie, 2009).

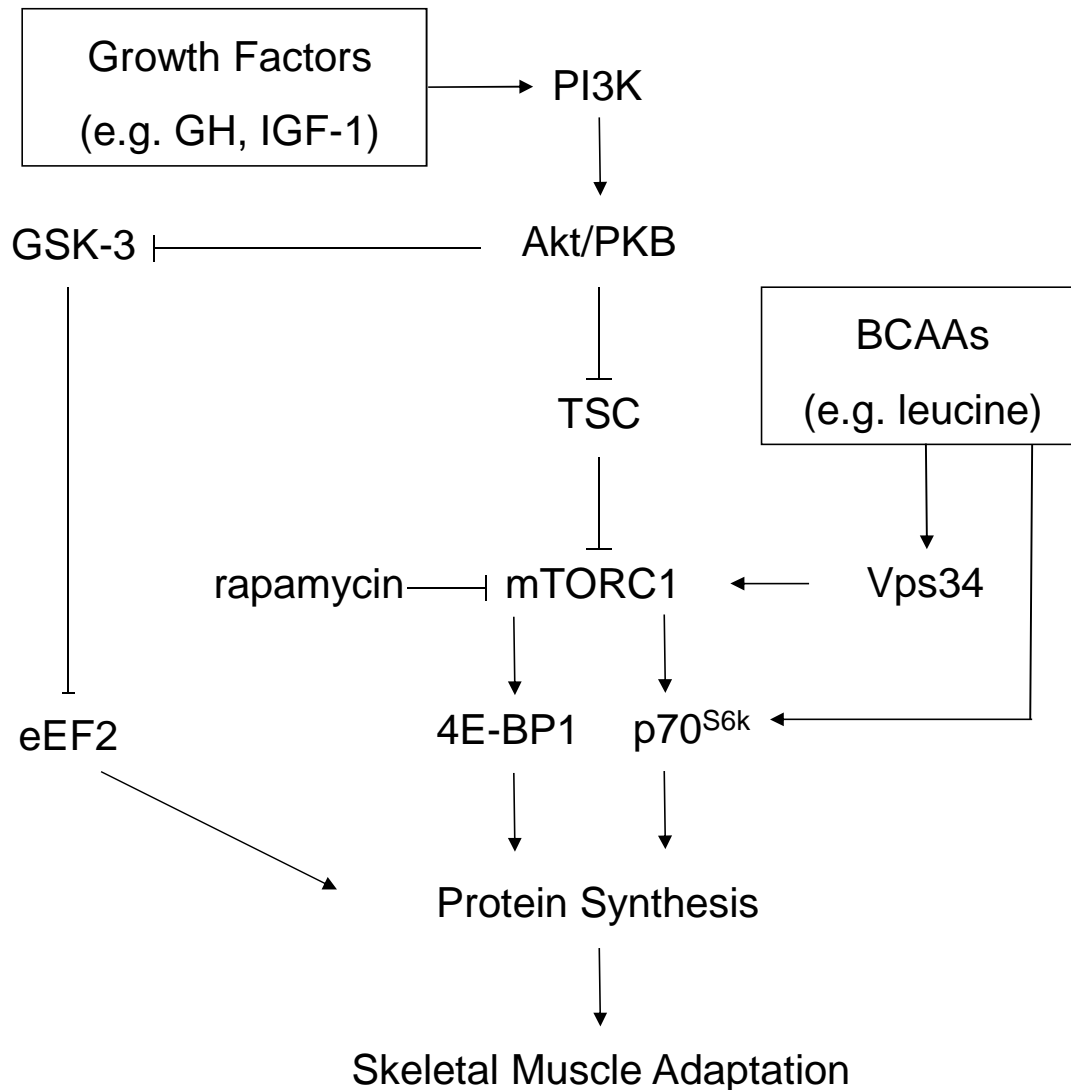


Figure 5. Growth factor and amino acid stimulation of protein synthesis.

GH=human growth hormone; IGF-1=insulin-like growth factor 1; PI3-K=phosphoinositide 3-kinase; Akt/PKB=protein kinase B; GSK-3=glycogen synthase kinase 3; TSC=tuberous sclerosis complex; BCAAs=branched-chain amino acids; Vps34=vacuolar protein sorting mutant 34; mTORC1=mammalian target of rapamycin complex 1; p70^{S6k}=ribosomal S6 kinase; 4E-BP1=eukaryotic initiation factor 4E binding protein-1; eEF2=eukaryotic elongation factor 2. Arrows represent an activating step and a blocked line represents an inhibition.

Interestingly, oral leucine administration promotes p70^{S6k} phosphorylation (Anthony et al., 2000; Deldicque, et al., 2005). The major pathway by which amino acids regulate mTOR downstream signaling is distinct from the insulin-initiated PI3K pathway and is not dependent on TSC inhibition (Figure 5) (Nobukuni et al., 2005). These authors have

demonstrated that the effect of amino acids on mTORC1 complex is mediated by the vacuolar protein sorting mutant 34 (Vps34). Importantly, mammalian Vps34 is increased following resistance exercise and may act as an internal amino acid sensor to mTOR (MacKenzie, et al., 2007).

However, the phosphorylation of downstream targets of mTORC1 resulting from leucine ingestion is only partially inhibited by rapamycin suggesting the contribution of an mTOR-independent pathway (Deldicque, et al., 2005). In human studies, branched-chain amino acid ingestion, in combination with resistance exercise, has a “striking” effect on p70^{S6k} phosphorylation (Karlsson et al., 2004) but not GSK-3 (Blomstrand, et al., 2006). Furthermore, the ability of insulin to initiate protein gene expression is inhibited in human skeletal muscle when amino acids are restricted (J. A. Bell, Fujita, Cadenas, & Rasmussen, 2005), while protein synthesis is enhanced when amino acids are co-administered with glucose (Rasmussen & Phillips, 2003; Bird, et al., 2006b).

In summary, growth factors are capable of activating the Akt/PKB pathway and initiate protein gene expression. An important role of amino acids in actualising the effects of resistance exercise via translation initiation and elongation is also evident. Indeed, Deldicque and colleagues (2005) concluded that amino acid ingestion was “necessary to fully activate protein muscle synthesis signaling in muscle” (Deldicque, et al., 2005). Thus, the signaling pathways for growth factors and amino acids are distinct and complementary.

2.2 Other Adaptive Mechanisms

It should be noted that the aforementioned mechanical, metabolic, and growth factor responses are by no means the only physiological mechanisms for strength, power, and muscular development. For example, human growth hormone (Kraemer et al., 1990; Kraemer et al., 1991; Goto, Sato, & Takamatsu, 2003; Smilios, Pilianidis, Karamouzis, & Tokmakidis, 2003; Goto, Ishii, & Takamatsu, 2004a), and other hormones such as prolactin (Bosco, Colli, Bonomi, Von Duvillard, & Viru, 2000) and Vitamin D (Buitrago, Vazquez, De Boland, & Boland, 2001; Montero-Odasso & Duque, 2005; Cannell, Hollis, Sorenson, Taft, & Anderson, 2009) have been studied in order to understand the mechanisms that control muscular adaptation. The inflammatory response of cytokines such as leukemia inhibitory factor and prostaglandins are also vital for muscle regeneration and adaptation (Cantini et al., 2002; Shen, Li, Zhu, Schwendener, & Huard, 2008; Veliça & Bunce, 2008; Spangenburg, 2009). β_2 adrenergic receptor agonists are also capable of eliciting muscular hypertrophy and changes in muscle fibre phenotype (Chang, 2007). The protein myostatin is known to play an important role as an inhibitor of muscular hypertrophy (Roth & Walsh, 2004; Walker, Kambadur, Sharma, & Smith, 2004; Favier, et al., 2008). The transcription factor forkhead box O (FOXO) and proteolytic muscle-specific ubiquitin ligases also play key roles in regulating muscular atrophy (Bassel-Duby & Olson, 2006; Chang, 2007; Coffey & Hawley, 2007; Urso, 2009). Therefore, it is apparent that the remodeling of skeletal muscle tissue is modulated by a plethora of interactions mediated by chemical milieu, gene interactions, and other myogenic regulatory factors. The exact mechanisms are complex and assimilate a range of biological signals which remain to be fully elucidated.

2.3 Integrated Molecular Signaling Model

As a result of this review of the mechanical, metabolic, and growth factor responses that are initiated by resistance exercise, it is evident that steroid hormones play an important role in modulating muscular adaptation to resistance exercise (Figure 6). Downstream effects of testosterone include the activation of extracellular-regulated kinases and phosphorylation of mTOR to induce hypertrophy (Altamirano, et al., 2009). It has also been demonstrated that glucocorticoids can mediate the effects of resistance exercise by inhibiting $p70^{S6k}$ (Shah, et al., 2000d) and upstream targets of extracellular-regulated kinases (Honda, et al., 2000).

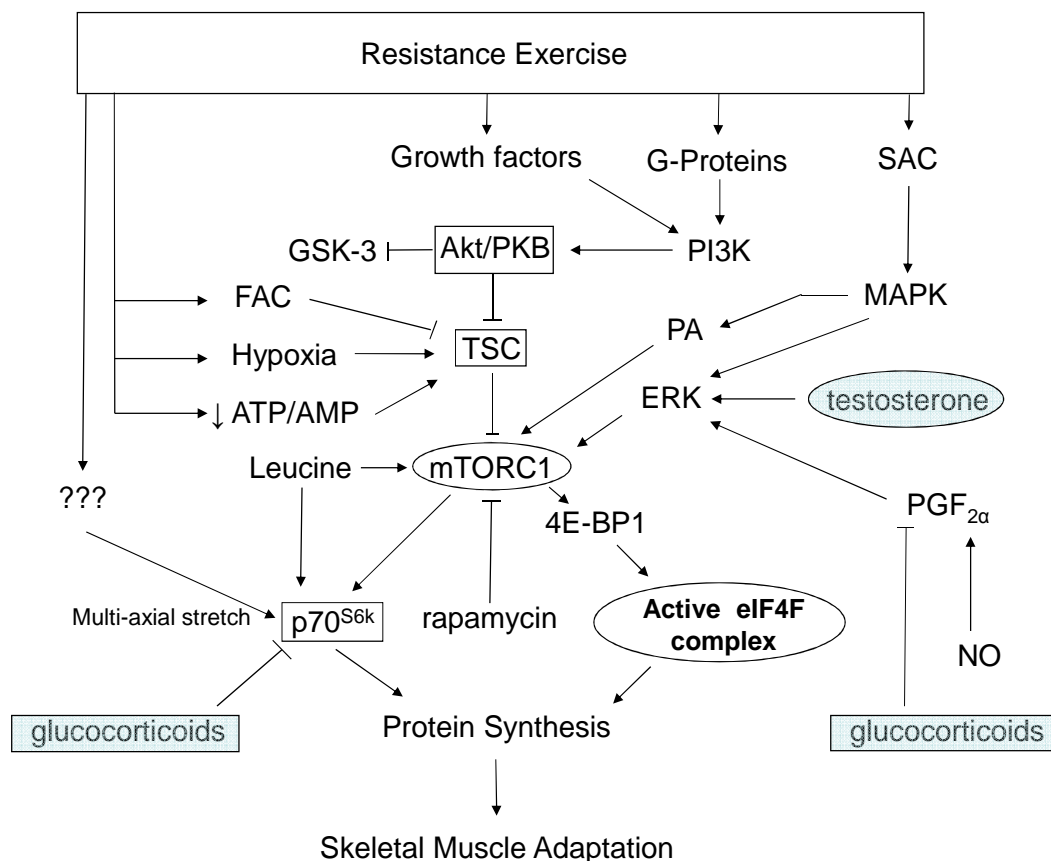


Figure 6. Integrated model of hormonal interaction with molecular signaling pathways associated with skeletal muscle adaptation in response to resistance exercise.

G-proteins=guanine nucleotide-binding proteins; SAC=stretch-activated ion channels; GSK-3=glycogen synthase kinase 3; Akt/PKB=protein kinase B; PI3-K=phosphoinositide 3-kinase; FAC=focal adhesion complexes; MAPK=mitogen-activated protein kinases; TSC=tuberous sclerosis complex; PA=phosphatidic acid; ATP=adenosine triphosphate; AMP=adenosine monophosphate; ERK=extracellular signal-regulated kinase; ??? = undefined mechanism; mTORC1=mammalian target of rapamycin complex 1; 4E-BP1=eukaryotic initiation factor 4E binding protein-1; eIF4F=eukaryotic initiation factor 4F; $PGF_{2\alpha}$ =prostaglandin $F_{2\alpha}$; $p70^{S6k}$ =ribosomal S6 kinase; NO=nitric oxide. Arrows represent an activating step and a blocked line represents an inhibition.

2.4 Steroid Hormones

Steroid hormones are relatively low-molecular weight lipophilic compounds that originate from cholesterol via a common series of biosynthetic pathways (Figure 7) (Miller, 1988). The steroid hormone end products are determined by the cell type and a complement of biosynthetic enzymes. These hormones are released into venous circulation as soon as they are synthesised by endocrine glands such as the gonads and the adrenal cortex, and are capable of acting on target tissues such as skeletal muscle and the central nervous system. Steroid hormones are generally divided into groups based upon their biological actions; mineralocorticoids, which regulate the salt balance and maintain blood pressure; glucocorticoids, which regulate carbohydrate metabolism and stress responses; estrogens, which are primarily produced by the ovaries and regulate reproductive function and the secondary sex characteristics of the female; and the androgens, which are mainly synthesised in the testes (Heikkilä, 2002).

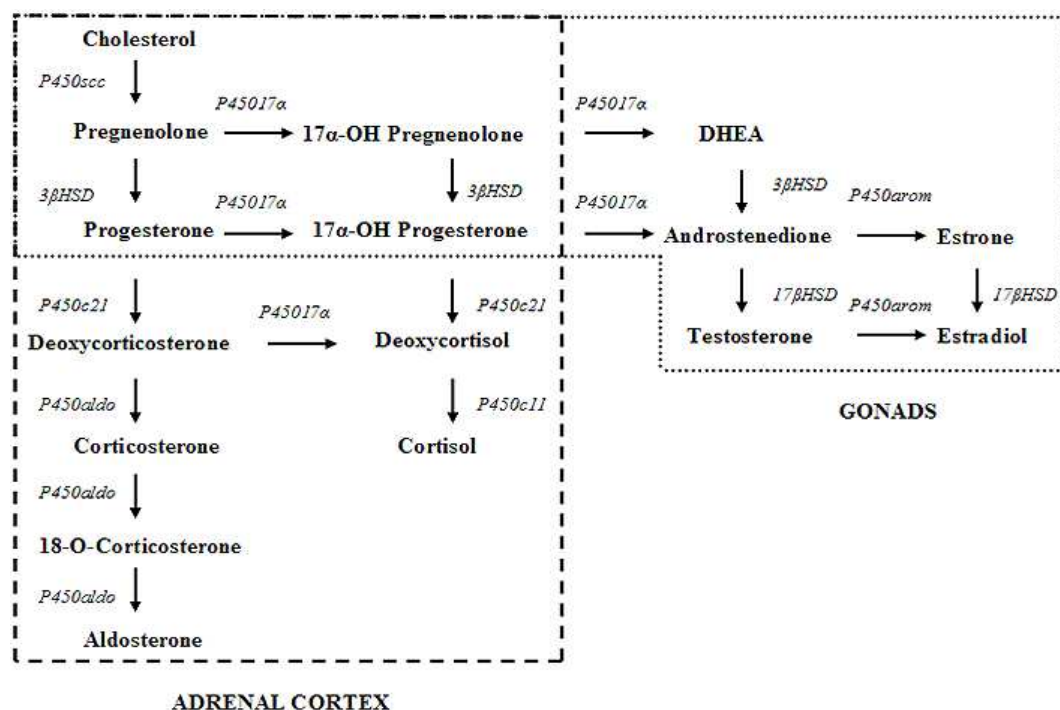


Figure 7. Steroid hormone synthesis pathways.

DHEA=Dehydroepiandrosterone; P450_{17 α} =17- α -hydroxylase; 3 β HSD=3- β -hydroxysteroid dehydrogenase; 17 β HSD=17- β -hydroxysteroid dehydrogenase

The androgen testosterone and the glucocorticoid cortisol are steroid hormones that play a key role in regulating protein metabolism and muscle accretion, and as such are considered crucial to the adaptive responses to resistance exercise. The lipophilic properties of testosterone and cortisol mean that the majority of these hormones are bound to carrier proteins in blood. This binding is both 'specific' and 'non-specific'. Low affinity steroid binding to albumin is unspecific and accounts for ~50% of the bound steroid, whereas specific plasma proteins, more commonly referred to as binding globulins, account for ~40% of the bound fraction. Sex hormone-binding globulin (SHBG) and cortisol-binding globulin (CBG) are the specific carriers for testosterone and cortisol respectively. The remaining unbound steroid circulates freely and is referred to as the free steroid concentration (Bagatell & Bremner, 1996; Marshall-Gradisnik, Green, Brenu, & Weatherby, 2009).

Only the unbound steroid is able to diffuse through the cell membrane to initiate the classical (genomic) hormone actions, and is thus termed biologically active (Rosner, 1991; Excoffon, Guillaume, Woronoff-Lemsi, & André, 2009). Genomic actions are so called as they involve the steroid molecule entering the cell, associating with a specific receptor, and influencing protein transcription and translation via interaction with the genome (Lösel et al., 2003). These actions are characterised by a relatively long time frame and their sensitivity to inhibitors of transcription. It is noteworthy that both testosterone and cortisol are capable of exerting physiological effects via both genomic and non-genomic effects. Non-genomic effects occur independently of classical steroid-androgen receptor interactions and these actions are often rapid (Zaki & Barrett-Jolley, 2002; Estrada, et al., 2003; Lösel, et al., 2003). Steroid that is weakly bound to albumin is able to rapidly dissociate in order to increase the pool of free steroid depending on requirements. Indeed, research by Obminski (1998) has demonstrated that the unbound fraction of testosterone in human serum is synergistically increased by hyperthermia and acidosis, conditions common to intensive exercise (Obminski,

1998). The combined concentration of albumin-bound and free steroid is often termed the 'bioavailable' steroid concentration.

Circulating concentrations of testosterone are characterised by a circadian rhythm, with the highest levels observed in the morning and a nadir in the late afternoon (Lévi et al., 1988; Cooke, McIntosh, & McIntosh, 1993; Kraemer et al., 2001; Diver, Imtiaz, Ahmad, Vora, & Fraser, 2003). Kraemer (1988) has suggested that the elevated morning testosterone concentration may serve an anti-catabolic role helping to protect the muscle against the proteolytic effect of cortisol on skeletal protein (Kraemer, 1988). Circulating concentrations of cortisol are also characterised by a diurnal rhythm, with highest levels in the morning and a nadir in the late afternoon (Lévi, et al., 1988; Iranmanesh, Veldhuis, Johnson, & Lizarralde, 1990; Thuma, Gilders, Verdun, & Loucks, 1995; Dimitiou, Sharp, & Doherty, 2002; Spiering, et al., 2008a).

2.5 Testosterone

Testosterone (17-beta-hydroxyandrost-4-ene-3-one) is the primary androgenic steroid hormone and the most abundant naturally occurring androgen (Figure 8). Testosterone is responsible for normal growth and development of male sex organs, protein metabolism, and maintenance of secondary sex characteristics (Bhasin et al., 2001a). Some examples of these characteristics are growth of body hair, beard growth, deep voice, aggressiveness, and increased libido (Martini & Welch, 2001).

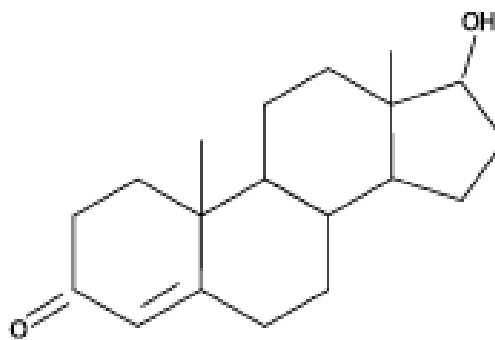


Figure 8. Structure of testosterone: 17-beta-hydroxyandrost-4-ene-3-one.

2.5.1 Regulation

The secretion of testosterone is regulated by the Hypothalamic-Pituitary-Gonadal (HPG) axis (Figure 9). The hypothalamus integrates peripheral and central signals and acts to control blood testosterone concentration via secretion of gonadotropin-releasing hormone (GnRH), also known as luteinizing hormone-releasing hormone (Martini, 2001). Hypothalamic GnRH containing neurons project to the median eminence and release GnRH pulses into the hypophysial portal blood (Jackson, Kuehl, & Rhim, 1991). GnRH stimulates the secretion of luteinizing hormone (LH) from the gonadotroph cells of the anterior pituitary into the blood in a pulsatile manner (Keenan et al., 2006; M. L. Johnson et al., 2008). Luteinizing hormone in turn, stimulates the secretion of testosterone from the Leydig cells located in the interstitium of the testes (Martini & Welch, 2001; Stocco, 2001). The secretion of testosterone is also known to be pulsatile with a tight coupling observed between LH and

testosterone secretory events in young men (Veldhuis et al., 1987b; Pincus et al., 1996; J. Veldhuis, Keenan, Liu, & Iranmanesh, 2009). In the blood, up to 98% of secreted testosterone is bound in complexes with sex hormone binding globulin or albumin (Loebel & Kraemer, 1998; Hayes, 2000). Elevated blood levels of testosterone inhibit subsequent HPG activity via multiple negative feedback mechanisms. These include a direct inhibition of GnRH secretion from hypothalamic neurosecretory cells (Baechle, 1994; Viru & Viru, 2001) and the direct inhibition of LH production from the anterior lobe of the pituitary gland (Figure 9) (Viru & Viru, 2001). Glucocorticoids are also capable of exerting negative feedback on the HPG axis by suppressing the pulsatile release of GnRH and LH (Waite, Spiga, & Lightman, 2009).

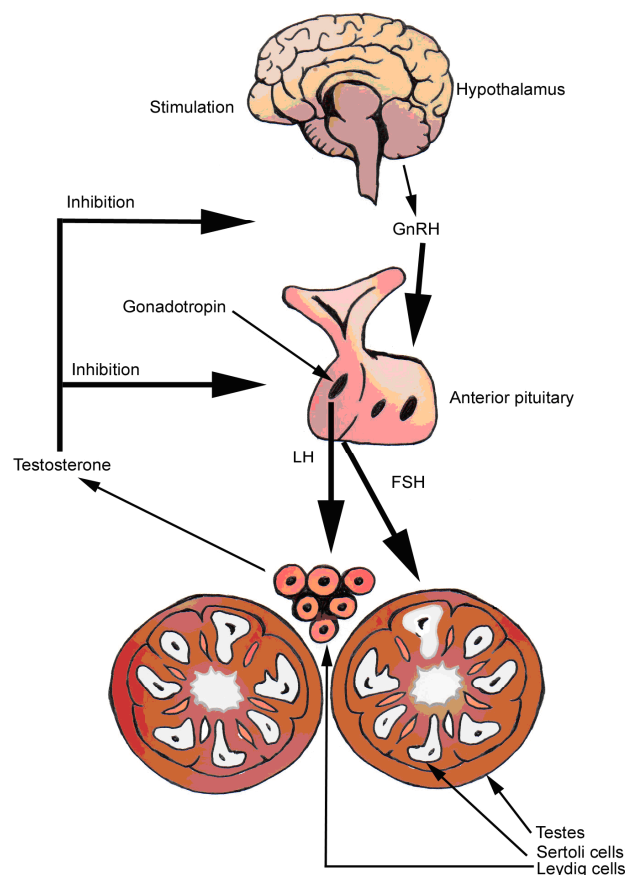


Figure 9. Schematic representation of the male hypothalamic-pituitary-gonadal axis.

GnRH= gonadotropin-releasing hormone; LH= luteinizing hormone; FSH= follicle stimulating hormone

It should be noted that, both direct neural pathways between the hypothalamus and the testes (Selvage, Parsons, & Rivier, 2006), and the direct actions of neurotransmitters and neuropeptides on the testes, have been shown to be involved in the regulation of testosterone production. These include catecholamines (adrenaline and noradrenaline), which stimulate testosterone production via activation of α_1 and β_1 adrenergic receptors; and potent inhibitors of testosterone biosynthesis such as corticotropin releasing hormone (CRH) and the neurotransmitter serotonin (Frungieri, Zitta, Pignataro, Gonzalez-Calvar, & Calandra, 2002).

2.5.2 Actions and Effects

Testosterone mediates protein biosynthesis via binding to specific androgen receptors in the cytoplasm. These activated receptors then translocate to the nucleus where they bind to androgen response elements in the promoter regions of specific genes to induce RNA transcription (Griggs et al., 1989; Marshall-Gradisnik, et al., 2009). By increasing the transcription of genes responsible for the synthesis of contractile proteins many of the anabolic effects of testosterone are realised (Deschenes, Kraemer, Maresh, & Crivello, 1991; Kadi, 2008). A metabolite of testosterone, dehydrotestosterone, is also a potent androgen as it possesses a high affinity for the androgen receptor (Bagatell & Bremner, 1996). Testosterone induces the transcription of genes responsible for the formation of red blood cells (Zitzmann & Nieschlag, 2001), repair of cellular damage (Viru & Viru, 2001), increased bone density (Gilsanz, Gibbens, & Roe, 1988), increases nitrogen retention, and is associated with increases lean body mass and strength (Kochakian, 1950; Bagatell & Bremner, 1996; Bhasin, et al., 1997).

2.5.3 Adaptation and Athletic Performance

Testosterone as the primary anabolic steroid hormone, has been linked to strength and muscle gain in humans. This relationship is based upon a number of observations including: an increase in muscle growth in males at puberty when testosterone production increases (Ramos, et al., 1998); the gradual loss of muscle mass and strength in ageing males that can

be reversed by testosterone supplementation (Anawalt & Merriam, 2001); testosterone replacement increased fat-free mass and muscle size in hypogonadal men (Bhasin, et al., 1997); exogenous testosterone administration in conjunction with resistance exercise positively influenced aspects of muscle architecture associated with force production (Blazevich & Giorgi, 2001); ingestion of a moderately supraphysiologic dose of a synthetic androgen in intact males resulted in greater strength and muscle gains from resistance exercise (Strawford, et al., 1999); and pharmacologic blockade of testosterone-specific receptors suppressed exercise-induced hypertrophy of skeletal muscle (Inoue, et al., 1994). Such observations have led Viru and Viru (Viru & Viru, 2005) to conclude that endogenous testosterone is “essential for myofibrillar hypertrophy” and a “vital instigator” in the remodeling of skeletal muscle (Allen, Roy, & Edgerton, 1999).

2.5.3.1 Genomic Actions

One proposed mechanism by which testosterone facilitates the hypertrophy of muscle fibres is the activation of satellite cells and the promotion of myonuclear accretion when existing myonuclei become unable to sustain further enhancement of protein synthesis (Niesler, Myburgh, & Moore, 2007; Kadi, 2008). Research that has investigated testosterone-induced muscle fibre hypertrophy, reported a dose-dependent increase in muscle cross-sectional area (Sinha-Hikim et al., 2002; Sinha-Hikim, Roth, Lee, & Bhasin, 2003). These authors also observed an association between testosterone and an increase in the number of satellite cells and myonuclear number. These data suggest a role for testosterone in satellite cell regulation either via an increase in satellite cell replication, an inhibition of satellite cell apoptosis, or an increase in the differentiation of stem cells towards a myogenic lineage.

Indeed, an article published in ‘Endocrinology’ demonstrated that testosterone regulated body composition by promoting the commitment of mesenchymal pluripotent cells into myogenic lineage and inhibiting the differentiation into adipogenic lineage via an

androgen receptor mediated pathway (Singh, Artaza, Taylor, Gonzalez-Cadavid, & Bhasin, 2003). The regulation of pluripotent cells by testosterone provides a unifying explanation for the reciprocal effects of testosterone on muscle and fat mass. Indeed, hypogonadism is associated with decreased muscle mass and increased fat mass (L. Katznelson et al., 1996; L. Katznelson et al., 1998). Conversely, several studies have linked testosterone supplementation with increases in fat-free mass (Bhasin, et al., 1997; Bhasin, et al., 2001a), as well as power and muscular strength (Bhasin, et al., 1996; C. Wang et al., 1996; Bhasin, Woodhouse, & Storer, 2001b; Herbst & Bhasin, 2004).

Interestingly, a 2004 review illustrated that, although testosterone improves maximal muscle strength and power, it does not improve the contractile properties of the muscle (Herbst & Bhasin, 2004). That is, the functional gains attributable to testosterone administration are proportional to the degree of muscular hypertrophy. Furthermore, it has been noted that, unlike the known effects of resistance training, testosterone administration did not alter the relative proportions of type I and type II muscle fibres (Sinha-Hikim, et al., 2002). These observations suggest that resistance training and testosterone administration operate via complementary, but distinct, mechanisms.

2.5.3.2 Non-Genomic Actions

Importantly, and in contrast to the androgen receptor-dependent pathway described by Singh *et alia*, an androgen receptor-independent mode of action of testosterone on skeletal muscle has also been described. As early as 1998, the importance of oscillations in calcium ion concentration $[Ca^{2+}]$ was reported as conferring specificity on intracellular messaging signals (Dolmetsch, Xu, & Lewis, 1998). Data presented by Estrada two years later recognised that testosterone and aldosterone initiated hormone-specific, rapid increases and oscillations in both cytosolic and nuclear $[Ca^{2+}]$ that were independent of an androgen receptor interaction (Estrada, Liberona, Miranda, & Jaimovich, 2000). In 2003, it was

confirmed that the rapid calcium signals elicited by androgenic steroid hormones led to an increase in inositol 1,4,5-trisphosphate via a G-protein linked receptor at the plasma membrane, and that the observed $[Ca^{2+}]$ oscillations originated from intracellular calcium stores (Estrada, et al., 2003).

Dunn, Burns and Michel (1999) had earlier shown that cells are unable to hypertrophy in the presence of calcineurin inhibitors (Dunn, Burns, & Michel, 1999). Calcineurin is a cytoplasmic, calcium-regulated phosphatase that mediates growth and differentiation via action on transcription factors. Indeed, in an article published in 1999 it was reported that $[Ca^{2+}]$ mediated skeletal muscle hypertrophy was “critically regulated” by calcineurin via the dephosphorylation and nuclear translocation of the transcription factor Nuclear Factor of Activated T-cell (NFAT) (Semsarian et al., 1999). Calcineurin signaling is only initiated by high frequency $[Ca^{2+}]$ oscillations, and a 2008 paper has proposed that calcineurin/NFAT act as integrators of the Ca^{2+} signal capable of decoding alterations in the frequency of $[Ca^{2+}]$ oscillations (Colella et al., 2008). Importantly, it has been shown that testosterone “systematically induces” $[Ca^{2+}]$ oscillations (Jaimovich & Espinosa, 2004). Such observations provide compelling evidence that calcineurin signaling is a necessary prerequisite for hypertrophy and that testosterone specifically induces $[Ca^{2+}]$ oscillations sufficient to activate this mechanism via an androgen receptor-independent pathway.

2.5.3.3 Response to Resistance Exercise

A great deal of research has been dedicated to investigating the links between the responses of the steroid hormones to resistance exercise and training regimes (Kraemer, et al., 1990; Kraemer, et al., 1991; Fry et al., 1993; K. Häkkinen & Pakarinen, 1993; Gotshalk et al., 1997; Ostrowski, Wilson, Weatherby, Murphy, & Lyttle, 1997; Giorgi, Wilson, Weatherby, & Murphy, 1998; Bosco, et al., 2000; K. Häkkinen, Pakarinen, & Kraemer, 2000; Pullinen, Mero, Huttunen, Pakarinen, & Komi, 2002; Smilios, et al., 2003; Crewther,

2004; Kraemer & Ratamess, 2005; Bird, et al., 2006a; Beaven, et al., 2008a; Beaven, Gill, & Cook, 2008b; Uchida et al., 2009). Generally, acute exercise has been shown to increase free and total testosterone levels in young adult males (Kraemer, et al., 1990; J. P. Ahtiainen, Pakarinen, Alen, Kraemer, & Häkkinen, 2003a; Beaven, 2004). However, some studies have reported no change (McCall, Byrnes, Fleck, Dickinson, & Kraemer, 1999; Nindl et al., 2001) or, in the case of body builders, a decrease in testosterone concentration across a resistance exercise session (Bosco, et al., 2000). Generally, protocol loads and volumes have been shown to influence the magnitude of testosterone responses and high-volume combined with a relatively low-load has been shown to induce larger testosterone responses than high-load, low-volume protocols (Kraemer, et al., 1991; Crewther, Cronin, Keogh, & Cook, 2008).

Equivocal hormone response data can in part be attributed to inter-individual variation, small sample sizes, as well as differences in experimental design and protocol variables. Many authors have commented on the individual variability of hormonal responses when measured in blood (Jensen et al., 1991), saliva (McGuigan, Egan, & Foster, 2004; Beaven, et al., 2008b) and urine samples (Ostrowski, et al., 1997; Giorgi, et al., 1998). Jensen and co-workers (1991) even suggested that these individual differences may have important consequences for individualised training (Jensen, et al., 1991).

In terms of functional outcomes, Hansen and colleagues (2001) described larger relative increases in isometric strength when the anabolic hormonal response was enhanced by additional leg exercises (Hansen, et al., 2001). Furthermore, Staron and co-workers (1994) linked increases in strength and muscle fibre transformation in males to elevated serum testosterone levels (Staron, et al., 1994). More recent data have demonstrated that suppression of endogenous testosterone attenuates strength and muscle mass gains observed over an eight-week strength training period (Kvorning, Anderson, Brixen, & Madsen, 2006). Furthermore, previous work has demonstrated enhanced strength and body weight gains when rugby players are prescribed resistance exercise that maximises testosterone response

(Beaven, et al., 2008a; Beaven, et al., 2008b). These data, taken together, suggest a link between acute exercise-induced elevations of testosterone and functional adaptation.

2.5.4 Rapid Effects

Evidence for a rapid neural pathway between the brain and the testes independent of the pituitary (Selvage & Rivier, 2003) suggests that rapid changes in testosterone concentrations may have the potential to modulate training and subsequent performance. Testosterone has been shown to be positively correlated to explosive performance and power tests (Cardinale & Stone, 2006; Winchester, 2008), and negatively correlated with endurance performance (Bosco, Tihanyi, & Viru, 1996b). It has been proposed that the short-term preconditioning of testosterone could be associated with neuromuscular function (Viru & Viru, 2005). Indeed, at an individual level, resting salivary testosterone and cortisol concentrations have been associated with lower body strength and power performance in semi-elite rugby players (Crewther, Lowe, Weatherby, & Gill, 2009a; Crewther, et al., 2009b). As such, the preconditioning of the steroid hormones prior to training or performance may provide a mechanism for enhancing performance.

2.5.5 Supplementation

As a result of the association between testosterone and functional adaptations, supplements claiming to enhance both basal testosterone levels and acute testosterone response to training are widely available. Furthermore, abuse of testosterone and testosterone analogues to enhance muscle size and athletic performance is not uncommon despite adverse health effects and the illegal nature of its use in the sporting environment (Kadi, 2008). The supplement Muscle FuelTM has been reported to augment human growth hormone, IGF-1, and free testosterone responses to a resistance exercise protocol, as well as enhance exercise performance and subsequent recovery (Kraemer et al., 2007). These authors suggested that as a result of the enhanced hormonal responses, chronic supplementation could result in long-term increases in muscle size and strength.

Supplementation with the leucine metabolite β -hydroxy- β -methylbutyrate (HMB) combined with amino acids, in the form of Muscle ArmorTM, has also been associated with enhanced resistance exercise adaptations (Kraemer et al., 2009). The herb *Tribulus terrestris* that contains steroidal saponins (Y. Wang, Ohtani, Kasai, & Yamasaki, 1997) that have traditionally been used as a treatment for impotence, is also suggested to raise testosterone levels (Poprzecki, Zebrowska, Cholewa, Zajac, & Waskiewicz, 2005). However, studies to date have provided little evidence to suggest that *Tribulus terrestris* supplementation can enhance changes in body composition and strength in competitive basketball (Poprzecki, et al., 2005) or rugby league (Rogerson et al., 2007) athletes.

Zinc-Magnesium Aspartate (ZMA) is another supplement that has been reported to enhance natural testosterone levels. Zinc deficiency has been associated with reduced testosterone levels via the impaired action of GnRH, LH and FSH (McClain, Gavalier, & van Thiel, 1984) and modified enzymatic conversion (Om & Chung, 1996). An eight-week, placebo-controlled study reported an improved anabolic profile and greater increases in lower body isokinetic strength in American football players when taking ZMA (Brilla & Conte, 2000). However, subsequent studies have failed to show differences in muscular adaptation in resistance trained males (Wilborn et al., 2004) or hormone status in subjects with a diet containing sufficient zinc (Koehler, Parr, Gayer, Mester, & Schänzer, 2009). These observations suggest that ZMA is ineffective in elevating baseline testosterone levels in cases where zinc deficiency is not evident.

Interestingly, a recent French study reported that magnesium ions decreased the binding affinity between testosterone and SHBG suggesting that physiologically relevant concentrations of magnesium could increase the levels of free testosterone (Excoffon, et al., 2009). These authors also reported that further work investigating the effect of zinc and calcium ions on the testosterone–SHBG binding complex were “in progress”. Another interesting study has reported that vegetable oils, and specifically olive oil, was able to

increase the activity of key enzymes (specifically 17- β -HSD and 3- β -HSD) involved in final steps of testosterone biosynthesis from DHEA (Figure 7), as well as testosterone levels in rats (Hurtado de Catalfo, de Alaniz, & Marra, 2009).

The ubiquitous and regularly consumed drug caffeine is also known to influence aspects of gonadal function (Ramlau-Hansen, Thulstrup, Bonde, Olsen, & Bech, 2008). In male mammalian animal studies, acute (Pollard, 1988) and chronic (Ezzat & el-Gohary, 1994; Celec & Behuliak, 2009) caffeine dosing has been associated with increases in testosterone. As a result Celec and Behuliak (2009) concluded that an “additional mechanism” of caffeine action beyond the known effects of caffeine on adenosine receptors was responsible for modulating the HPG. In human studies caffeine consumption has also been reported to be positively associated with increased testosterone levels (Ferreni & Barrett-Conner, 1998; Svartberg et al., 2003; Ramlau-Hansen, et al., 2008). Indeed, Ramlau-Hansen and colleagues (2008) reported that Danish men with a high dietary caffeine intake (defined as < 175 mg daily) had a ~14% higher testosterone concentration than men with a low caffeine intake (defined as > 25 mg daily). Thus, it appears that caffeine can modulate aspects of testosterone regulation and production and may have the potential to enhance resistance exercise gains.

2.5.6 Summary

The corollary of studies investigating the effects of testosterone on skeletal muscle adaption to exercise is that testosterone is necessary for skeletal muscle adaptation and is able to modulate muscle accretion via both genomic and non-genomic mechanisms. The importance of testosterone in strength and power adaptation has been demonstrated although testosterone administration does not appear to influence muscle fibre type transformation. The use, and abuse, of testosterone and supplements designed to enhance testosterone are a consequence of the established efficacy of testosterone actualising beneficial functional outcomes. Indeed, testosterone has been positively associated with explosive performance

and acute responses have been shown to enhance strength and body mass adaptations. As such, strength and conditioning practitioners should be aware that strategies that elevate testosterone are likely to prove beneficial to enhance resistance training and performance outcomes.

2.6 Cortisol

Cortisol (11-beta, 17-alpha, 21-trihydroxypregnen-4-ene-3, 20-one) is the most active of the glucocorticoid hormones and accounts for approximately 95% of all glucocorticoid activity (Figure 10) (Viru & Viru, 2001). Cortisol secretion increases in response to any acute stressor, whether physical (such as exercise, illness, surgery etc) or psychological (Dallman et al., 2004). One of the primary roles of cortisol is to direct the body's energy processes, preparing an individual to deal with stress and ensure that adequate glucose sources are maintained (Martini & Welch, 2001).

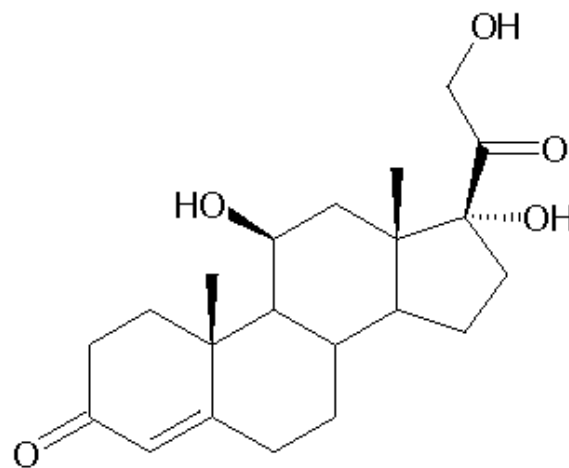


Figure 10. Structure of cortisol: 11-beta, 17-alpha, 21-trihydroxypregnen-4-ene-3, 20-one.

2.6.1 Regulation

Cortisol production is regulated by the Hypothalamic-Pituitary-Adrenal (HPA) axis (Figure 10) (Cone, Low, Elmquist, & Cameron, 2002). The HPA axis maintains the basal and stress-related homeostasis of the central nervous, cardiovascular, metabolic, and immune systems (Chrousos, 1995). When a threat to homeostasis is encountered, neurosecretory neurons in the medial parvocellular and magnocellular divisions of the paraventricular nucleus (PVN) of the hypothalamus act to integrate signals from the central nervous system (Ferrini, Grillo, Piroli, de Kloet, & De Nicola, 1997; Ziegler & Herman, 2002). Stimulation of the PVN results in the release of adrenocorticotrophic hormone (ACTH) secretagogues, the

most important of which are corticotropin releasing hormone (CRH) and arginine vasopressin which have a synergistic effect on ACTH release. The production of CRH by the PVN is controlled by a diverse range of inputs including the amygdala and hippocampus (E. O. Johnson, Kamilaris, Chrousos, & Gold, 1992). The pulsatile release of CRH from the paraventricular nucleus of the hypothalamus into the hypophyseal portal blood via the median eminence stimulates the secretion of ACTH from corticotroph cells in the anterior pituitary gland (Herman & Cullinan, 1997).

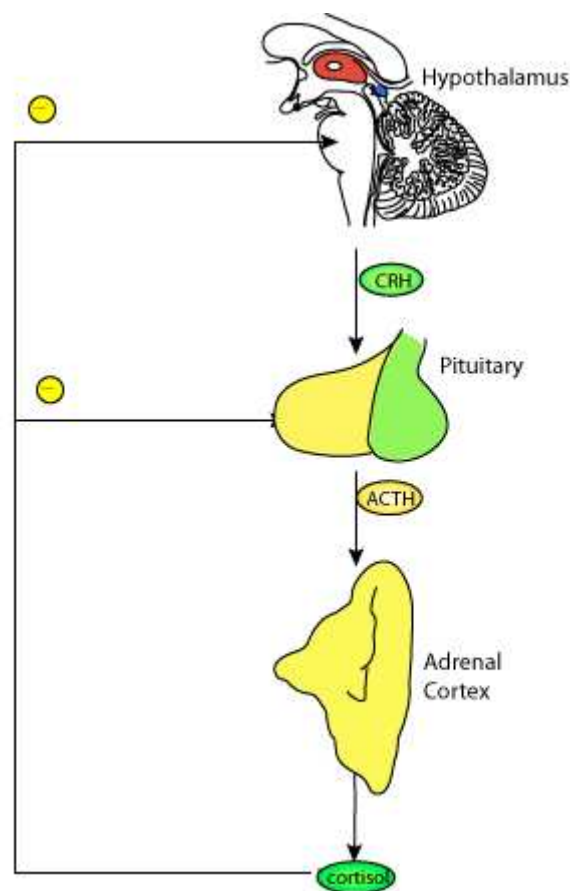


Figure 10. Schematic representation of the hypothalamic-pituitary-adrenal axis (Cone, et al., 2002).

CRH=corticotropin-releasing hormone; ACTH=adrenocorticotropic hormone; Inhibition is denoted by the yellow circles with minus symbols.

ACTH is transported via systemic circulation to the adrenal gland where it stimulates steroid biosynthesis by binding to receptors located in the plasma membrane of adrenocortical cells (Stocco & Clark, 1996). Steroid biosynthesis within the adrenal cortex can also be directly innervated by catecholamines derived from the splanchnic nerve (Erhart-Bornstein, Hinson, Bornstein, Scherbaum, & Vinson, 1998). Following activation, the

adrenal zona fasciculata and reticularis cells synthesise and secrete the glucocorticoid cortisol into the blood in a pulsatile manner (Keenan & Veldhuis, 2003). There is a tight coupling of ACTH and cortisol secretion, with ACTH secretory events consistently preceding cortisol secretion in human plasma (Henley et al., 2009). This pulsatility in glucocorticoids is an essential component of their biological activity (Conway-Campbell et al., 2007). Additionally, recent research *in vivo* has identified direct neural links between the paraventricular nucleus of the hypothalamus and cortisol secretion (Bujis et al., 1999; Zaki & Barrett-Jolley, 2002). This link suggests that hormone secretion is also regulated through mechanisms that bypass the pituitary gland.

In the blood, approximately 94% of secreted cortisol is bound in complexes with corticosteroid binding globulin or albumin (Keenan, Roelfsema, & Veldhuis, 2004; Lewis, Bagley, Elder, Bachmann, & Torpy, 2005; Gagliardi, Ho, & Torpy, 2010). The concentration of cortisol in the blood exerts a negative inhibitory feedback on CRH production in the hypothalamus and ACTH secretion from the pituitary (Martini & Welch, 2001). Interestingly, testosterone can also exert negative feedback on aspects of the HPA axis by enhancing glucocorticoid negative feedback efficacy, and inhibiting the activation of the PVN motor neurons and ACTH secretagogue synthesis (M. Williamson, Bingham, & Viau, 2008; M. Williamson & Viau, 2008).

2.6.2 Actions and Effects

The genomic actions of cortisol are actualised through the binding to specific cellular receptors in the cytoplasm and subsequent transportation into the nucleus where it induces the transcription of enzyme proteins. These actions of glucocorticoids are mediated by two types of receptors: the mineralocorticoid (high affinity) receptor; and the glucocorticoid (low affinity) receptor (Rosenfeld, Eekelen, Levine, & de Kloet, 1993). Glucocorticoid binding to the mineralocorticoid receptors exerts proactive feedback on the HPA axis thus maintaining basal activity and promoting coordination of circadian events (de Kloet, Vreugdenhil, Oitzl,

& Joëls, 1998). The main function of the glucocorticoid receptors is to respond to stress-induced glucocorticoid levels and restrict HPA axis hyperactivity through inhibition of CRH and arginine vasopressin synthesis (Orchinik, 1998). Activated glucocorticoid receptor complexes bind to short specific sequences of DNA in hormone-responsive genes termed glucocorticoid response elements, and receptor binding stimulates transcription. Importantly, rapidly changing intra-nuclear concentrations of activated mineralocorticoid and glucocorticoid receptors are likely to convey differential information and determine specific glucocorticoid responses to fluctuations in hormone levels (Conway-Campbell, et al., 2007).

Glucocorticoids increase the availability of glucose by stimulating gluconeogenesis and inhibiting glucose uptake by cells not essential to the stress response (de Kloet & Veldhuis, 1985). Cortisol acts to maintain homeostasis through lipolysis and increasing the rate of synthesis of gluconeogenic enzymes in the liver (Martini & Welch, 2001). Cortisol secretion also modulates proteolytic enzymes and can cause muscle proteolysis leading to the mobilisation of amino acids into the bloodstream and an increase in the release of 3-methylhistidine from muscle tissue (Sapolsky, Romero, & Munck, 2000; Viru & Viru, 2001). Further effects of cortisol include: the metabolic control of the glucose-alanine cycle where it catalyses the formation of alanine (Viru & Viru, 2004); and the inhibition of immune and inflammatory mediators such as IL-6, TNF- α , histamine, and nitric oxide (Sapolsky, et al., 2000; Ziegler & Herman, 2002). These inhibitory functions can become damaging if left unchecked (Munck, Guyer, & Holbrook, 1984).

2.6.3 Rapid Response

Some of the metabolic effects of cortisol occur too rapidly to be directly attributable to enzyme transcription or amino acid augmentation as these effects require a 2-3 hour time period to evolve (Shah, Anthony, Kimball, & Jefferson, 2000b). In such cases it is postulated that cortisol affects post-receptor processes and enhances the activity of protein kinases. As such, cortisol can be thought of as a biological amplifier in some cases. This

“permissive action” of cortisol was initially proposed in the fifties (Ingle, 1952). Non-genomic actions of cortisol have been described by Edwardson and Bennett (1974) with cortisol administration able to modulate the release of CRF from mammalian hypothalamic synaptosomes via direct membrane effects (Edwardson & Bennett, 1974). Glucocorticoids have also been reported to rapidly inhibit arginine vasopressin release in the hypothalamic cells of rats in a non-genomic manner (X. Liu, Wang, & Chen, 1995). In addition to these non-genomic roles in HPA regulation, glucocorticoids are also known to enhance glutamate uptake in a dose-dependent manner via G-protein activation (Zhu, Zhu, & Chen, 1998) and rapidly effect neural (Feldman, Dalith, & Conforti, 1973; Avanzino, Ermirio, Ruggeri, & Cogo, 1984; Zaki & Barrett-Jolley, 2002) and locomotor activity (Sandi, Venero, & Guaza, 1996) of rats.

2.6.4 Adaptation and Athletic Performance

The HPA axis is rapidly activated by physical exercise with an initial ACTH response eliciting a rapid increase in blood cortisol concentration. The initial ACTH response is generally short-lived and returns to basal levels while cortisol levels continue to rise, presumably due to the time lag involved in transporting ACTH to the adrenal cortex via the circulatory system (Virus & Virus, 2004). Indeed, the exercise-induced increase in glucocorticoids has been reported to be vital to endurance performance in rats (T. L. Sellers, Jaussi, Yang, Heninger, & Winder, 1988). A relationship has also been reported between resting salivary cortisol and box squat strength in trained male athletes (Crewther, et al., 2009a) although it is possible that this observation was an artifact of enhanced sympathetic activation / fight or flight-type response.

Cortisol causes a reduction in cellular protein stores by suppressing protein synthesis, enhancing protein degradation, and is known to cause an increase in nitrogen excretion (Simmons, Miles, Gerich, & Haymond, 1984). At the same time amino acids are being produced from the catabolism of protein thus mobilising amino acids from non-hepatic tissue

and creating a free amino acid pool (Viru & Viru, 2001). However, during exercise the catabolic effects of cortisol on the muscle appear to be limited by contractile activity (Hickson & Davis, 1981; Seene & Viru, 1982) with indicators of muscle catabolism, such as 3-methylhistidine levels, remaining relatively constant in active muscles (Varrik, Viru, Ööpik, & Viru, 1992). Due to the catabolic nature of cortisol, its increase following exercise has generally been interpreted as a negative response. This conclusion would be particularly true in the case of resistance exercise where anabolic responses are considered ideal to promote exercise-induced hypertrophy. However, this interpretation ignores the important roles cortisol performs in terms of metabolic control. The catabolic action of cortisol is essential in post-translational control of protein molecules as well as creating an increased pool of free amino acids. Thus, in the absence of sufficient amino acid concentrations, cortisol production is necessary to provide the energy and substrates for adaptive protein synthesis and can be considered an integral component in the remodeling of skeletal muscle tissue (Viru & Viru, 2001).

Further, an increase in the availability of amino acids, and in particular leucine, shows a positive curvilinear relationship with muscle protein synthesis via phosphorylation of elements of the mTOR pathway (Rennie, Bohe, Smith, Wackerhage, & Greenhaff, 2006). As mentioned earlier though, cortisol is also known to inhibit downstream targets of the mTOR pathway. Exogenous cortisol has been shown to reduce the rate of protein synthesis via modulating effects on the activities of eukaryotic initiation factors (Shah, et al., 2000b; Shah, et al., 2000c, 2000d). Exercise-induced elevation in cortisol has also been reported to attenuate the phosphorylation of p70^{S6k} (Spiering, et al., 2008a). Indeed, the administration of synthetic glucocorticoids such as prednisone or dexamethasone is known to increase muscle atrophy, suppress protein synthesis, and antagonise the anabolic action of amino acids (Z. Liu, Li, Kimball, Jahn, & Barrett, 2004; Short, Nygren, Bigelow, & Nair, 2004). Glucocorticoids have been shown to directly inhibit Leydig cell function and testosterone

production in rats (Bambino & Hsueh, 1981), guinea pigs (Fenske, 1997), and humans (Cumming, Quigley, & Yen, 1983). Furthermore, in humans negative relationships have been reported between cortisol response and post-exercise testosterone levels (Brownlee, Moore, & Hackney, 2005; W. Daly, Seegers, Rubin, Dobridge, & Hackney, 2005). The most likely mechanism for this inhibition is via inhibition of the activity of the testicular 17 α -hydroxylase (Welsh, Bambino, & Hsueh, 1982; Fenske, 1997).

2.6.5 Supplementation

As cortisol can negatively impact protein synthesis, strategies designed to attenuate cortisol responses to resistance training have been investigated. Tarpenning and colleagues (2001) showed that the ingestion of 8.5 mL·kg⁻¹ of bodyweight of a 6% carbohydrate solution during a resistance exercise protocol elevated plasma insulin and blunted the exercise-induced cortisol response compared to a placebo condition (Tarpenning, et al., 2001). Importantly, these authors tracked adaptive changes over a 12 week training program and established that, not only did carbohydrate supplementation continue to attenuate the catabolic response to exercise, but this attenuation was associated with greater gains in both type I and type II muscle fibre area as measured by muscle biopsy taken from the vastus lateralis muscle of the dominant thigh.

Further work by Bird and colleagues (Bird, et al., 2006b, 2006a; Bird, Tarpenning, & Marino, 2006c) confirms that carbohydrate ingestion suppresses exercise-induced cortisol responses and that cortisol responses are further attenuated by amino acid supplementation. In addition, it was evident that combined carbohydrate and amino acid supplementation dampened indices of muscle damage and enhanced hypertrophy of type I and type II muscle fibres compared to either supplement in isolation or a control group (Bird, et al., 2006b). Furthermore, essential amino acid and carbohydrate supplementation have been shown to reduce muscle catabolism resulting from immobility and bed-rest (Paddon-Jones et al., 2004). Two supplements assessed by Kraemer and colleagues, 'Cortitrol' (Kraemer et al.,

2005) and Muscle ArmorTM (Kraemer, et al., 2009) have shown potential for “improved muscular adaptation” by decreasing pre-exercise cortisol concentrations. Adequate hydration has also been demonstrated to decrease the cortisol responses to resistance exercise and as such, the hydration state of athletes is an “important consideration” as it can affect the catabolic response to resistance exercise (Judelson et al., 2008).

2.6.6 Summary

The corollary of studies investigating the effects of cortisol is that cortisol activates cellular mechanisms that promote catabolism and skeletal muscle remodeling in response to specific functional demands. Indeed, appropriate exercise-induced cortisol response has been reported to be essential to endurance performance in rats due to its ability to mobilise endogenous energy stores (T. L. Sellers, et al., 1988). The ability of cortisol to increase the availability of amino acids necessary for muscular adaptation suggests that it is vital for skeletal muscle adaptation in the absence of adequate nutritional support. However, the inhibitory effect of cortisol on the mTOR signaling pathway demonstrates that cortisol production is not ideal from the perspective of muscular adaptation in strength and power athletes. Importantly, the use of supplements designed to attenuate acute cortisol responses by supplying carbohydrates and amino acids to the exercising athlete, has demonstrated the efficacy of glucocorticoids in limiting the beneficial resistance training outcomes. As such, is important for strength and conditioning practitioners to be aware of the effects of cortisol on specific training outcomes.

2.7 Research Focus

As the roles of the steroid hormones in strength and power adaptation have become apparent, the importance of understanding hormonal responses to exercise stimuli has increased. Strength and power training are necessary components of preparing for optimal athletic performance, and maximising resistance training gains will contribute to successful competitive outcomes. Strategies by which hormonal responses, and therefore functional outcomes, can be manipulated are becoming more widespread with protein and carbohydrate supplementation obvious examples of attempts to modulate the adaptive response to training.

This following section of the literature review is intended to introduce three broad areas whereby hormone-mediated strategies may enhance adaptation and ultimately performance. These areas are: modulation of prescribed exercise variables; hormonal biorhythms and; supplementation. Specifically, a concise background is provided for the four areas that constitute the experimental chapters within this thesis:

- Combined strength and power-type exercise in a single session and hormone responses (Complex Training).
- The effects of the circadian biorhythms on hormones and resistance training.
- The influence of ultradian pulsatility on hormonal response to exercise.
- The ability of caffeine to modulate hormone response to resistance exercise.

The rationale behind investigating a range of strategies is two-fold. The strategies may influence hormone responses via distinct mechanisms raising the possibility of a cumulative effect. Additionally, from a practical perspective it may not be feasible to change the time of day at which you train or make use of ultradian rhythms. Therefore, investigating a range of possible strategies allows options that may be able to be utilised in a practical setting.

2.7.1 Modulation of Prescribed Exercise Variables: Combined Strength and Power Training

The necessity for power development in sports is unquestionable and as such, athletes and certified strength and conditioning specialists dedicate a great deal of time working on muscular power and strength development. Current training programs to improve power are comprised of a combination of resistance and plyometric exercises. Researchers have investigated the effects of both forms of training individually, and in combination, on performance indicators such as vertical jump height, and maximal strength. Literature to date suggests that a combination of resistance training and plyometric training yields superior results than either method in isolation (K. Adams, O'Shea, & O'Shea, 1992; Baker, Nance, & Moore, 2001; Cronin, McNair, & Marshall, 2002; Mangine et al., 2008). It is hypothesised that the combination of power and strength training maximises power output by increasing both muscle fibre hypertrophy and neuromuscular adaptations (Blakey & Southard, 1987; K. Adams, et al., 1992; Fatouros et al., 2000; Kotzamanidis, Chatzopoulos, Michailidis, Papaiaikovou, & Patikas, 2005).

For the purpose of this thesis 'Complex' training is defined as a combination of heavy resistance exercise with a biomechanically similar plyometric exercise within an exercise session. Complex training has been reported to be more effective in enhancing power production than other training program designs due to the development of an enhanced neuromuscular environment (Masamoto, Larson, Gates, & Faigenbaum, 2003). One study examined the effect of a complex training protocol on vertical jump height over a 4-week period in college aged volleyball players (Mihalk, Libby, Battaglini, & McMurray, 2008). Findings from this American study showed that the complex training protocol improved vertical jump height and power to a greater extent than a protocol that involved performing the same amount of work but on different days. These results support the majority of existing literature in suggesting that combining exercise modalities is effective for improving muscular power (Polhemus, Burkhart, Osina, & Patterson, 1981; K. Adams, et al., 1992; Newton, Kraemer, & Häkkinen, 1999; Fatouros, et al., 2000; Kotzamanidis, et al., 2005; Tricoli, Lamas, Carnevale, & Ugrinowitsch, 2005; Faigenbaum et al., 2007). Further, an

Australian training study concluded that complex training produced load-power, load-force and load-velocity results that were “superior” to power training alone (Cormie, McCaulley, & McBride, 2007).

Workout variables such as load intensity, rest periods, and volume are determinative in activating the endocrine system and stimulating cellular processes that lead to muscle tissue remodeling following resistance exercise (Kraemer, et al., 1990; J. P. Ahtiainen, Pakarinen, Kraemer, & Häkkinen, 2003b; Smilios, et al., 2003; J. P. Ahtiainen, Pakarinen, Alen, Kraemer, & Häkkinen, 2005). Typically, researchers have found that hypertrophy protocols elicit relatively large increases in serum total testosterone concentration (Häkkinen & Pakarinen, 1993 ; Kraemer, Fleck, Dziados, Harman, & Marchitelli, 1993; Kraemer et al., 1998; Smilios, et al., 2003). Furthermore, in these same studies, the use of weight training with lower total work and longer rest periods resulted in lesser or non-significant increases in testosterone concentration. Generally power protocols produce only modest changes in the circulating levels of steroid hormones (Mero et al., 1993; Volek, Kraemer, Bush, Incledon, & Boetes, 1997; Pullinen, Mero, MacDonald, Pakarinen, & Komi, 1998; Linnamo, Pakarinen, Komi, Kraemer, & Häkkinen, 2005). A New Zealand study reported that a hypertrophy protocol increased salivary testosterone and cortisol, whereas power and maximal strength protocols produced little to no change in these hormonal measures (Crewther, et al., 2008). The power and strength protocols were matched by volume and displayed similar hormonal responses. As a result these authors argued that differences in load, intensity, rest periods, and technique are secondary to volume in terms of eliciting hormonal responses. It has previously been demonstrated that the propensity to respond to an exercise stimulus differs at an individual level and that this response is modulated by the volume and intensity of an exercise bout (Beaven, et al., 2008b).

Training studies have not yet determined the combination of resistance and plyometric exercises needed to elicit optimal muscular power adaptation. Furthermore, there seems to be a paucity of data surrounding the anabolic and catabolic hormonal responses associated with

complex training despite an awareness of the important influence of exercise variables in modulating hormonal responses and adaptive outcomes. New knowledge regarding the effects of complex exercise protocols on acute hormonal responses may provide an insight into the mechanisms behind the effectiveness of combination training protocols and demonstrate an effective strategy for hormone manipulation.

2.7.2 Hormonal Biorhythms: Hormone Circadian Rhythmicity and Exercise Training

It is accepted that circulating concentrations of the steroid hormones testosterone and cortisol in all forms (total, bioavailable and free) are characterised by a circadian rhythm, with highest levels in the morning and a nadir in the late afternoon (Lévi, et al., 1988; Cooke, et al., 1993; Diver, et al., 2003). The master pacemaker that controls the circadian clock in mammals is located in the suprachiasmatic neurons located in the ventral hypothalamus (Ralph, Foster, Davis, & Menaker, 1990; Gachon, Nagoshi, Brown, Ripperger, & Schibler, 2004). Early experiments in rats demonstrated that bilateral lesions in the suprachiasmatic nuclei abolished circadian rhythms in behaviour and activity (Stephan & Zucker, 1972) as well as neuroendocrine rhythms (Halász, 1969). Indeed, it has been suggested that alterations in this brain region account for altered circadian rhythms in testosterone in elderly human males (Bremner, Vitiello, & Prinz, 1983; Wise, Walovitch, Cohen, Weiland, & London, 1987). Such circadian rhythms are dependent on the transmission of zeitgebers, or synchronizing signals, to the suprachiasmatic nuclei in order to remain in sync with geophysical time (Gachon, et al., 2004; Froy & Miskin, 2010).

The circadian rhythm is responsible for coordinating vital functions of a range of tissues and controlling the timing of daily secretory events (Winget, DeRoshia, & Holley, 1985). The elevated morning testosterone levels typically observed are thought to stimulate protein synthesis, whereas the morning rise in cortisol accelerates metabolism (Florini, 1987) as well as stimulating gluconeogenesis and proteolytic activity (Dineen, Alzaid, Miles, & Rizza, 1993). The post awakening rise in cortisol has been advanced as a reliable biological

marker of adrenocortical activity as well as perceived stress (Pruessner et al., 1997; Pruessner, Hellhammer, & Kirschbaum, 1999). Kraemer (1988) has also suggested that the early morning increase in testosterone may serve an anti-catabolic role thus counteracting the stimulatory effect of cortisol on skeletal protein degradation (Kraemer, 1988). Indeed, several instances of opposing interactions between the HPA and HPG axis have been reported (Waite, et al., 2009). For example, in rats castration increases the circadian secretion of corticosterone (Seale et al., 2004) and testosterone levels are suppressed by stress (Garcia-Bonacho, Esquifino, Castrillón, Toso, & Cardinali, 2000). In humans, stress associated with simulated wartime interrogations as part of military survival training, has been reported to promote drastic reductions in salivary and serum testosterone concentrations (Morgan et al., 2000).

Deschenes and co-workers (Deschenes et al., 1998) reported that the functional capacity of the muscle was at its lowest in the early morning (0800 h) and peaks in the early evening (2000 h). However, these researchers also stated that the early morning could be considered the “optimal time for resistance exercise” due to the greater pre- and post-exercise plasma testosterone levels observed relative to other times of the day (Deschenes, et al., 1998). Furthermore, these authors suggested that, if the ratio of testosterone to cortisol provided a more accurate reflection of the anabolic potential for protein accretion, then the early evening may be more “conducive to resistance exercise”. More recently researchers have reported that “the optimal time for resistance training is in the evening in order to alter the balance between hormone-mediated anabolic and catabolic activities and enhance anabolic/catabolic status” (Bird & Tarpenning, 2004).

It is apparent that more research is required to elucidate the effect of training at different times over the circadian rhythm. Despite the similar circadian profiles of testosterone and cortisol, the change in the ratio of these hormones shifts from a predominantly catabolic state to an anabolic state across the day. This dynamic change in the steroid milieu may be

capable of mediating differential responses to resistance exercise. Indeed, an anabolic hormonal milieu is known to be associated with favourable skeletal muscle adaptations following resistance exercise. New knowledge of the effects of hormonal biorhythms on adaptation may provide strength and conditioning practitioners with valuable information regarding the best time of day to train to enhance the effectiveness of specific training protocols.

2.7.3 Hormonal Biorhythms: Ultradian Pulsatility of Hormones and Exercise Timing

An ultradian rhythm is defined as a recurrent cycle with a periodicity of less than 24 hours. Ultradian rhythms in plasma concentrations are the result of discrete episodic secretion and ultradian pulsatility is inherent in the activity of both the HPA and HPG axes (Van Cauter, 1990; Ingram, Crockford, & Matthews, 1999; Veldhuis, Keenan, & Pincus, 2008; Droste, de Groote, Lightman, Reul, & Linthorst, 2009). Episodic secretion of steroid hormones has been identified in a variety of mammalian species including deer (Sarnyai et al., 1995; Windle, Wood, Shanks, Lightman, & Ingram, 1998; Ingram, et al., 1999; Young, Carlson, & Brown, 2001; Schlatt, Pohl, Ehmcke, & Ramaswamy, 2008), and humans (Winters & Troen, 1986; Veldhuis, Carlson, & Johnson, 1987a; Schürmeyer, Brademann, & Von Zur Mühlen, 1996; Young, et al., 2001; Young, Abelson, & Lightman, 2004). Pulsatile release and rapid clearance of the steroid hormones has the effect of exposing target tissues to large fluctuations of hormone and are a critical component of biological actions (Young, et al., 2001).

The principal pulse generators for both axes are located in the hypothalamus (Gachon, et al., 2004). The PVN is responsible for pulse generation in the HPA axis while the arcuate nucleus performs an analogous role in the HPG axis. In both cases pulsatile release of secretagogues from the median eminence entrains episodic release throughout the respective hormonal cascades. It is proposed that such pulsatile release may function to

prevent down regulation of the HPA and HPG axes enabling the axis to maintain maximal responsiveness (Young, et al., 2004).

Knowledge of ultradian rhythmicity is necessary in order to understand the control of both the HPA and HPG axes and factors that influence these systems. Pulsatility itself appears to be a key factor in communicating different information to target cells. At the very least, knowledge of ultradian rhythmicity should suggest that single point sampling without an understanding of the underlying basal pattern, may not supply an accurate picture of HPA and HPG axes activity. Indeed, pulsatility could partially account for the characteristic variability observed in human hormone responses both between and within individuals.

Models of hormone ultradian rhythmicity have been proposed suggesting periods of system activation interspersed with subsequent feedback inhibition and a dormant or refractory period (Lightman et al., 2008). In support of this model, Windle and co-workers (1998) found periods of post-pulse quiescence that were associated with non-responsiveness of the HPA axis to an audio stressor in a rat model (Windle, et al., 1998). Further work has shown that male rats displayed less threatening behaviours ($p < 0.03$) when confronted by a male intruder when in a decreasing phase of their corticosteroid ultradian rhythm (Haller et al. 2000). The results from these animal studies demonstrate that the underlying pattern of HPA activity may be a determinant of both behavioural and physiological reactivity to acute stress. The fact that constant intra-peak intervals were observed over a 24 h period in male and female Sprague-Dawley rats suggests that the HPA axis is being actively driven over the entire circadian cycle. Thus, periods of quiescence following acute stress may be regarded as periods of active inhibition.

Though the ultradian rhythmicity has been shown to influence both behavioural and physiological responses to external stimuli in an animal model, no such data appears to exist in humans, or in the HPG axis. The ramifications of an interaction between steroid hormone

ultradian biorhythms and physiological responses could be substantial and have the potential for widespread consequences in the field of exercise prescription and functional adaptation.

2.7.4 Caffeine Supplementation as a Hormone Modulator

Caffeine is known to have ergogenic benefits and enhance neuromuscular function (Kalmar & Cafarelli, 1999). Positive effects of caffeine in time-to-exhaustion and endurance tests have been demonstrated in laboratory conditions (Tarnopolsky, 1994; Tarnopolsky & Cupido, 2000; Norager, Jensen, Madsen, & Laurberg, 2005). Caffeine-modulated performance enhancements have also been reported in simulated performance conditions for cycling (Ivy, Costill, Fink, & Lower, 1979; Kovacs, Stegen, & Brouns, 1998), swimming (MacIntosh & Wright, 1995) and running (D. G. Bell, McLellan, & Sabiston, 2002; Bridge & Jones, 2006). However, despite its widespread use and acknowledged performance benefits, the effects of caffeine as a training aid, and its effects on training responses, have largely been neglected.

The mechanism of action of caffeine to actualise endurance exercise enhancement remains to be fully elucidated. One theory that caffeine elicits lipolysis, sparing muscle glycogen, and thus realising an ergogenic effect now seems unlikely (Jackman, Wendling, Friars, & Graham, 1996; Graham, Helge, MacLean, Kiens, & Richter, 2000). Indeed, plasma β -endorphin levels have been demonstrated to increase 1.8-fold when $6\text{mg}\cdot\text{kg}^{-1}$ caffeine was ingested 90 minutes prior to two hours of cycling at 65% of VO_2 peak without a detectable glycogen sparing effect (Laurent et al., 2000). The β -endorphin elevation implicates a pain suppressing mechanism for caffeine as one possible mechanism for enhancing athletic performance.

It is also apparent that caffeine ingestion leads to a reduction in perceived exhaustion which can translate into an enhancement in exercise performance (Norager, et al., 2005; Burke, 2008; Tarnopolsky, 2008). Caffeine is known to antagonise adenosine receptors, and suppression of adenosine's inhibitory function on neurotransmitter release and neurological

firing frequency could account for some of caffeine's effects during exercise (Nehlig, Daval, & Debry, 1992; Greer, Morales, & Coles, 2006). The physiological relevance of adenosine in exercise proposed by Biaggioni (2004) is that the increasing concentrations of adenosine produced during exercise act to inhibit sympathetic efferents and activate afferent nerves (Biaggioni, 2004). The net effect of this inhibition is to protect the muscle tissue during ischemia or exhaustive exercise. Thus, the antagonistic actions of caffeine on adenosine receptors have the potential to reduce the normal inhibitory effect of adenosine on motor efferents. Indeed, pharmacologic antagonism of the adenosine receptor prevents the ergogenic effects of caffeine in rats (Davis et al., 2003). Interestingly, this 2003 study provided evidence for a central nervous system mechanism for caffeine efficacy as only intracerebroventricular injection of caffeine resulted in increased endurance performance in rats whereas intraperitoneal injection was ineffective.

The ergogenic effects of caffeine cannot be solely attributed to sympathetic nervous system enhancement however, as electrically-induced contractions in tetraplegic patients, who are incapable of sympathetic activation, elicit increases in plasma free fatty acids and glycerol without a concomitant increase in catecholamines (Van Soeren & Graham, 1998). This observation suggests a direct ergogenic effect of caffeine on skeletal muscle. Thus, there is evidence for effects of caffeine on both the central nervous system and peripheral neuromuscular function (Tarnopolsky, 2008).

Caffeine has also been shown to have beneficial effects on the physical and skill activities required in an intermittent high-intensity team sport (Stuart, Hopkins, Cook, & Cairns, 2005). Furthermore, data from this 2005 study suggested that ingested caffeine may elevate endogenous testosterone levels (CJ Cook, unpublished observations). Previous experiments have demonstrated that high doses of caffeine increased plasma levels of testosterone in rodents (Pollard, 1988). In humans, cross-sectional studies have reported a positive association between caffeine intake and elevated testosterone levels in adult males

(Ferreni & Barrett-Conner, 1998; Svartberg, et al., 2003; Ramlau-Hansen, et al., 2008). Specifically, Ferreni and Barrett-Conner (1998) found a significant correlation ($r = -0.54$; $p < 0.001$) between caffeine intake and bioavailable testosterone in 810 Californian men. Similarly, the Danish study of 343 men (Ramlau-Hansen et al. 2008) showed a significant positive relationship ($p < 0.007$) between testosterone and caffeine intake reporting that men with a high caffeine intake had approximately 14% higher testosterone concentration than those with a low caffeine intake ($p = 0.008$). However, caffeine has been reported to inhibit aspects of the mTOR signaling pathway through interactions with the mTORC1. For example, caffeine has been shown to closely resemble rapamycin in terms of its effects on gene expression (Reinke et al. 2006), block the ability of insulin to stimulate protein kinase B (Foukas et al. 2002), and inhibit mTOR by acting as a low affinity ATP analog (Ballou and Lin 2008). As a result, caffeine would be expected to negatively impact muscle growth and adaptation.

Research into the effects of caffeine as a training aid has largely been neglected as studies have generally focused on attenuation of fatigue and performance benefits. Indeed, the effect of varied caffeine doses on endogenous testosterone and cortisol responses to resistance exercise are unknown. The demonstration of a link between caffeine ingestion and both acute and chronic testosterone elevation suggests that caffeine could provide strength and conditioning practitioners with a strategy for modulating resistance exercise outcomes.

2.8 Salivary Hormone Sampling

The hormone levels reported in the experimental chapters of this thesis all refer to concentrations determined from whole saliva samples. Saliva collected from the oral cavity is a complex biological medium produced by the major salivary glands (parotid, sub-mandibular and sub-lingual) with minor secretions from a number of labial, buccal, and palatal glands (Vining & McGinley, 1986). It should be noted that differences in composition and action of saliva emanating from these major glands has been reported in rats (Bodner, 1991; Grossman, Binyamin, & Bodner, 2004) and humans (Chu & Ekins, 1988; Höld, de Boer, Zuidema, & Maes, 1995).

Due to their lipophilic nature, steroid hormones can enter saliva via rapid diffusion through the acinar cells (Quissell, 1993; Lewis, 2006). It has also been reported that unbound steroid concentrations are not influenced by the rate of saliva production due to these lipophilic properties (Vining, McGinley, & Symons, 1983; Arregger, Contreras, Tumilasci, Aquilanos, & Cardoso, 2007). This property of the steroid hormones is important as it allows for the use of saliva to assess endocrine response to exercise. Indeed, such usage is becoming more common and has been used in exercise trials, including that of Kraemer and colleagues (2001) in which the authors conclude that “salivary testosterone provides a good indication of fluctuations in free testosterone” (Kraemer, et al., 2001). This conclusion was reached after referencing work conducted by Dabbs (Dabbs, 1990) and Vittek and co-workers (1985). Indeed, Vittek reported a correlation of $r = 0.97$ between serum free testosterone and salivary testosterone (Vitteck, L'Hommedieu, Gordon, Rappaport, & Southren, 1985).

The non-invasive nature of salivary sampling is a major benefit when collecting multiple samples from athletes in a practical situation. The use of relatively non-invasive salivary sampling is a less stressful imposition than venepuncture, and may in fact be a more useful measure that is able to avoid any HPA activation associated with collecting blood. Not only does the assessment of cortisol in saliva avoid the confounding physiological or

psychological effects of venepuncture-induced stress (Merran, Hattersley, Mould, & Bloom, 1993; Suay et al., 1999; Kirschbaum & Hellhammer, 2000; Gerra et al., 2001), but it allows a single researcher to collect samples from a number of subjects in a short time period. Indeed, the speed of collecting blood samples is likely to be prohibitive when working in an applied setting such as a gymnasium, unless a large number of resources are available and dedicated to this task. Also important is the fact that hormones in saliva are relatively stable at room temperature for hours to days (Gröschl, 2008) and for up to three months when stored at -20°C (Levin, 2008).

Salivary measures are reported to correlate with physiological and psychological measures that are known to be responsive to hormonal regulation. Indeed, work published in the 'Journal of Research in Personality' reported high test-retest reliability for salivary testosterone when measuring its temporal stability in humans and observed a relationship between salivary testosterone and traditional measures of personality (J. G. Sellers, Mehl, & Josephs, 2007). Interestingly, Wilke and Utley (1987) reported that free testosterone was a superior diagnostic tool than serum testosterone when assessing hirsutism in women (Wilke & Utley, 1987). Similarly for cortisol, Gozansky and colleagues reported that salivary cortisol was significantly correlated with serum values ($r = 0.81$) providing "greater diagnostic accuracy" and that it allowed researchers to obtain "more physiologically relevant data" compared to serum total cortisol (Gozansky, Lynn, Laudenslager, & Kohrt, 2005). Therefore, saliva may offer a more valuable measure of free steroid levels, as it empirically reflects levels, even during intensive exercise when the established assumptions regarding carrier protein concentration and binding constants used to calculate bioavailable steroid levels may breakdown due to haemoconcentration and blood pH changes.

Indeed, earlier work with elite athletes stated that, as salivary values "reflected plasma free cortisol concentrations", saliva was a "very valuable tool for assessing glucocorticoid activity in exercise" (Stupnicki & Obminski, 1992). This article concluded

that salivary cortisol concentration was “more suitable” than serum cortisol in exercise situations (Stupnicki & Obminski, 1992). In further work with athletes, these authors reported a significant correlation between salivary and serum values ($r = 0.874$) and identified a two-component relationship with a breakpoint at approximately $600 \text{ nmol}\cdot\text{L}^{-1}$ (Obminski & Stupnicki, 1997). Saliva has also been proposed as being a superior method to urine sampling as steroids are not generally converted into water-soluble metabolites as they are in the kidneys (Gröschl, 2008). These data suggest that the ability to monitor free hormone values, albeit in saliva where possible fractionation may occur as the molecules equilibrate into the salivary biocompartment, has merit. It is possible that salivary monitoring has the capacity to observe the free hormone concentration increases that lead to subsequent hormone-receptor interaction.

Saliva contains the unbound form of the steroid hormones which is established as the form capable of actualising physiologic effects. A range of research from a variety of sources has formed a solid basis for the use of salivary hormones in assessing endocrine status (Obminski & Stupnicki, 1997; Pruessner, et al., 1997; Kraemer & Ratamess, 2005; Erskine, Smillie, Leiper, Ball, & Cardinale, 2007; Celec & Behuliak, 2009; Manuck et al., 2010). Investigations of whole body vibration (Erskine, et al., 2007), water-based exercise (Cadore et al., 2009), and a 90-minute “medium-high intensity” exercise (Di Luigi et al., 2006) have successfully utilised salivary hormone sampling to assess exercise-induced alterations in hormone concentrations. Salivary hormone responses to a resistance exercise session along with a relationship between hormone levels and strength and power production have recently been reported (Crewther, et al., 2009a). Importantly, previous work published in 2008 strongly suggests that salivary responses are consistent within an individual, and that the magnitude of acute salivary hormone responses are indicative of positive adaptive changes at an individual level (Beaven, et al., 2008a; Beaven, et al., 2008b).

2.9 Conclusion

Muscular strength and explosive power are critical to success in a range of sporting endeavours. As a result, a significant portion of an athlete's training time is dedicated to resistance and plyometric training programs. The acute testosterone and cortisol responses to resistance exercise play an important role in cellular signaling, protein metabolism, and functional adaptation. Various training strategies can modify hormonal responses and have the potential to enhance functional adaptation that would likely be translated into improved athletic performance. The aim of this work was to utilise salivary collection as a non-invasive method to assess the effectiveness of distinct strategies in modulating free steroid hormone concentrations. In doing so, it is intended to provide exercise practitioners with an improved understanding of the mechanisms underlying resistance exercise adaptation.

CHAPTER THREE: STUDY ONE – COMPLEX TRAINING

3.0 Acute Salivary Hormone Responses to Complex Exercise Protocols

3.1 Prelude

As identified in the literature review steroid hormones have the potential to modulate resistance exercise outcomes. In particular, the prescribed exercise variables are an integral determinant of subsequent adaptation and hormone responses. Complex training that incorporates a strength-focussed exercise was followed by a biomechanically similar dynamic exercise has been reported to enhance training gains in well-trained athletes. In the context of this thesis, it was intended to identify how complex training prescription influenced steroid hormone responses. This chapter compares the acute salivary testosterone and cortisol responses of complex training bouts with strength and power bouts. The implications of this information for researchers and practitioners are discussed.

3.2 Introduction

Testosterone and cortisol are steroid hormones that respond to resistance exercise (Kraemer, et al., 1990; Smilios, et al., 2003) and modulate subsequent neuromuscular adaptation. Exercises designed to improve maximal strength via primarily morphological adaptation (hypertrophy-type bouts: moderate load; high volume; short rest periods) generally produce larger relative increases in testosterone than those designed to enhance strength through neural adaptation (maximal strength-type bouts: heavy load; low volume; long rest periods) (Kraemer, et al., 1991; Crewther, Keogh, Cronin, & Cook, 2006; Beaven, et al., 2008b). Dynamic power bouts designed to maximise power, in which weights are lifted with explosive intent, have also produced significant androgen responses (Mero, Komi, Kyllonen, Pullinen, & Pakarinen, 1991; Pullinen, et al., 1998) similar to those seen in hypertrophy-type bouts (Crewther, et al., 2006).

As the primary anabolic hormone, testosterone has been linked to gains in strength and muscle mass. For example, Staron and co-workers (Staron, et al., 1994) have linked increases in strength and muscle fibre transformation in males to elevated serum testosterone levels. Hansen and colleagues (Hansen, et al., 2001) also concluded that increases in isometric strength were related to the magnitude of testosterone response to resistance exercise in young men. Furthermore, suppression of endogenous testosterone attenuates strength and muscle mass gains (Kvorning, et al., 2006). Recent data suggests that prescribing resistance exercise based on salivary testosterone responsiveness produced superior gains in both upper and lower body strength (Beaven, et al., 2008a; Beaven, et al., 2008b).

Training that combines both power and strength stimulus has been reported to be superior to more conventional weight training bouts in actualizing strength and power gains (K. Adams, et al., 1992; Fatouros, et al., 2000; Baker, et al., 2001; Cormie, et al., 2007). In their 2007 paper, Cormie and colleagues (Cormie, et al., 2007) concluded that combining strength and power produced greater improvements in jump height or related power output compared to a power workout that was matched for work performed. Indeed, the use of a combination of high force and high power appears to be superior to classical exercise prescriptions in terms of functional benefits (Harris, Stone, O'Bryant, Proulx, & Johnson, 2000; Goto et al., 2004b; Rahimi & Behpur, 2005).

In view of the association between acute testosterone responses to resistance exercise stimuli and enhanced functional training benefits, it was decided to investigate a potential role of hormones in actualizing the observed benefits of complex training. Thus, four distinct exercise bouts were designed that included strength, power, or a combination of the two stimuli. Salivary samples were collected throughout in order to assess testosterone and cortisol and ascertain whether complex training resulted in enhanced hormonal milieu for adaptation.

3.3 Methods

3.3.1 *Experimental Approach to the Problem*

Rugby players were recruited and assigned to complete each of four exercise bouts twice in a balanced order. Bouts were performed twice a week over a four week period and incorporated box squats and/or jump squats. Saliva samples were collected before, during and after exercise to determine free testosterone and cortisol concentrations. Differences in steroid hormone responses between four distinct bouts were assessed as well as changes in one-repetition maximum (1-RM) and body composition over the study period.

3.3.2 *Subjects*

Sixteen semi-professional male rugby union players with squat lifting experience (> one year) were recruited for this study. They were involved in a four week pre-season training period (approximately 10 hours per week) as part of a High Performance Academy that was set up for talented players identified in their region. Prior to this training period the players had a scheduled two-week detraining phase. Player characteristics are provided in Table 1. Players were informed of the experimental risks and signed an informed consent document prior to the investigation. The investigation was approved by the Human Subject Ethics Committee of the Waikato Institute of Technology for the use of human subjects and saliva sampling (Application #210605).

Table 1. Physical characteristics of the players recruited.	
	Mean \pm SD
Age (yr)	20.6 \pm 1.4
Height (cm)	187 \pm 6
Body mass (kg)	100 \pm 12
Soft lean mass (kg)	82.0 \pm 8.1
Maximum vertical jump (cm)	67 \pm 7
1-RM (kg)	150 \pm 15
Jump-squat peak power (W)	4830 \pm 540
Body fat (%)	13.3 \pm 3.7
Thigh muscle circumference (cm)	56.7 \pm 2.7
Combined leg volume (L)	20 \pm 2
RM= Repetition Maximum	

3.4 Procedure

3.4.1 Player Assessment

Each player's body composition, vertical jump height, 1-RM and jump-squat peak power were assessed in the week prior to the start of the experimental period and again at the end of the final training week. Body composition (body mass, soft lean mass, body fat, thigh muscle circumference and leg volume) was estimated using a reliable eight point, tetra-polar, segmental bioelectrical impedance analyser (*InBody 3.0*, Biospace Seoul, South Korea), according to the manufacturers' guidelines (Medici & Bedogni, 2005). For the purposes of this article, soft lean mass is defined as the sum of the total body water and the protein mass as estimated by the *InBody 3.0* bioelectrical impedance device.

Vertical jump was assessed using a yardstick with one-cm intervals (Swift Performance Equipment, Lismore, Australia) with the player instructed to extend their arm above their head while standing flat on the floor. The bottom finger of the yardstick was adjusted to this height to set the zero value and the player then leapt as high as possible from a two-footed standing position and knocked away the fingers of the yardstick. Players were allowed to use their arms and hands as desired to maximise jump height. Each player performed three jumps with a 20 second rest between efforts. The best jump was recorded for analysis. The vertical jump assessment was selected as it has high validity (0.8) and reliability (0.93) coefficients (Fatouros, et al., 2000).

Box squat strength (1-RM) was assessed using a squat rack and weighted box, an Olympic barbell and free weights (Fitness Works, New Zealand) and following a procedure similar to Cormie and colleagues (Cormie, et al., 2007). The player would start in a standing position with the load above the posterior deltoids at the base of the neck. Players would lower themselves to a sitting position on the box and then return to a standing position. The box height was adjusted for each athlete to allow the top of the thighs to be parallel to the

floor while in the seated position. Trained professionals monitored all sessions, providing encouragement and spotting lifts when necessary.

Jump squat power was assessed using a Smith machine and free weights (Fitness Works, New Zealand) and required the player to start in a standing position with a load equivalent to 50% of their box squat 1-RM above the posterior deltoids at the base of the neck. This load was based on work conducted by Baker, who reported loads in the range of 47-63% of 1-RM maximised power output (Baker, et al., 2001). Players would lower themselves into a half-squat position, before explosively jumping in the concentric phase to achieve maximal height. Players were encouraged to jump as high and as explosively as possible. On landing, players were instructed to sink to absorb their mass and the loaded bar. Peak power was calculated for the concentric phase using the GymAware system (Kinetic Performance Technology, Australia). The GymAware system consists of a linear encoder, attached to the bar via a retractable cord, and provides a valid ($r = 1.00$) and reliable (coefficients of variation $CV = 1.08\%$) measurement of concentric squat power (Drinkwater, Galna, McKenna, Hunt, & Pyne, 2007).

3.4.2 Exercise Bouts

Players completed two exercise bouts each week (Tuesday and Thursday) between 1500 and 1700 h in a randomised and balanced manner. Each bout was completed at least 48 hours apart and repeated twice over a four week period. No competitive rugby matches were scheduled during this training period and no other lower-body resistance training was performed. Players would arrive at the gymnasium where they were accustomed to training and warm up on an exercycle at a self-selected intensity for five minutes. After a supervised technique tutorial and stretching, the players completed a standardised warm-up of four sets of back squat exercise consisting of 15, 12, 10, 6 repetitions (reps) at ~50, 60, 70 and 80% of their 1-RM respectively, with a 60-s rest between sets. Subjects were assessed at the same

time of day to account for diurnal variation in performance and hormonal concentrations (Bird & Tarpenning, 2004; Atkinson, Todd, Reilly, & Waterhouse, 2005).

After a 4-min rest period, players performed one of two exercise blocks (1) power: jump squat exercise, 3 sets of 3 reps at 50% 1-RM with 3-min rest between sets; or (2) strength: box squat exercise, 3 sets of 3 reps at a 3-RM load with 3-min rest between sets. Each player then performed a second exercise block (either power or strength) after a further 4-min rest. Thus, there were four exercise bout combinations: power followed by power (power-power); power followed by strength (power-strength); strength followed by power (strength-power); and strength followed by strength (strength-strength). Each player performed each of these four exercise bout combinations on two separate occasions. Note that the power-strength and strength-power sessions have identical loads and volumes with only the order of the exercise differentiating these two bouts. Subsequent analysis of total weight lifted confirmed that the difference in loading between the power-strength and strength-power was very likely trivial ($0.9; \pm 1.7\%$). Figure 11 shows an example power-strength bout.

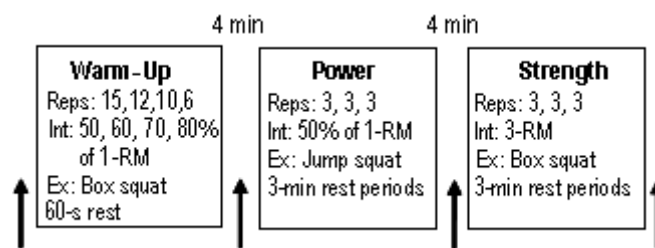


Figure 11. An example power-strength protocol indicating rest periods, exercise volume and intensity.

Reps: Repetitions, Int: Exercise intensity, Ex: Exercise, RM: Repetition maximum. Arrows represent saliva sample collection times.

All strength and power training was performed following the procedures outlined in the player assessment section above. The 3-RM load was adjusted throughout the course of the training period such that players were performing three lifts at maximal intensity within the exercise bouts. An estimated 1-RM load was calculated from this 3-RM data (Lander, 1985). The 50% 1-RM load for the power block was also adjusted throughout the course of

the training period to account for strength changes. Instant power feedback was delivered to the player after each jump squat effort along with verbal encouragement.

3.4.3 Hormone Assessment

Whole saliva samples were collected before and after the standard warm up, and at the completion of 3 and 6 sets of squat exercises (see Figure 11). Time-matched samples were also collected on a control day when no exercise was performed. Players expectorated a 2 mL sample into polyethylene tubes which was stored at -20°C until assayed. Sugar free gum was used to stimulate saliva flow before collection. Testosterone and cortisol concentrations were determined in triplicate using commercially available radioimmunoassay kits (Diagnostic Systems Laboratories, USA) modified for salivary measurement (Granger, Schwartz, Booth, & Arentz, 1999; Mörelius, Nelson, & Theodorsson, 2004). Testosterone assay sensitivity was 3.5 pmol·L⁻¹ with intra- and inter-assay CV of <8%. Cortisol assay sensitivity was 0.14 nmol·L⁻¹ with intra- and inter-assay CV of <10%. Saliva samples for each participant were analysed in the same assay to eliminate inter-assay variance. Salivary sampling was selected due to the fact that it is relatively stress free method of sample collection and because salivary steroid concentrations provide a good indication of fluctuations in the bioavailable free steroid concentrations in plasma (Sannika, Terho, Suominen, & Santti, 1983; Granger, et al., 1999; Kraemer, et al., 2001).

3.5 Statistical Analyses

Testosterone and cortisol concentrations were analysed with the mixed-model procedure (Proc Mixed) in the Statistical Analysis System (Version 9.1, SAS Institute, Cary, NC). The dependent variables were log-transformed before analysis; plots of residuals versus predicted showed that this transformation produced acceptable uniformity of error, and no observations were excluded as outliers. Back-transformation provided estimates of mean effects as percentages and errors as coefficients of variation. For qualitative assessment of magnitude, the log-transformed effects were standardised by dividing by the between-subject

standard deviation in the rest condition. In keeping with recent trends in inferential statistics, magnitude-based inferences about true (population) values of effects were made by expressing the uncertainty in the effects as 90% confidence limits. For brevity, confidence limits are shown as $\pm x$, where x represents half the confidence interval. Magnitudes of the standardised effects were interpreted using thresholds of 0.2, 0.6, and 1.2 for small, moderate and large, respectively. Standardised effects of between -0.19 and 0.19 were termed trivial. An effect was deemed unclear if its confidence interval overlapped the thresholds for substantiveness (that is, if the chances of the effect's being substantially positive and negative were both $>5\%$); otherwise the effect was termed clear and the magnitude of the effect was reported as the magnitude of its observed value (Hopkins, Marshall, Batterham, & Hanin, 2009). Thresholds for assigning the qualitative terms to chances of substantial effects were: $<1\%$, almost certainly not; $<5\%$, very unlikely; $<25\%$ unlikely; $25 - 75\%$, possibly; $>75\%$ likely; $>95\%$ very likely; and $>99\%$ almost certain.

3.6 Results

Of the sixteen players, thirteen completed the strength and power assessments and each of the eight exercise bouts over the four-week period. Three players were unable to complete the training program due to other commitments, illness, or unrelated injuries. Performance and body composition changes observed over the four-week training period are reported in Table 2.

Table 2. Performance and body composition percentage changes in 13 rugby players over four weeks.

	Mean; \pm CL	Effect Size
Box squat 1-RM (kg)	22; ± 5	Large
Peak Power (W)	7.6; ± 3.6	Moderate
Maximum vertical jump (cm)	3.5; ± 3.0	Small
Body fat (kg)	-2.9; ± 3.9	Small
Body mass (kg)	0.9; ± 1.1	Trivial
Soft lean mass (kg)	-0.6; ± 1.1	Trivial
Combined leg volume (L)	0.5; ± 0.6	Trivial

RM= Repetition maximum; CL= 90% Confidence limits.

For qualitative assessment of magnitude, effects were standardised by dividing by the between-subjects SD in the rest condition. Effect size was interpreted using thresholds of 0.2, 0.6, and 1.2 for small, moderate and large, respectively.

Figure 12 shows the hormonal responses to the four exercise bouts. The largest exercise-induced testosterone change was a small, clear increase (13%; 90% confidence limits $\pm 7\%$) observed at the conclusion of the strength-power bout. The strength-strength, power-power and power-strength bouts induced clear but trivial increases in testosterone. A small, clear increase in testosterone concentration in the strength relative to the power exercise blocks was seen at the mid-point of the exercise (9%; $\pm 6\%$). A small, clear increase in testosterone (10%; $\pm 8\%$) was also observed in the strength-power bout relative to the power-power bout immediately after exercise. No change was observed (0%; $\pm 15\%$) in testosterone over the same time period on a control non-training day.

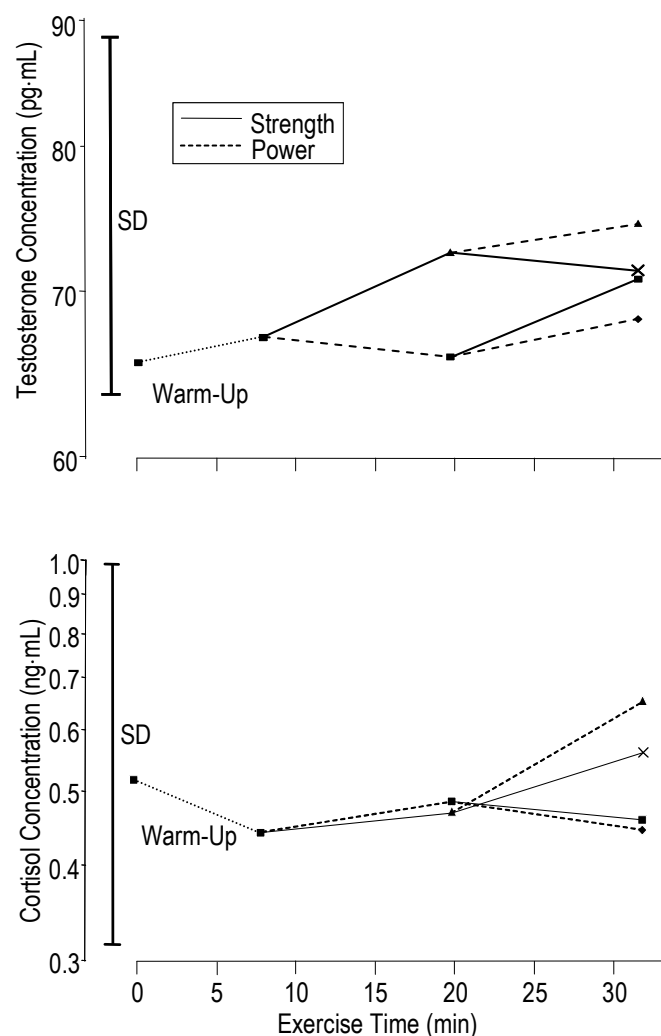


Figure 12. Testosterone and cortisol responses to four exercise protocols on a logarithmic scale.

SD= Between subject standard deviation in the resting condition, Warm-Up= Four sets of 15, 12, 10 and 6 repetitions at ~50, 60, 70 and 80% of individual one-repetition maximal weight with a 60-sec rest interval, Strength= Three sets of three repetitions at individual three-repetition maximum load with a three-minute rest interval, Power= Three sets of three repetitions at 50% of individual one-repetition maximum load with a three-minute rest interval.

In contrast to testosterone, cortisol showed little difference between the strength and power exercise blocks at the mid-point of the exercise. The overall exercise-induced cortisol response with each bout was also clearly trivial. As with testosterone, there was a small but clear cortisol difference (47%; $\pm 39\%$) between the strength-power and the power-power bouts immediately after exercise, and there was a similar difference (43%; $\pm 31\%$) between the strength-power and power-strength bouts after exercise. There was little change (10%; $\pm 38\%$) in salivary cortisol over the same time period on a control non-training day.

3.6 Discussion

The steroid hormone data presented are consistent with a favorable anabolic response following the strength-power bout compared with the power-power bout. To our knowledge, no other studies have reported that acute testosterone responses are enhanced following a complex training bout. Although the strength-power bout had an identical load and volume to the power-strength bout, only a trivial testosterone increase was observed following the power-strength bout. There was no substantial difference between the strength-power and power-strength bouts, however.

No other lower-body resistance training was performed over the experimental period and body composition data showed very little change, with only trivial changes in fat-free mass, leg volume, and total body mass. Similarly, Harris and colleagues (Harris, et al., 2000) reported a lack of evidence for lower-body hypertrophy over a nine-week complex training period despite performance increases in parallel squat strength and vertical jump height. The results of the current study also showed performance gains and demonstrated large 1-RM strength gains. Moderate increases in peak power and small, but clear, increases in vertical jump height were also observed despite a lack of discernable muscular hypertrophy. However, the design of the study prevents us from attributing these gains to a specific exercise bout.

Research by Fatouros and co-workers (Fatouros, et al., 2000) demonstrated that, in terms of strength gains, a combination training group that performed both plyometric and weight training improved to a greater extent than a plyometric training group, but gains were similar in a weight-training group. Similar results were reported by Rahimi and Behpur (Rahimi & Behpur, 2005) when comparing complex training with plyometric only training. Interestingly, the testosterone data in the current study followed a similar pattern to the performance data reported by Rahimi and Behpur, with a clear difference between the strength-power and the power-power bouts. Indeed, in terms of vertical jump height, power and flight time outcome measures, the previous study also found combination training superior to resistance training and plyometric training performed separately (Rahimi & Behpur, 2005). It should be mentioned that, in the study by Fatouros (Fatouros, et al., 2000), the plyometric training was performed three hours before the resistance training in the combination group, and there was no attempt to equate the total workload between groups. Many studies that examine complex training suffer from this limitation, as it is practically difficult to equate training duration and volume between complex and non-complex training groups.

A recent study (Mihalk, et al., 2008) that did equate total work reported similar improvements in vertical jump height when combining resistance and plyometric training within a workout (complex training), or on alternate training days (compound training). However, there was no control or 'standard' training group to compare the observed performance increases against as it was the intention of the authors to determine performance differences, as well as examine the rate of change of performance differences, between groups. Dodd and Alvar (Dodd & Alvar, 2007) used a counterbalanced rotation design to compare complex training with heavy resistance and plyometric training and equated the total volume in each intervention. These researchers found that when heavy resistance sets were immediately followed by a biomechanically similar exercise at <30% of 1-RM, sprint

times and standing broad jump improved to a greater extent. As a result, these authors concluded that complex training provided “equal, if not greater” functional benefits. Indeed, although the calculated effect sizes for the interventions were generally reported as being trivial (Dodd & Alvar, 2007), further examination of the data revealed small improvements in sprint speeds and agility when complex training was compared with resistance-only and plyometric-only training over a 15 week period.

The strength-power bout employed in the current study produced the greatest elevation in salivary testosterone. In other acute studies, Goto and colleagues have shown that an additional set at 50% of 1-RM following a strength session stimulated growth hormone secretion to a greater extent than additional sets at 20, 30, 70 or 90% of the strength session alone (Goto, et al., 2003; Goto, et al., 2004a). Furthermore, the addition of these low-intensity sets in a four week training period was associated with increased 1-RM strength, maximal isokinetic strength, and a trend towards an increase in muscle cross-sectional area (Goto, et al., 2004b).

The short rest periods and high repetitions performed as part of the standardised warm-up protocol in this study approximate hypertrophy-type training. Hypertrophic exercise bouts generally result in large increases in both testosterone and cortisol (Kraemer, et al., 1990; Kraemer, et al., 1998). Only a trivial hormonal increase was observed after the warm-up, although the short duration (approximately 8 min) of the warm-up is likely to explain this discrepancy. In contrast, the small increase in testosterone observed in response to the strength block is less common in literature, although salivary testosterone is seldom reported. It is possible that some of this testosterone response is due to the prior warm-up protocol; however, the lack of an increase following the power block performed on the same timescale would suggest otherwise.

In contrast to the testosterone data, there was no discernable difference between the cortisol levels at the exercise mid-point. Indeed, the relatively large variability in the cortisol

concentration meant that changes from resting values were insubstantial in all four exercise bouts. The average cortisol levels at all time points were below 1 ng·mL which is not surprising, as the early evening collection time points coincide with the nadir in the circadian rhythm (Bird & Tarpenning, 2004). Furthermore, the lack of substantial responses to the resistance exercise bouts is consistent with data suggesting that the circadian rhythm of cortisol influences the cortisol response to exercise (Kanaley, Weltman, Pieper, Weltman, & Hartman, 2001). Diurnal rhythms and individual responses in steroid secretion may mask responses to exercise if not accounted for. The control data in the current study however, indicates that hormone concentrations were relatively stable over the early evening sampling time period.

The current study, in combination with earlier growth hormone work, is suggestive of a hormonal mechanism that can mediate superior functional gains when resistance exercise is combined with low-intensity power-type exercises. Such findings reinforce the importance of understanding the hormonal training responses and the resulting training outcomes. A longitudinal study equating volume between complex and traditional training bouts that specifically addresses the influence of testosterone responses on functional outcomes would be beneficial

3.7 Practical Applications

The greatest testosterone response was observed when a power-type exercise was applied after a heavy resistance training stimulus. Therefore, a complex exercise sequence provided a favourable anabolic response for adaptation compared to the other training methods examined. As recent research has linked salivary testosterone responses with enhanced strength and body weight gains (Beaven, et al., 2008a), it is suggested that the superior functional gains associated with complex training are related to the improvements in the hormonal environment. These data have practical implications for exercise and training prescription designed to increase or maintain strength.

CHAPTER FOUR: STUDY TWO – CIRCADIAN RHYTHMICITY

4.0 Lower-body Strength and Power Development during Different Phases of the Circadian Rhythm

4.1 Prelude

The review also highlighted that hormonal biorhythms are responsible for coordinating a range of vital functions in the human body. In particular, testosterone and cortisol are characterised by diurnal fluctuations. Steroid hormones can affect multiple aspects of the neuromuscular system and regulate performance factors such as maximal force and power output which likely contribute to strength and power adaptation. In the context of this thesis, it was intended to assess whether the time at which resistance exercise was performed within the circadian biorhythms of salivary testosterone and cortisol could influence strength and power adaptive gains. The implications of this hormonal and adaptational information for researchers and practitioners are discussed.

4.2 Introduction

It is recognised that circulating concentrations of testosterone and cortisol are characterised by a circadian rhythm, with highest levels in the morning and a nadir in the late afternoon (Lévi, et al., 1988; Cooke, et al., 1993; Kraemer, et al., 2001; Diver, et al., 2003). It is proposed that this circadian rhythm is responsible for coordinating the vital functions of a range of tissues and controlling the timing of daily secretory events (Winget, et al., 1985). The elevated testosterone levels typically observed are thought to stimulate protein synthesis, whereas the morning rise in cortisol accelerates metabolism (Florini, 1987), and stimulates gluconeogenesis and proteolytic activity (Dineen, et al., 1993). Kraemer (1988) has suggested that the observed early morning increase in testosterone may serve an anti-catabolic role helping to protect the muscle against the proteolytic effect of cortisol on skeletal protein (Kraemer, 1988).

It is also known that maximal voluntary strength also exhibits a circadian pattern with higher values observed in the afternoon than in the morning (Drust, Waterhouse, Atkinson, Edwards, & Reilly, 2005). Researchers have also demonstrated that peak torque, peak anaerobic power (Souissi, Gauthier, Sesboüe, Larue, & Davenne, 2002; Souissi et al., 2007), and time trial performance (Atkinson, et al., 2005) are higher in the evening than in the morning. Thus, many factors associated with muscular performance are increased when exercise is performed in the afternoon. Indeed, Deschenes and co-workers have reported that the functional capacity of muscle was at its lowest in the early morning and peaks in the early evening (Deschenes, et al., 1998). However, they also stated that the early morning could be considered the “optimal” time for resistance exercise due to the greater pre- and post-exercise plasma testosterone levels observed relative to other times of day (Deschenes, et al., 1998). They did suggest though, that if the ratio of testosterone to cortisol provided a more accurate reflection of the anabolic potential for protein accretion then in fact, the early evening may be more “conducive to resistance exercise”.

The time of day that resistance exercise is performed has been shown to modulate cortisol responses with significantly greater responses observed when exercise is performed in the morning (Kanaley, et al., 2001). Indeed, researchers have reported that the optimal time for resistance training is in the evening due to the enhanced “anabolic/catabolic status” (Bird & Tarpenning, 2004). In terms of resistance training outcomes, one study demonstrated that maximal strength gains from two daily sessions (performed morning and afternoon) were superior to one session of equal volume performed in the morning (A. Häkkinen & Kallinen, 1994). No hormonal data was presented by these Finnish researchers; however it is possible that the enhanced anabolic environment and/or anabolic responsiveness in the afternoon contributed to the superior functional outcome observed.

Hansen and colleagues (Hansen, et al., 2001) have described a larger relative increase in isometric strength when the anabolic hormonal response was enhanced by additional leg

exercises. Furthermore, Staron and co-workers (Staron, et al., 1994) linked increases in strength and muscle fibre transformation in males to elevated serum testosterone levels. More recent data have demonstrated that suppression of endogenous testosterone attenuates strength and muscle mass gains observed over an eight-week strength training period (Kvorning, et al., 2006). Beaven and colleagues (2008) have reported enhanced strength and body weight gains when rugby players were prescribed resistance exercise that maximised the acute salivary testosterone response (Beaven, et al., 2008a). Nutritional supplementation that blunts the cortisol response to resistance exercise has also been shown to enhance the functional adaptations associated with resistance exercise (Bird et al. 2006). These data, taken together, may suggest a link between acute exercise-induced modification of the anabolic/catabolic status of the body and functional adaptation.

The response of cortisol to resistance exercise is known to be influenced by the circadian rhythm and testosterone responses have been reported to enhance functional outcome of resistance exercise. Responses of semi-professional rugby players to resistance exercise performed either in the early morning or early evening were investigated. It was believed that the anabolic/catabolic status of the body post exercise would differ in a training time-specific manner due to the effect of the circadian rhythm. Identification of differential responses would provide valuable information regarding an optimal time to train in order to maximise the post-exercise anabolic milieu.

4.3 Methods

4.3.1 Subjects

Eight semi-professional male athletes from a single rugby team (see Table 3 for characteristics) were recruited to participate in the study which involved performing a back squat protocol either in the morning (0700-0800 hrs) or late afternoon (1600-1700 hrs). The athletes were involved in two 4-wk pre-season training blocks (approximately 10 hours per week) as part of a high-performance academy for talented players.

Table 3. Physical characteristics of the 8 players monitored.

	Mean \pm SD
Age (yr)	21.1 \pm 1.7
Height (cm)	188 \pm 7
Body mass (kg)	100 \pm 14
Soft lean mass (%)	82.1 \pm 3.0
Body fat (%)	12.9 \pm 4.9
1-RM (kg)	151 \pm 17
Jump-squat peak power (W)	4530 \pm 720
RM= Repetition maximum	

Each pre-season training block was separated by a full rugby season and off-season and immediately preceded by a two week enforced break from training. No games were scheduled during either of these two pre-season training blocks. All athletes were fully informed of the nature and possible risks of the study before giving written consent. Athletes were also informed that they could cease their participation in the trial at any time without giving a reason, and with no repercussions. Ethical approval was obtained from the Waikato Institute of Technology Ethics Committee.

4.3.2 Procedure

Each athlete's body composition, 1-RM and jump-squat peak power were assessed in the week prior to the start of the experimental block and again at the end of the fourth training week. Body composition (body mass, soft lean mass, body fat) was estimated using a reliable eight point, tetra-polar, segmental bioelectrical impedance analyser (*InBody 3.0*, Biospace Seoul, South Korea), according to the manufacturers' guidelines (Medici & Bedogni, 2005). For the purposes of this study, soft lean mass is defined as the sum of the total body water and the protein mass as estimated by the *InBody 3.0* bioelectrical impedance device. Throughout each four week block, athletes completed two exercise bouts each week (Tuesday and Thursday) in a gymnasium where they were accustomed to training. Thus, each bout was completed eight times over each four week training block, and at least 48 hours apart.

During the first block the athletes performed the squat exercise bouts in the morning. In the second block, the same athletes performed the squat exercise bouts in the late afternoon. The two blocks were incorporated into the pre-season trainings for two consecutive rugby seasons and no other lower body resistance exercise was performed during either training block. Each bout commenced with a warm up on an exercycle at a self-selected intensity for 5 min and supervised stretching. The athletes then completed four sets of back-squat exercise comprising 15, 12, 10, 6 repetitions (reps) at ~50, 60, 70 and 80% of each individual's 1-RM (repetition maximum) respectively, with a 60-s rest between sets. Athletes subsequently performed three sets of 3-RM box squat and three sets of jump-squat at a load equivalent to 50% of their box squat 1-RM.

Box squat strength (1-RM) was assessed using a squat rack and weighted box, an Olympic barbell and free weights (Fitness Works, New Zealand) and following a procedure similar to Cormie and colleagues (Cormie, et al., 2007). The player would start in a standing position with the load above the posterior deltoids at the base of the neck. Players would lower themselves to a sitting position on the box and then return to a standing position. The box height was adjusted for each athlete to allow the top of the thighs to be parallel to the floor while in the seated position. Trained professionals monitored all sessions, providing encouragement and spotting lifts when necessary.

Jump squat power was assessed using a Smith machine and free weights (Fitness Works, New Zealand) and required the player to start in a standing position with a load equivalent to 50% of their box squat 1-RM above the posterior deltoids at the base of the neck. This load was based on work conducted by Baker, Nance and Moore who reported loads in the range of 47-63% of 1-RM maximised power output (Baker, et al., 2001). Players would lower themselves into a half-squat position, before explosively jumping in the concentric phase to achieve maximal height. Players were encouraged to jump as high and as explosively as possible. On landing, players were instructed to sink to absorb their mass and

the loaded bar. Peak power was calculated for the concentric phase using the GymAware system (Kinetic Performance Technology, Australia). The GymAware system consists of a linear encoder, attached to the bar via a retractable cord, and provides a valid ($r = 1.00$) and reliable (coefficients of variation $CV = 1.08\%$) measurement of concentric squat power (Coutts, Reaburn, Piva, & Murphy, 2007; Drinkwater, et al., 2007).

Immediately prior to, and following the exercise bout, the athletes were asked to provide a whole saliva sample of approximately 2 mL. Sugar-free gum (Extra, Wrigleys, Auckland, New Zealand) was provided to assist with saliva stimulation (Höld, et al., 1995). Saliva samples were stored at -20°C until assay and were analysed in triplicate for testosterone and cortisol using commercially available radioimmunoassay kits (Diagnostic Systems Laboratories, USA) modified for salivary measurement (Granger, et al., 1999; Mörelius, et al., 2004).

Testosterone assay sensitivity was $3.5 \text{ pmol}\cdot\text{L}^{-1}$ with intra- and inter-assay coefficients of variation (CV) of $<8\%$. Cortisol assay sensitivity was $0.14 \text{ nmol}\cdot\text{L}^{-1}$ with intra- and inter-assay CV of $<10\%$. Saliva samples for each participant were analysed in the same assay to eliminate inter-assay variance. Salivary sampling was selected due to the fact that it is relatively stress free method of sample collection and because salivary steroid concentrations provide a good indication of fluctuations in the bioavailable free steroid concentrations in plasma (Sannika, et al., 1983; Granger, et al., 1999; Kraemer, et al., 2001).

4.4 Statistical Analyses

All steroid hormone data were log transformed prior to analysis to reduce non-uniformity of error and to express effects as percent changes. Back-transformation provided estimates of mean effects as percentages and errors as coefficients of variation. For qualitative assessment of magnitude, the log-transformed effects were standardised by dividing by the pre-exercise, between-athlete standard deviation. The magnitude of an effect was deemed unclear if its'

confidence interval overlapped positive and negative thresholds for substantiveness. When this overlap did not occur, the magnitude of the observed effect is reported using thresholds of 0.2, 0.6 and 1.2 for small, moderate and large respectively, a modification of Cohen's thresholds of 0.2, 0.5 and 0.8 (Cohen, 1988). These modifications are based primarily on congruence with Cohen's thresholds for correlation coefficients (Hopkins 2009). Thresholds for assigning the qualitative terms to chances of substantial effects were: <1 %, almost certainly not; <5 %, very unlikely; <25 % unlikely; 25 – 75 %, possibly; >75 % likely; >95 % very likely; and >99 % almost certain. Statistical comparisons of the hormonal responses to resistance exercise sessions were made based on the time of day that they were performed using a pre-post crossover trial spreadsheet available at www.sportsci.org.

4.5 Results

Pre- and post- exercise salivary testosterone and cortisol concentrations were clearly higher in the morning than in the late afternoon (Figure 13). For testosterone, samples taken in the morning (0700 to 0800 hrs) were a factor of 1.8 ($\times/\div 1.1$; 90% confidence limits) greater before exercise, and 1.4 ($\times/\div 1.1$) greater after exercise, than samples taken in the afternoon (1600 to 1700 hrs). For cortisol, samples taken in the morning were a factor of 5.7 ($\times/\div 2.3$) greater before exercise, and 3.6 ($\times/\div 2.3$) greater after exercise, than samples taken in the afternoon. In contrast, the ratio of testosterone to cortisol was clearly lesser in the morning by a factor of 0.3 ($\times/\div 2.4$) (Figure 14). Testosterone and cortisol almost certainly decreased over the morning exercise bouts by 19% ($\pm 8\%$; 90% confidence limits) and 48% ($\pm 24\%$), respectively. However, only trivial hormone changes were seen in response to the afternoon training. There was a moderate increase of 60% ($\pm 20\%$) in the testosterone to cortisol ratio during the morning exercise bouts with only trivial increases in the afternoon (23; $\pm 25\%$).

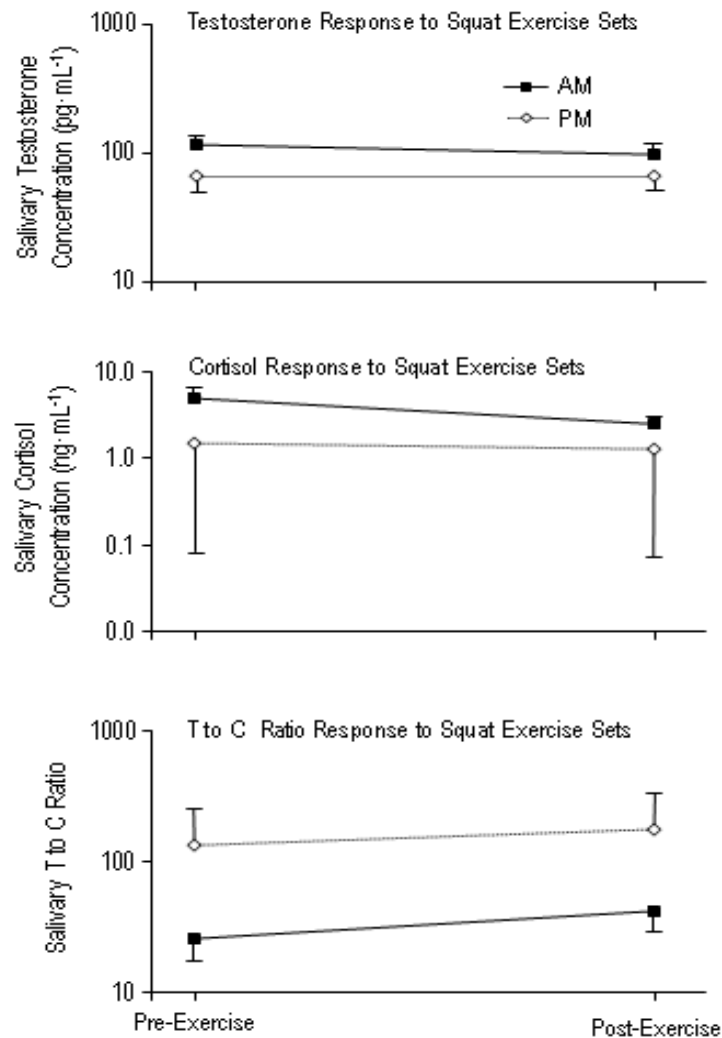


Figure 13. Average hormonal responses to squat exercise sets performed either in the morning or the afternoon (n=8).

T= testosterone; C=cortisol. Error bars are standard deviations.

Figure 14 indicates that a small increase in jump-squat peak power produced at a load equivalent to 50% 1-RM was evident after the four week afternoon training block ($8.5 \pm 11.2\%$). The change over the same time period when bouts were performed in the morning was trivial ($3.8 \pm 6.7\%$). Box squat 1-RM strength moderately increased over both four week training blocks (PM: $17.4 \pm 4.1\%$; AM: $16.6 \pm 2.5\%$) with no difference between the strength gains observed. No clear differences in the measures of body composition were observed over either of the four week training blocks.

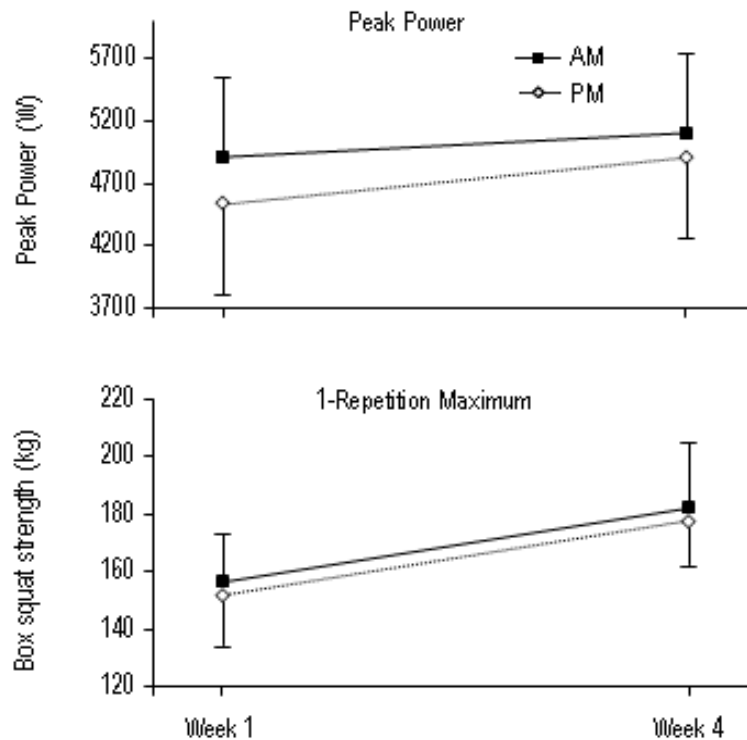


Figure 14. Change in jump-squat peak power and box squat strength over 4-wk training period (n=8). Error bars are standard deviations.

4.6 Discussion

One clear outcome was a training time-dependent difference between salivary testosterone and cortisol, and their ratio, both before and after resistance exercise bouts. Circadian differences have previously been described (Lévi, et al., 1988; Cooke, et al., 1993; Kanaley, et al., 2001; Kraemer, et al., 2001; Diver, et al., 2003; Bird & Tarpenning, 2004) however some researchers have failed to observe significant circadian variation in salivary cortisol levels (Dimitiou, et al., 2002). The current paper utilizing salivary sampling shows clear differences between the anabolic/catabolic environment in the body at different times of day.

The levels of salivary testosterone and cortisol measured in the current study are comparable to those observed elsewhere (Kraemer et al. 2001; Di Luigi et al. 2006; Crewther et al. 2009; Cadore et al. 2009; Argus et al. 2009) and fall within the normal range (Ellison et al. 2002). The importance of monitoring both testosterone and cortisol concomitantly is due to the fact that cortisol can potentially act in an antagonistic and inhibitory manner to that of testosterone. Glucocorticoids have been reported to suppress the secretion of testosterone's

signaling peptide, luteinising hormone, and thus production of cortisol can suppress the secretion of testosterone (Doerr & Pirke, 1976; Nindl, et al., 2001). Glucocorticoids are also known to inhibit Leydig cell steroidogenesis (Welsh et al. 1982) and protein synthesis by affecting translational initiation (Shah, et al., 2000c, 2000d). Cortisol has also been reported to elevate serum free fatty acid levels which may attenuate testosterone secretion (Meikle et al. 1989) and growth hormone responses to resistance exercise (Wu & Lin 2010). Furthermore, Chen and colleagues provided evidence of a direct physical interaction between androgen and glucocorticoid receptors at the transcriptional level contributed to their opposing physiological effects (Chen, Wang, Yu, Liu, & Pearce, 1997). Indeed, exercise studies have reported negative correlations between cortisol and testosterone concentrations (Brownlee et al., 2005; Daly et al. 2005). Thus, the ratio of testosterone to cortisol may provide an indication of the anabolic/catabolic status of the endocrine system and the capacity of the system to realise adaptive outcomes.

The greater ratio of testosterone to cortisol observed in the afternoon session did not appear to affect the strength gains observed over the four week training block as similar increases were observed over the two distinct training blocks. Indeed, the training gains in box squat strength observed in the current study are of a similar magnitude to those observed in a study of elite rugby players over an equivalent time period (Argus, Gill, Keogh, Hopkins, & Beaven, 2010). This observation demonstrates that positive, moderate improvements in lower body strength are achievable within a relatively short time frame in well-trained athletes in a de-trained state. As the eight bouts in each block were the only lower body resistance exercise performed, and exercise volume and intensity was matched, it can be concluded that each exercise block was equally effective in increasing 1-RM box squat strength regardless of the time of day performed. This conclusion is similar to that reached by other authors (Souissi, et al., 2002; Sedliak, Finni, Cheng, Kraemer, & Häkkinen, 2007) although Sedliak and colleagues did note a “slightly greater” increase in maximal voluntary contraction peak torque after a ten-week

program when lower-body exercise was performed between 1700-1900 hrs compared to 0700-0900 hrs.

In contrast to maximal strength, peak power produced at a load equivalent to 50% of 1-RM during a countermovement jump-squat was improved to a greater extent during the four week block when training was performed in the afternoon. In a similar study with Super 14 rugby players conducted by Argus and colleagues, a small decrease in lower-body power was observed over an identical time frame (Argus, et al., 2010). It is possible that the lower volume of resistance training session performed in the current study allowed an adequate recovery according to the fitness-fatigue model (Chiu & Barnes, 2003). It is also worth noting that the majority of the resistance exercise performed by the Super 14 players was in the morning. Our results showed only trivial increases in lower-body power when training was performed in the morning, in contrast to the small increase observed when training was performed in the late afternoon. One possible explanation for this observation is that the elevated testosterone to cortisol ratio contributes to the realisation of gains in the functional ability of the muscle to produce force rapidly. However, it is also possible that the greater initial levels of power production in the second training block limited the potential to see gains of a similar magnitude to those of the first training block.

Cross-sectional studies have identified associations between endogenous testosterone levels and vertical jump power output (Bosco et al., 1996a), maximal anaerobic power (Mero, Jaakkola, & Komi, 1990), squat-jump power (Crewther, et al., 2009a), counter-movement jump height, and 30-m running speed (Bosco, et al., 1996b). Indeed, several studies have reported relationships between testosterone and/or cortisol concentrations, and power or strength adaptation in trained athletes (K. Häkkinen, Pakarinen, Alen, & Komi, 1985; K. Häkkinen, Pakarinen, Alen, Kauhanen, & Komi, 1988; J. P. Ahtiainen, et al., 2003a). Importantly, an anabolic endocrine environment has been suggested to be “decisive” for the development of fast twitch muscle fibres (Viru & Viru, 2005).

It is clear however, that testosterone and cortisol are not the sole determinants of muscular adaptation. Adaptation is the result of a raft of mechanical, endocrine, cellular, and molecular processes that are essential to the adaptive process (Favier, et al., 2008) in which the intensity, volume, and type of muscular contractions are important determinants (Kraemer & Ratamess, 2005; Tidball, 2005). Indeed, individual differences in acute hormone response to resistance training are known modulate adaptive outcomes (Beaven et al., 2008b). It is also possible that other circadian factors would influence the optimal time to perform resistance exercise such as core temperature which influences neural conduction velocity (de Jong, et al. 1966) and lower body power production (Racinais et al. 2005; Taylor et al. 2009). It is also possible that normal locomotor activity may prime lower body exercise performed later in the day. Ultimately, the improvements in lower body strength observed by Häkkinen and Kallinen in 1994 cannot be explained by the current hormonal data as no difference in strength gain was observed in our rugby athletes. It is possible that the lower acute training volume, and thus a decrease in fatigue resulting in more effective cumulative training adaptation, contributed to the strength improvements observed in these Finnish athletes.

4.7 Conclusion

There are obvious differences between the hormonal environments experienced by an individual exercising at different times within the circadian rhythm. The current data suggests that these differences have the potential to modulate adaptation to exercise. Specifically, greater gains in peak power produced at a load equivalent to 50% of 1-RM during a countermovement jump-squat were observed when training was performed in the late afternoon compared to training in the morning. As such, the testosterone to cortisol ratio, rather than absolute testosterone and cortisol concentrations, may work in conjunction with other mechanisms to modulate power gains and underlie the performance gains observed in these semi-professional rugby athletes.

CHAPTER FIVE: STUDY THREE – ULTRADIAN RHYTHMICITY

5.0 Ultradian Rhythmicity and Induced Changes in Salivary Steroid Hormones

5.1 Prelude

In assessing the daily biorhythms within endogenous hormone production, it was apparent that underlying ultradian rhythms played a key role in hormone regulation. Interestingly, research in an animal model demonstrated that the pulsatile nature of steroidal stress hormone production had the potential to modulate the physiological response to an applied stressor. Due to the comparable regulation of steroid hormones in humans, it was hypothesised that a biorhythm may be identifiable in human saliva. In the context of this thesis, it was intended to investigate steroid hormone pulsatility and the potential of such a biorhythm to influence response to an exercise stimulus. The implications of this information regarding the influence of ultradian rhythms on exercise responses for researchers and practitioners are discussed.

5.2 Introduction

In humans, testosterone and cortisol are known to respond to exercise stimuli (Kraemer, et al., 1990; J. U. Ahtiainen, Pakarinen, Kraemer, & Häkkinen, 2004). Indeed, maximal exercise is known to elicit rapid hormonal responses in humans with the magnitude of the response related to the intensity of the exercise (Kraemer, et al., 1990; Busko & Opaszowski, 2005; Crewther, Lowe, & Weatherby, 2007). Video games have also been used as a purely psychological intervention to evoke acute hormonal responses (Mazur, Susman, & Edelbrock, 1997; Sharma, Khera, Mohan, Gupta, & Ray, 2006). Cortisol promotes the mobilisation of energy reserves during physical activity by stimulating gluconeogenesis, promoting lipolysis and increasing protein catabolism (Viru & Viru, 2004). Stimulation of testosterone secretion promotes muscular hypertrophy via activation of satellite cells (Kadi, 2008) and administration has been associated with positive changes in muscle architecture

(Blazevich & Giorgi, 2001), strength (Strawford, et al., 1999), and fat-free mass (Bhasin, et al., 1997). Importantly, putative sites of action within the mammalian target of rapamycin signaling pathway, which is intimately involved in skeletal muscle adaptation, have been demonstrated for both testosterone (Altamirano, et al., 2009) and cortisol (Shah, et al., 2000c). Indeed, the contribution of testosterone and cortisol in realizing the adaptive responses to resistance exercise have been previously reported (Hansen, et al., 2001; Bird, et al., 2006b; Kvorning, et al., 2006; Beaven, et al., 2008a).

Both cortisol and testosterone exhibit circadian rhythms in both basal levels, which are elevated in the morning hours with a nadir in the late afternoon, and responsiveness to stimuli (Kanaley, et al., 2001; Kraemer, et al., 2001). The basal concentrations of both testosterone and cortisol are regulated by the hypothalamus and are characterised by pulsatile secretion that defines both ultradian and circadian rhythmicity. Pulsatile release and rapid clearance of endogenous steroids overlay the circadian rhythm and characterise an ultradian rhythm that has the effect of exposing target tissues to large fluctuations of hormone and is a critical component of biological actions (Young, et al., 2001; Veldhuis, et al., 2008). Episodic secretion of cortisol and testosterone have been identified in a variety of species (Sarnyai, et al., 1995; Windle, et al., 1998; Ingram, et al., 1999; Young, et al., 2001; Schlatt, et al., 2008) including humans (Winters & Troen, 1986; Veldhuis, et al., 1987a; Schürmeyer, et al., 1996; Young, et al., 2001; Young, et al., 2004) and it is proposed that pulsatile release may function to prevent down regulation of the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes enabling the maintenance of maximal responsiveness to stressors (Veldhuis, et al., 1987a; Young, et al., 2004).

Knowledge of such ultradian rhythmicity is necessary in order to understand the control of the HPA and HPG axes and factors that influence these systems. At the very least, knowledge of ultradian rhythmicity should suggest that single point hormonal sampling, without an understanding of the underlying basal pattern, may not provide an accurate

depiction of endocrine activity. Pulsatility itself may communicate different information and the rapidly changing concentrations of hormones may result in differential occupation of receptors throughout the circadian rhythm (Young, et al., 2004). Indeed, periods of post-pulse quiescence have been associated with hypo-responsiveness of the HPA axis to an audio stressor (Windle, et al., 1998). These results in rats clearly demonstrated that the underlying pattern of HPA activity can be a determinant of reactivity to acute stress. Indeed, pulsatility and the resultant periods of post-pulse quiescence may partially account for the characteristic variability observed in individual hormone responses (Jensen, et al., 1991; Ellison et al., 2002; Beaven, et al., 2008a; Beaven, et al., 2008b). However, few researchers have addressed the role ultradian rhythms play in on exercise-induced hormone responses.

An appreciation of pulsatility suggests the necessity for multiple samples to build a dynamic profile of hormonal concentrations rather than a single point 'snap-shot'. Such a sampling regime benefits from a minimally-invasive sampling approach which avoids stress that may confound results. To avoid HPA axis activation due to the stress associated with venepuncture (Merran, et al., 1993; Kirschbaum & Hellhammer, 2000) salivary sampling was utilised. This method allowed the collection of a large number of serial samples on consecutive days. A range of research from a variety of sources has formed a solid basis for the use of salivary hormones in assessing endocrine status (Vittekk, et al., 1985; Dabbs, 1990; Obminski & Stupnicki, 1997; Pruessner, et al., 1997; Kraemer, et al., 2001; Gozansky, et al., 2005; Kraemer & Ratamess, 2005; Erskine, et al., 2007). Salivary sampling also offers the advantage of assessing the free steroid concentration that is available to interact with appropriate receptors to actualise physiologic effects (Erskine, et al., 2007; Wood, 2009).

The interaction between dynamic basal rhythms and physiological responsiveness of the HPA axis in the rat indicates a mechanism that has the potential to be an important factor in determining differential responses of individuals to exercise. As the ultradian rhythmicity has been demonstrated to influence physiological responses to external stimuli, it was

proposed that applying an intervention at a time point that may coincide with a secretory event could provide a greater physiological response than during a period of quiescence. Thus, the current study aimed to establish: whether episodic secretion could be detected in the saliva of healthy human males and; whether the response to stimuli was modulated by ultradian rhythmicity.

5.3 Methods

5.3.1 Procedure

Seven healthy and recreationally active males who were not currently participating in an organised training regime ([Mean \pm SD] age: 27 ± 7 y; body mass 83.5 ± 20.1 kg; height 174.1 ± 4.9 cm) volunteered to participate in this study. Fasted subjects arrived at the study venue at 0745 h and completed informed consent forms and were given instructions for the day. Subjects were also informed that they could cease their involvement in the study at any time without repercussion. Sample collection commenced at 0800 h thereafter subjects were provided with a standardised breakfast that consisted of Weetbix®, milk and orange juice (2104 kJ, 15% protein, 17% fat, 68% carbohydrate). The first day was a non-intervention day and subjects remained at the study venue until 1600 h providing a saliva sample every ten minutes when prompted by an audible alarm. At midday subjects were provided with a standardised meal that consisted of ham, cheese and tomato wholegrain sandwiches, and orange juice (2879 kJ, 18% protein, 24% fat, 58% carbohydrate). Subjects were permitted to drink water *ad libitum*.

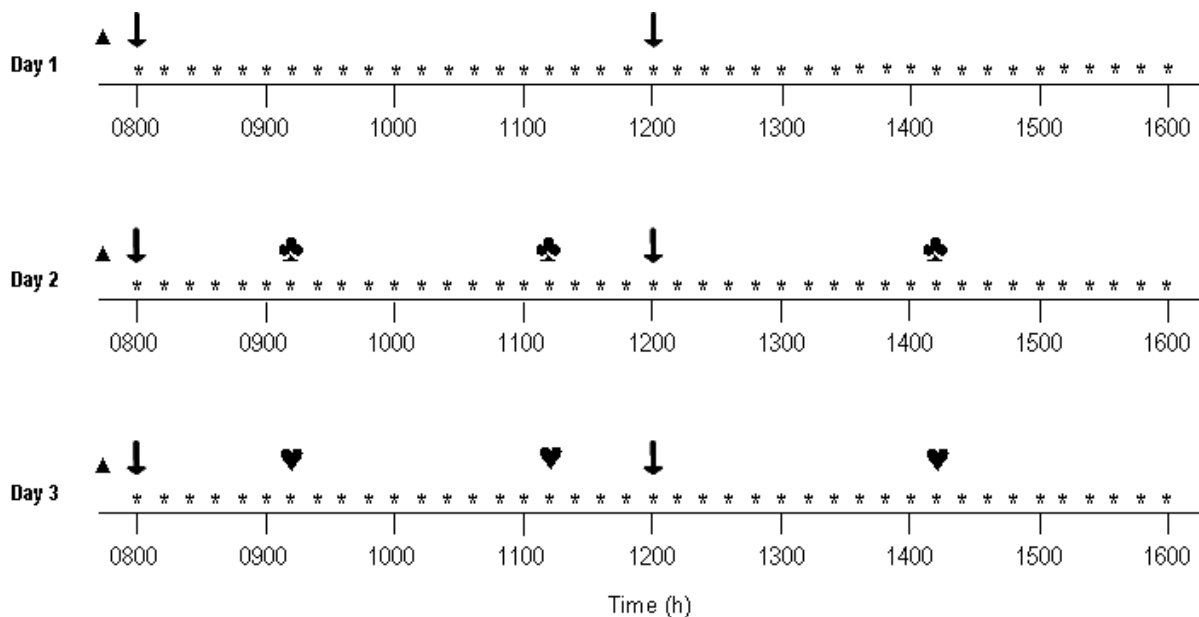


Figure 15. Methodological timeline depicting the sequence of events on three consecutive study days.

▲= Arrive at study venue, ↓= Consume standardised meal, *= Provide saliva sample, ♣= First set of subjects perform one of three interventions at random, ♥= Remaining subjects perform one of three interventions at random.

Subjects randomly performed three interventions on one of the two remaining study days. The second and third days were identical to the first day apart from the superimposition of the interventions. Thus, each subject was monitored over two non-intervention days and one intervention day (Figure 15). The interventions were: completion of two 30 sec cycle sprints; completion of two 30 sec boxing efforts and; participation in the violent, player versus player, video combat game. The interventions were performed in a randomised order with at least two hours separating each challenge. The sprints were performed on a friction-loaded cycle ergometer (Monark Ergonomic 834E, Sweden), using a resistance equivalent to 10% of an individual's body mass. Seat height was adjusted for each individual and the feet fixed to the pedals with toe-clips. After warming up for two minutes, subjects began unloaded pedaling and, once the load was applied, were required to pedal at maximum effort for 30 seconds. A further 30 sec effort was performed one minute after the completion of the first effort with subjects instructed to continue unloaded pedaling during the intermediary period. Subjects remained seated throughout and were given strong verbal encouragement. The boxing intervention also consisted of two maximal 30 second efforts with a one minute

recovery period and involved wearing boxing gloves and striking hand-held focus pads. The video game intervention consisted of a 10 minute first-person shooter scenario where two subjects were encouraged to shoot, maim and kill each other in a simulated futuristic wartime environment ('Killzone'® PS2, Sony, USA). Ethical approval was obtained from the Waikato Institute of Technology Ethics Committee.

5.3.2 Saliva Collection and Analyses

Whole saliva samples of approximately 5mL were collected every 10 min via expectoration into a graduated 10-ml centrifuge tube (LB SCT1002; Labserve, Auckland, N. Z.). Sugar-free gum (Extra®, Peppermint, Wrigley's, N.Z.) was provided to assist with saliva stimulation. To prevent blood contamination of saliva, resulting in an overestimation of hormone concentrations, subjects were advised to avoid brushing their teeth and drinking hot fluids in the two hours prior to reporting to the study venue. Saliva samples were stored on ice and transported to a freezer at the end of each study day where they remained at -20°C until assay. Saliva samples were analysed in triplicate for testosterone and cortisol using radioimmunoassay (RIA). The methods were modified from those described by Granger and colleagues (Granger, et al., 1999). Briefly, standards from serum diagnostic kits (Diagnostic Systems Laboratories, USA) were diluted in phosphate buffer saline (Sigma P4417) to cover the ranges of 0-18.56 and 0-1.73 nmol·L⁻¹, for cortisol and testosterone respectively. Saliva sample sizes of 50 and 100µL were used for cortisol and testosterone respectively. The supplied antibody solutions were diluted (1:5 and 1:13 for cortisol and testosterone, respectively) in a phosphate buffered saline solution containing 0.05% bovine serum albumin. This dilution resulted in approximately 50% binding in the zero standard compared to the total counts (10,000 and 4,500 counts per minute for cortisol and testosterone, respectively). Detection limits for the assays were 0.013 and 0.002 nmol·L⁻¹, for cortisol and testosterone respectively. The intra- and inter-assay coefficients of variation were <9% for cortisol and <10% for testosterone.

5.3.3 Statistical Analyses

Data from all subjects were included in the analysis. All intervention-induced hormone responses were log transformed before analysis to reduce non-uniformity of error and effects and expressed as percent changes (Hopkins, 2002). In keeping with trends in inferential statistics (e.g., (Sterne & Smith, 2001)), magnitude-based inferences about true (population) values of effects were made by expressing the uncertainty in the effects as factor 90% confidence limits. For brevity, confidence limits are shown as \times/\div 'x'. An effect was deemed unclear if its confidence interval overlapped the thresholds for substantiveness (that is, if the chances of the effect being substantially positive and negative were both $>5\%$); otherwise the magnitude of the effect was reported as the magnitude of its observed value (Batterham & Hopkins, 2006). The smallest standardised change was assumed to be 0.20 (Cohen, 1988). Pearson correlations provided estimates of linear associations between the subject characteristics and aspects of their hormonal profiles. The reported magnitudes for correlations are based on the thresholds of Cohen (1988) and the 90% confidence intervals derived via the Fisher z transformation to estimate the likely range of the true value.

An automatic deconvolution method (Pulse_XP, Version 20080429, University of Virginia) was employed to derive quantitative estimates of testosterone secretory events on non-intervention days (Veldhuis, et al., 1987a). Single-component elimination half-life and Gaussian distribution of secretory rates were assumed. Concordance between the modeled 'best fit' and the observed temporal hormone profile was assessed using the root mean square weighted residual statistic provided in 'Pulse_XP' software and visual inspection in an iterative process. The measurement error at each datum point was estimated as recommended by Johnson and colleagues (M. L. Johnson, et al., 2008). Hormonal pulse half-duration, half-life of elimination, and distribution volume were assumed to be constant over the three day experimental period for all subjects. No outlying data points were identified by the

‘Pulse_XP’ software. Pulse detection was not attempted on the cortisol temporal profiles due to the presence of non-spontaneous increases invoked by feeding. Approximate entropy values were determined on non-intervention days using Pulse_XP to quantify the orderliness of sub-patterns in the sequential testosterone and cortisol measures (Veldhuis, et al., 2009). Approximate entropy is a sensitive and specific statistic that is a translation- and scale-independent measure of a data series and changes in this measure have been reported to be predictive of subsequent clinical changes (Veldhuis, et al., 2008). These authors have suggested that increased irregularity (approximate entropy) with a hormonal time series is commonly associated with attenuation of negative feedback.

5.4 Results

Deconvolution data confirmed the presence of pulses over the eight hour sampling period for testosterone with an inter-pulse interval of 47 ± 9 min. Figure 16 represents typical salivary testosterone profiles with a modeled best fit derived by deconvolution software. A very large negative linear association between age and mean testosterone concentration was observed (Pearson correlation $r = -0.72$, 90% confidence interval 0.39 to 0.89). Approximate entropy data which quantifies the degree of orderliness within serial data, also suggested a greater degree of relative randomness in the salivary testosterone data set than in salivary cortisol with the approximate entropy of testosterone being 0.3 ± 0.1 units higher than that determined in the cortisol data.

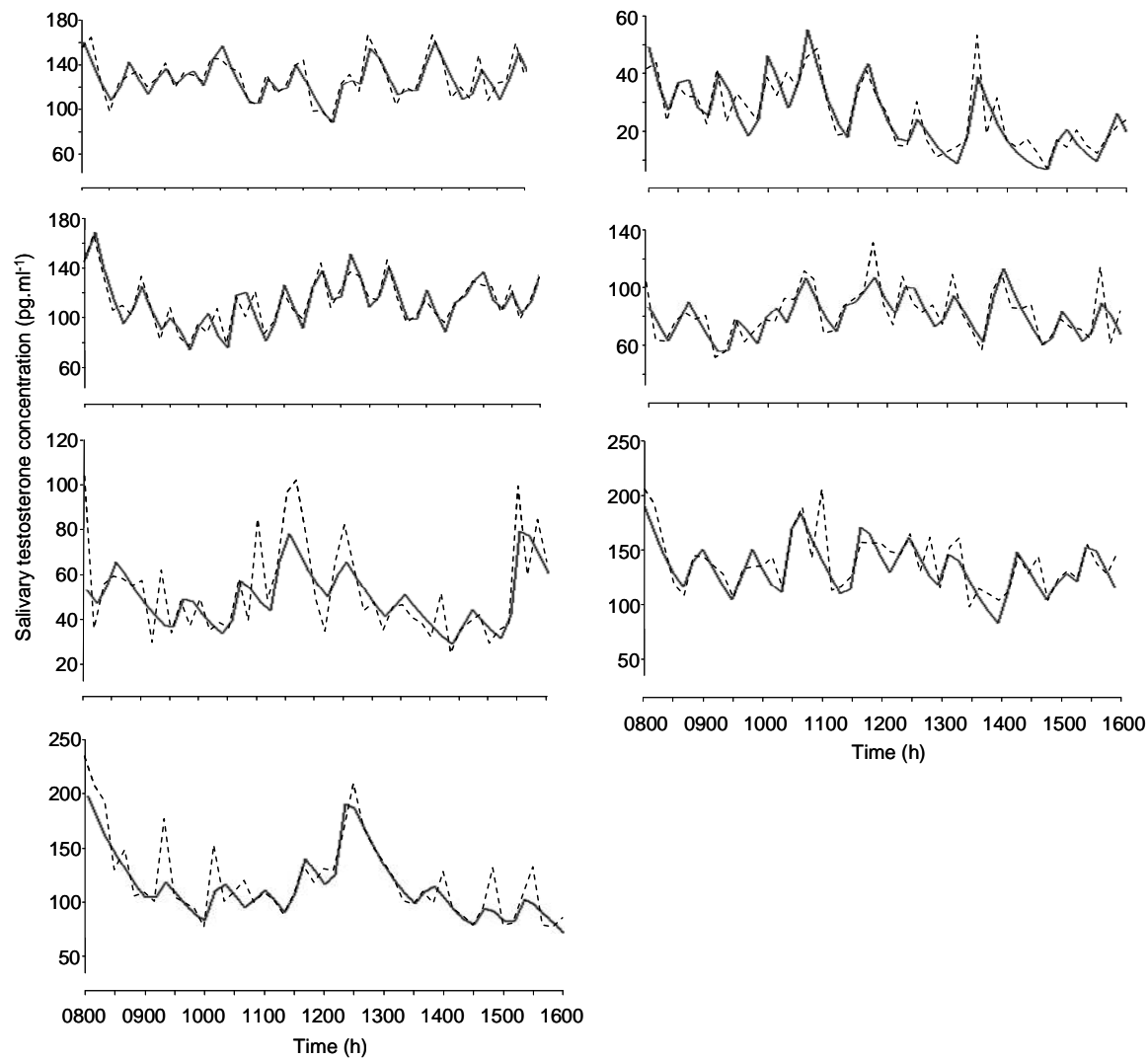


Figure 16. Eight hour time courses of salivary testosterone concentration in all subjects.

Samples are from a non-intervention day where subjects were sampled at 10-min intervals. The dashed line represents the observed data and the solid line is the modelled best fit as derived by the AutoDecon software.

Testosterone concentration decreased (by a factor of 0.77; factor 90% confidence limits $\times/\div 1.17$) over the period between 0800 and 1600 h, and cortisol concentration decreased a factor of 0.43 ($\times/\div 1.50$) over the same time period. Auto-correlational analyses of the testosterone residual data were consistent with transient episodic pulses lasting less than 20 min with a small mean correlation of 0.21; ± 0.17 . A greater correlation ($r = 0.64$; ± 0.16) was found between consecutive residual data points for cortisol, indicating cortisol levels were less labile. The standard deviations quoted compare with an expected variability in a sample of this size of 0.09 (Hopkins, 2007).

The sprint intervention elicited a small transient elevation in testosterone by a factor of 1.21 ($\times/\div 1.21$) 10 min after exercise (Figure 17). A cortisol response was observed as a result of the sprint intervention with a moderate elevation peaking at 50-min post-exercise (factor 2.3; $\times/\div 2.6$). A moderate cortisol elevation was also clear at the 60 min post-exercise time point and over the 60 min area under the curve (factor 1.79; $\times/\div 2.0$ and 1.63; $\times/\div 1.7$) respectively. The hormonal effects of the other interventions were generally trivial or unclear at all time points although a small transient elevation in testosterone (factor 1.20; $\times/\div 1.28$) was observed following the video game (Figure 17). The testosterone response to the video game tended to differ between winners and losers with changes of a factor of 1.38 ($\times/\div 1.60$) and 1.00 ($\times/\div 1.12$) respectively.

Correlational analysis revealed that the dynamic changes in testosterone prior to the sprints were associated with the magnitude of the testosterone response. The change in testosterone concentration in the 10 min immediately prior to the sprint intervention was positively associated with a testosterone response at 10-min post exercise ($r = 0.78$; 0.23 to 0.95). A large positive association between the change in testosterone prior to exercise and response was also observed at the 20-min post-exercise time point ($r = 0.93$; 0.70 to 0.99). In contrast, correlational data from non-intervention days demonstrated a negative relationship between analogous testosterone concentration changes ($r = -0.38$, -0.71 to 0.10; and $r = -0.35$, -0.70 to 0.13) for the 10- and 20-min post time points respectively. Interestingly, a positive correlation ($r = 0.83$; 0.35 to 0.96) was observed between an individual's approximate entropy value for basal secretion of testosterone and the magnitude of the testosterone response to the sprint intervention.

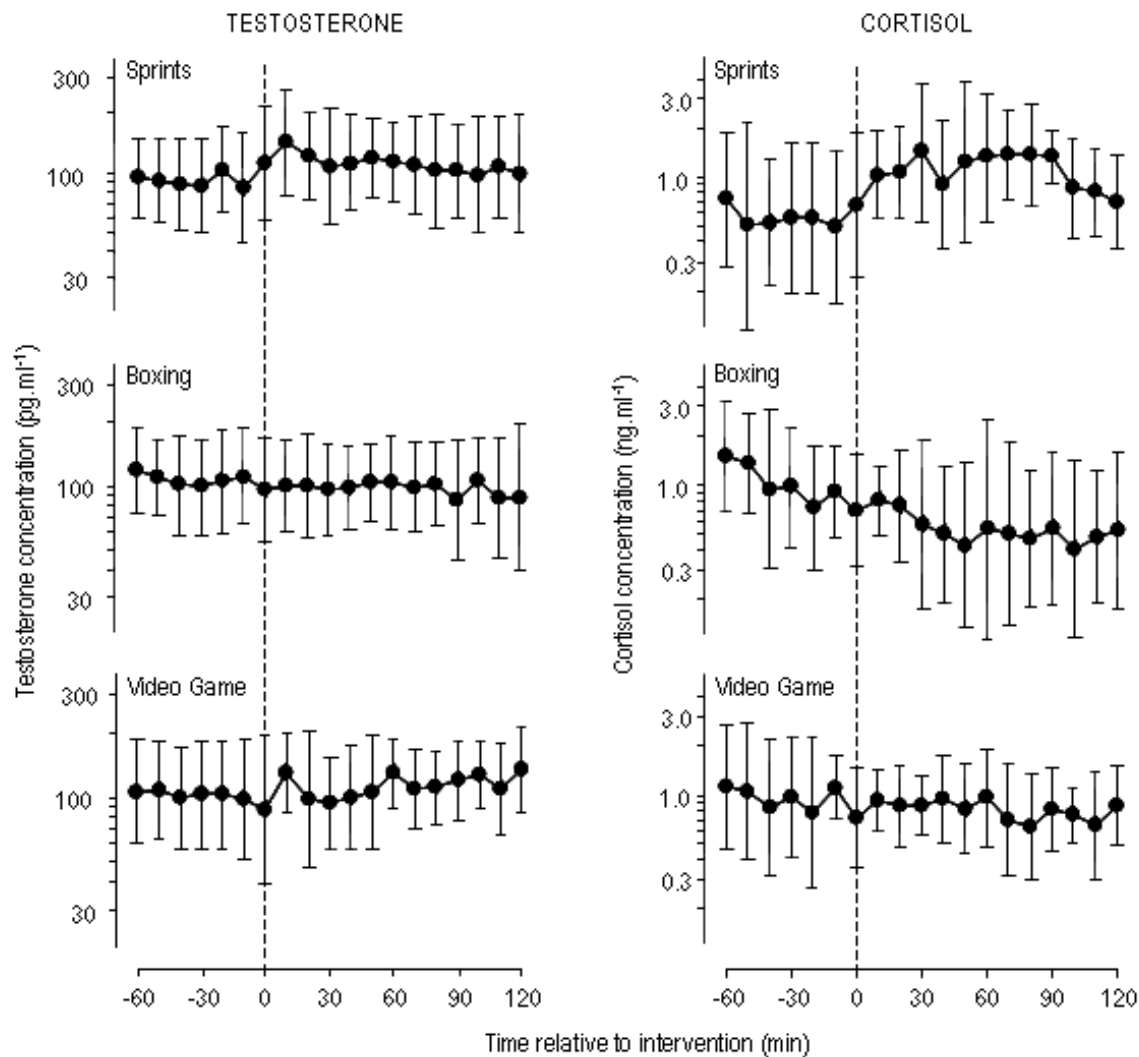


Figure 17. Group average salivary testosterone and cortisol concentrations before and after three interventions. Error bars represent standard deviations. Dashed line represents the commencement of the intervention.

5.5 Discussion

Deconvolution analysis of the testosterone data in the current study was able to discern circadian secretory events in salivary testosterone. Auto-correlational results in the testosterone data set suggested that these secretory events were transient with no relationship observed between consecutive data points. Previous work has identified episodic secretion of testosterone in the testicular vein at a circadian frequency, with testosterone levels ranging from 1 to 1,540 ng.mL⁻¹ (Winters & Troen, 1986). With respect to cortisol, the current study presents auto-correlational results showing a relationship between consecutive data points.

This observation contrasts that seen in salivary testosterone suggesting that, at least in the saliva partition, cortisol secretion is less labile than testosterone.

Analysis of the standardised approximate entropy data revealed that there was almost certainly a difference between the orderliness of the testosterone and cortisol values, with the testosterone data series being more complex. This difference has been previously reported in plasma with reported ranges of 0.8-0.9 and 1.26-1.57 for cortisol and testosterone respectively (Pincus, et al., 1996; van den Berg, Pincus, Veldhuis, Frölich, & Roelfsema, 1997; Veldhuis, 1999; Henley, et al., 2009). The novel finding of a relationship between the approximate entropy of basal testosterone secretion on non-intervention days and the testosterone response to the cycle sprint exercise may reflect attenuation of negative feedback. This observation suggests that the approximate entropy statistic provides data regarding the sensitivity of the HPG axis to physiological stimuli. As such, approximate entropy may be capable of distinguishing individuals who are susceptible to respond in a positive anabolic manner to exercise and provide insight into differential inter-individual responses to an identical stimulus. An expected association was also observed between age and mean testosterone concentration with a very large negative correlation reported. Age-related decreases in testosterone concentrations are commonly reported and have been associated with changes in fat mass, muscle mass, and bone density (Nahoul & Roger, 1990; Ellison, et al., 2002; Kaufman & Vermeulen, 2005).

In the current study, two consecutive cycle sprints were performed in an attempt to elicit a hormonal response to a physiological stress. The sprint intervention produced distinct anabolic and catabolic hormone responses. The small elevation in testosterone was transient, whereas the moderate cortisol elevation that was evident post-exercise followed a slower time course. These data are consistent with recent observations (Busko & Opaszowski, 2005; Crewther, et al., 2007) that have shown a rapid elevation in testosterone returning to baseline within 30 min and a gradual increase in cortisol 30 min post-exercise.

The boxing intervention was matched with the sprint intervention for exercise intensity and duration but was intended to incorporate a psychological component with subjects instructed to strike the hand-held focus pads in an aggressive manner. It has been reported that circulating testosterone in men is correlated with dominant or aggressive behaviour (Mazur & Booth, 1998). These authors have suggested a reciprocal model in which testosterone level is variable, acting as both a cause and effect of behaviour. However, in contrast to the observed hormonal response to the sprint intervention, no clear response of either testosterone or cortisol was apparent following the punch bag intervention. The lack of response may have been due to the smaller muscle mass recruited by this type of exercise and/or possibly a lack of maximal effort in some cases due to the unfamiliar nature of the exercise performed by these subjects. The lack of any observable psychological stimulation may also be due to the fact that the intervention lacked an adversarial component, in that no competitive outcome was measured.

The video game provided a purely psychological stimulus and simulated the two subjects shooting and killing one another in a wartime environment. Earlier research has suggested that competition affects male testosterone in two ways: an anticipatory testosterone rise prior to the challenge; and that testosterone increases in winners and declines in losers (Booth, Shelley, Mazur, Tharp, & Kittok, 1989). Indeed, winners of a non-physical, face-to-face competition have been shown to increase relative to losers (Mazur, Booth, & Dabbs, 1992). The current data showed a small testosterone response to the video game intervention, and supported the theory that post-competition levels were higher in winners compared to losers. It has been suggested that historically, winners are more likely to be faced by further challengers and that the high post-competition testosterone may prepare them for subsequent challenges (Mazur & Booth, 1998).

The rapid testosterone responses observed in the current study are unlikely to be explained by the 'typical' hormonal cascade of HPG axis activation, which involves the

release of secretagogues from the arcuate nucleus and anterior pituitary gland. Similar rapid responses have been reported in males in literature in response to various stimuli such as maximal exercise (Busko & Opaszowski, 2005; Crewther, et al., 2007) and exposure to potential mates (Roney, Lukaszewski, & Simmons, 2007). Research has described direct neural links between the brain and testes suggesting that hormonal secretion may also be regulated through a rapid neural mechanism (Selvage & Rivier, 2003; Lee, Selvage, Hansen, & Rivier, 2004). Although speculative, the high degree of sympathetic activation associated with supra-maximal exercise may promote testosterone production due to increased sympathetic tone to the Leydig cells (Frungeri, et al., 2002). However, it is possible that exercise-induced changes in plasma volume or transient changes in plasma binding capacity account for the observed elevation in salivary free hormone levels.

The second aim of the current study was to examine whether there was an interaction between ultradian rhythmicity and stress responsiveness. Interestingly, the salivary testosterone response to the sprint intervention was associated with the direction of the dynamic change in baseline testosterone levels prior to exercise. These findings support our hypothesis that responsiveness to stressors is dependent on the underlying dynamic state of the endocrine system. Indeed, an interaction between the rapid sympathetic activation of the HPG axis and coincident LH-induced up-regulation of testosterone production would conceivably result in an amplification of the physiological response.

It is known that strength and body weight gains can be enhanced by maximising the testosterone response to resistance training (Hansen, et al., 2001; Beaven, et al., 2008a). Therefore, the ability to ascertain the state of the endocrine system in near real-time has the potential to enhance training adaptation by maximizing the anabolic and/or minimising the catabolic response to a given stimulus. For example, the timing of a training session intended to increase muscle mass and strength could be manipulated to coincide with an individual's endogenous testosterone secretory event to maximise the anabolic responsiveness. It is

acknowledged that the low number of subjects in the current preliminary investigation very much limits extrapolation to the general population and correlational data should be viewed with this limitation in mind. Importantly, the requirement for retrospective analysis, and lack of repeated measures, also limits within-subject comparisons of the baseline activity and subsequent responsiveness to exercise. It is also important to acknowledge that the elevation in testosterone in response to exercise, while important, is only one of many temporal anabolic signals that work in unison to actualise positive skeletal muscle adaptation. Despite these limitations, confirmation of the intra-individual reliability of hormonal pulsatility could improve understanding of variability and the ability to modulate training outcomes in athletes of all levels. Indeed, the ability to harness the ultradian biorhythms and enhance the anabolic response to resistance exercise and subsequent functional adaptation has the potential to benefit athletes that is currently unrealised.

5.6 Conclusion

In addition to identifying pulsatile secretory events in salivary testosterone profiles, the current study has shown the expected circadian rhythm, an age-related decrease in testosterone concentration, and distinct anabolic and catabolic hormone responses to exercise. Thus, it would appear that salivary monitoring of steroid hormones is a valid measure of basal endocrine concentrations and stimulus response, as well as characterising the age-related decline in testosterone in males. Importantly, the anabolic response to this sprint intervention was associated with the dynamic change in testosterone levels prior to exercise and the approximate entropy of baseline secretion. The correlations observed may have implications for training protocols that attempt to maximise anabolic response in order to maintain or increase muscle mass.

CHAPTER SIX: STUDY FOUR – CAFFEINE

6.0 Dose Effect of Caffeine on Testosterone and Cortisol Responses to Resistance Exercise

6.1 Prelude

The literature review also identified that supplements could influence steroid hormones. Furthermore, supplements that affect steroid hormones have demonstrated the potential to modulate resistance exercise outcomes. Caffeine has been reported to increase plasma levels of testosterone in rodents and has been positively associated with increased testosterone levels in human cross-sectional studies. In the context of this thesis, this chapter assesses the dose-response of salivary testosterone and cortisol to physiologically tolerable doses of caffeine to modulate steroid hormone responses to exercise. The implications of this information regarding caffeine supplementation during resistance exercise for researchers and practitioners are discussed.

6.2 Introduction

Interest in the use of caffeine as an ergogenic aid has increased since the International Olympic Committee lifted the partial ban on its use in 2004. The positive effects of caffeine in time-to-exhaustion and endurance tests have been demonstrated in laboratory conditions (Graham & Spriet, 1995; Pasman, van Baak, Jeukendrup, & de Haan, 1995; Norager, et al., 2005), and in experiments that have simulated performance conditions. Caffeine-modulated performance enhancements have been reported in cycling (Kovacs, et al., 1998), swimming (MacIntosh & Wright, 1995) and running (D. G. Bell, et al., 2002; Bridge & Jones, 2006). Caffeine has also been shown to have beneficial effects on the physical and skill activities required in an intermittent high-intensity team sport (Stuart, et al., 2005). Furthermore, data from this 2005 study (unpublished observations) suggested that ingested caffeine may elevate

endogenous testosterone levels. Previous rodent experiments have also demonstrated that high doses of caffeine increased plasma levels of testosterone (Pollard, 1988).

Testosterone is also known to increase in response to resistance exercise with a relationship between total work and intensity and the degree of acute testosterone response (Kraemer, et al., 1990; J. U. Ahtiainen, et al., 2004; Kraemer & Ratamess, 2005). Indeed, testosterone as the primary anabolic hormone, has been linked to strength and muscle gain. The relationship in humans is based upon a number of observations which include: males show muscle growth at puberty when testosterone production increases (Ramos, et al., 1998); exogenous application of supraphysiologic doses of testosterone in intact males results in greater strength and muscle gains from resistance exercise (Bhasin, et al., 1996); and pharmacologic blockade of testosterone-specific receptors suppressed exercise-induced hypertrophy of skeletal muscle (Inoue, et al., 1994). In healthy adults Staron and co-workers (Staron, et al., 1994) linked increases in strength and muscle fibre transformation in males to elevated serum testosterone levels. Furthermore, Hansen and colleagues (Hansen, et al., 2001) concluded that increases in isometric strength were related to the magnitude of testosterone response to resistance exercise in young men. More recent data has demonstrated that suppression of endogenous testosterone attenuates strength and muscle mass gains observed over an eight-week strength training period (Kvorning, et al., 2006). It should also be noted that, testosterone has been reported to promote the differentiation of pluripotent stem cells towards a myogenic lineage (Singh, et al., 2003) and induce specific intracellular calcium oscillations in primary myotubes (Estrada, Espinosa, Gibson, Uhlen, & Jaimovich, 2005).

The stress hormone cortisol has also been shown to rise during resistance exercise (Smilios, et al., 2003; J. U. Ahtiainen, et al., 2004) due to the increase in metabolic demand. The catabolic nature of cortisol however suggests that an increase in this steroid hormone is not ideal from the perspective of a weight training athlete whose aim is to increase or

maintain muscle mass. Recent work has illustrated the detrimental effects of cortisol on resistance training outcomes by showing that supplementation that blunted cortisol release resulted in increased muscle mass gains (Bird, et al., 2006b). The ratio of testosterone to cortisol has been used as an index of the relative anabolic/catabolic state (R. M. Daly, Rich, & Klein, 1998) and positive changes in this index have been associated with an enhanced environment for muscular growth (Zakas, Mandroukas, Karamouzis, & Panagiotopoulou, 1994).

The effects of caffeine as a training aid have largely been neglected as studies have focused on performance benefits. The effects of caffeine on endogenous testosterone and cortisol responses to resistance exercise are unknown. As a result this placebo controlled, double-blind, crossover investigation was designed to examine the effect of caffeine ingestion on salivary testosterone and cortisol levels before, during, and after resistance exercise.

6.3 Methods

6.3.1 Subjects

Twenty-four professional rugby league athletes (age 22.3 ± 3.9 yr, mass 94.9 ± 9.8 kg; values are mean \pm SD) from a single team were recruited to participate in the study. All subjects were fully informed of the nature and possible risks of the study before giving written consent. The protocol was approved by the Auckland University of Technology Ethics Committee. All subjects were informed that they could cease their participation in the trial at any time without giving a reason with no repercussions.

6.3.2 Experimental Protocol

The study was a randomised, double-blind, placebo controlled, balanced trial. Subjects were assessed on four occasions where gelatine capsules containing either lactose (placebo) or caffeine were ingested 1 h before exercise. Four caffeine doses of 0, 200, 400

and 800 mg were utilised in the study and represent a range of dosages commonly reported in the literature (Graham & Spriet, 1995; Bridge & Jones, 2006). Subjects were instructed to refrain from ingesting dietary caffeine, such as coffee, tea, chocolate and caffeine-containing beverages prior to exercise on the experimental days. A dietary log for the preceding 24 h was collected to assess caffeine intake and reminders were given to ensure dietary compliance. Subjects also completed a short questionnaire at the conclusion of training to ascertain their perception of the caffeine dose that they had ingested.

Saliva samples were obtained from each subject at the time of caffeine ingestion, prior to resistance exercise, every 15 minutes during exercise, and at 15 and 30 minutes post-exercise. For each sample, subjects were required to expectorate 2mL of saliva into sterile containers (Labserve, Auckland, N.Z.). Saliva samples were stored at -20°C until assay. Salivary steroid samples were taken in this study as they are minimally invasive and have the advantage of reflecting free (or bioavailable) steroid concentrations which are reported to be more physiologically relevant than total blood levels (Vining, McGinley, Maksvytis, & Ho, 1983; Obminski & Stupnicki, 1997). To prevent blood contamination of saliva, resulting in an overestimation of hormone concentrations, subjects were advised to avoid brushing their teeth and drinking hot fluids in the two hours prior to assessment. Individual subjects performed each of the four sessions at the same time of day to control for the effects of circadian rhythm on hormonal concentrations.

6.3.3 Saliva Analyses

Saliva samples were analysed in triplicate for testosterone and cortisol using radioimmunoassay (RIA). The methods were modified from those described by Granger and colleagues (Granger, et al., 1999). Briefly, standards from serum diagnostic kits (Diagnostic Systems Laboratories, USA) were diluted in phosphate buffer saline (Sigma P4417) to cover the ranges of 0-500 and 0-51.2 nmol/l, for cortisol and testosterone respectively. Saliva sample sizes of 50 and 100µl were used for cortisol and testosterone respectively. The

antibodies were diluted in a phosphate buffered saline solution containing 0.05% bovine serum albumin. Kit standards were diluted so that approximately 50% binding was achieved compared to the total counts (10000 and 4500 counts per minute for cortisol and testosterone respectively). Detection limits for the assays were 0.55 and 0.035 nmol/l, for cortisol and testosterone respectively. The intra- and inter-assay coefficients of variation were 8.8 and 8.5 % for cortisol, and 10.0 and 6.3 % for testosterone.

6.3.4 Exercise Protocols

All exercises were performed at the stadium gymnasium where the subjects were accustomed to training. Subjects completed a five minute warm-up consisting of footwork ladder and cone based agility drills. A sport-specific skill component was included (catch and pass) in these drills. All players completed four primary lifts comprising the following movements: squat, dead-lift, lunge (or variations of) and some isolated hamstring strengthening (knee flexion). The core lifts were coupled in supersets / complex sets with stability and speed patterns. Speed consisted of 10-m sprints and players were given quantitative feedback on their sprint performance via timing gates. The primary lifting component of the workout averaged approximately 45 minutes in duration. All players then completed a circuit of core strengthening exercises (abdominal plus trunk and hip extensors) at the completion of the strength training session that lasted approximately ten minutes.

6.4 Statistical Analyses

Testosterone and cortisol concentrations and their ratio were analyzed with the mixed-model procedure (Proc Mixed) in the Statistical Analysis System (Version 9.1, SAS Institute, Cary, N.C.). The fixed effects in the model were clock time in hours (to adjust for any linear diurnal change) and the interaction of caffeine dose (four levels: 0, 200, 400 and 800), and exercise time (eight levels: -60, 0, 15, 30, 45, 60, 75, 90). The mean effects of exercise and caffeine were estimated by appropriate combinations of the levels of this interaction. The

random effects in the model were the residual (to estimate error of measurement within a session), athlete identity (representing mean differences between subjects), the interaction of athlete identity with exercise session (representing within-subject variation between sessions), and the interaction of athlete identity with dummy numeric variables to estimate within-subject variation (individual responses) associated with exercise and with caffeine. The dummy variable for caffeine had values of 0, 1, 2 and 3 to represent the four doses of caffeine, and the resulting individual responses were therefore estimated as if they increased linearly in magnitude with each step increase in caffeine dose.

The dependent variables were log transformed before analysis; plots of residuals vs. predicted values showed that this transformation produced acceptable uniformity of error, and no observations were excluded as outliers. Back transformation provided estimates of mean effects as percents and estimates of individual responses and errors as coefficients of variation. For qualitative assessment of magnitude, the log-transformed effects were standardised by dividing by the between-subject standard deviation at rest in the no-caffeine condition. The standard deviation was the square root of the sum of the variances for athlete identity; athlete identity interacted with session identity, and the residual. Small-sample bias in the standardised effects was adjusted by multiplying by a factor of $1-3/(4v-1)$, where v was the degrees of freedom of the standard deviation ($=15$). Magnitudes of the standardised effects were interpreted using thresholds of 0.2, 0.6 and 1.2 for small, moderate and large respectively, a modification of Cohen's thresholds of 0.2, 0.5 and 0.8 (Cohen, 1988); the modifications are based primarily on congruence with Cohen's thresholds for correlation coefficients (see <http://newstats.org/effectmag.html>).

The effect of caffeine on sprint performance was analysed with a mixed model. The dependent variable was log-transformed mean sprint time. The fixed effect was either the actual dose or the guessed dose, with values 0, 1, 2 or 3. The random effects were the residual, athlete identity, and the interaction of identity with the actual or guessed dose (to

allow for individual responses to dose). For qualitative assessment of magnitude, the threshold for a substantial change in sprint time was assumed to be 0.8% (Paton, Hopkins, & Vollebregt, 2001).

In keeping with recent trends in inferential statistics (e.g., (Sterne & Smith, 2001)), magnitude-based inferences about true (population) values of effects were made by expressing the uncertainty in the effects as 90% confidence limits. For brevity, confidence limits are shown as $\pm x$, where x represents half the confidence interval. An effect was deemed unclear if its confidence interval overlapped the thresholds for substantiveness (that is, if the chances of the effect being substantially positive and negative were both $>5\%$); otherwise the magnitude of the effect was reported as the magnitude of its observed value (Batterham & Hopkins, 2006).

6.5 Results

All of the subjects completed the trial. The mean salivary testosterone concentration measured before each session was $0.29 \text{ nmol}\cdot\text{L}^{-1}$ and ranged between 0.07 and $0.97 \text{ nmol}\cdot\text{L}^{-1}$ (21.6 and $281 \text{ pg}\cdot\text{ml}^{-1}$). Salivary cortisol ranged between 0.55 and $15.2 \text{ nmol}\cdot\text{L}^{-1}$ (0.2 and $5.5 \text{ ng}\cdot\text{ml}^{-1}$) with a mean of $4.14 \text{ nmol}\cdot\text{L}^{-1}$. A small diurnal decline in testosterone ($11 \text{ }\cdot\text{h}^{-1}$; $\pm 7 \text{ }\cdot\text{h}^{-1}$) occurred over the experimental period. After adjustment for this effect, testosterone concentration showed a small exercise-induced increase (15% ; $\pm 19\%$). The effect of caffeine dose on testosterone during exercise is presented in Figure 18.

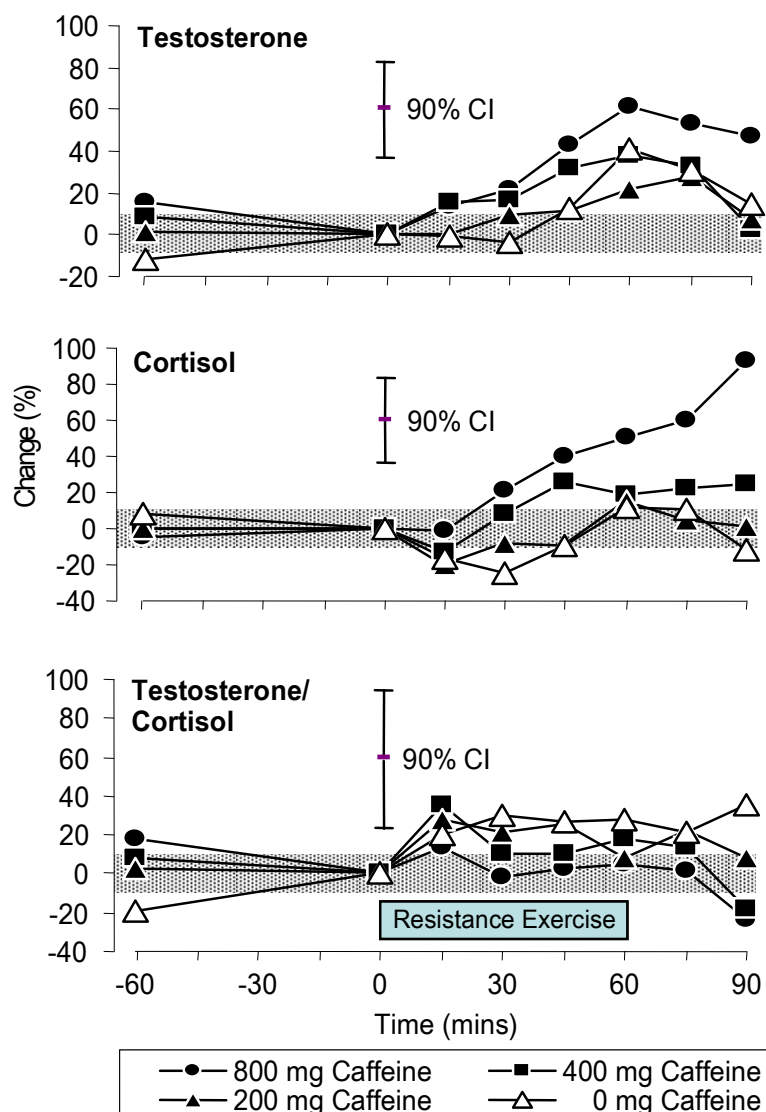


Figure 18. Temporal profile of hormonal response to four caffeine doses.

A Cohen effect size of less than 0.2 is represented by the shaded area indicating a trivial percentage change. The error bar represents an approximate 90% confidence interval (CI) for each point other than the zero time point.

Caffeine doses of ≥ 400 mg tended to cause a small decrease in testosterone after ingestion, followed by a rapid increase after the commencement of resistance exercise. The 800 mg caffeine dose produced a 61% ($\pm 33\%$) increase in testosterone after 60 min of resistance exercise. The 800 mg caffeine dose produced a 51% ($\pm 41\%$) increase in cortisol over the exercise period (Figure 18) that continued to increase to 93% ($\pm 62\%$) above baseline during the recovery period monitored. As a result of the elevated cortisol seen in the post-exercise period the percentage change in the testosterone/cortisol ratio at caffeine doses of ≥ 400 mg was negative compared to the placebo condition (Figure 18).

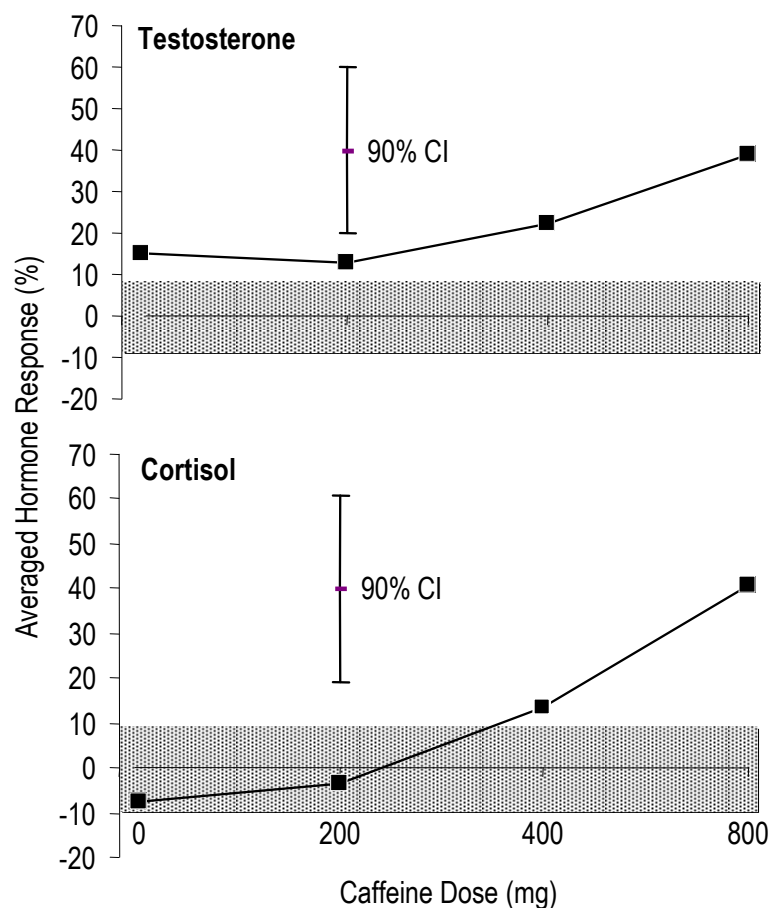


Figure 19. Hormonal response averaged over the exercise and recovery period.

A Cohen effect size of less than 0.2 is represented by the shaded area indicating a trivial change. The error bar represents an approximate 90% confidence interval (CI) for each point.

When hormonal responses were averaged over the exercise and recovery period, caffeine doses of ≥ 400 mg raised testosterone concentration in a dose-dependent manner and by 39% ($\pm 24\%$) at the highest dose (Figure 19). Of this 39% response, 21% ($\pm 24\%$) can be attributed to the caffeine dosage. The 800 mg dose of caffeine also caused a moderate 52% ($\pm 44\%$) increase in cortisol during exercise and recovery when compared to exercise alone (Figure 19).

Only 18% of subjects were able to correctly identify the caffeine dose they ingested on the questionnaire they completed following exercise. Furthermore only 10% of subjects correctly guessed the occasion on which they were prescribed the 800 mg caffeine dose.

Caffeine was associated with a small improvement in sprint performance, with the 800-mg dose decreasing sprint times (1.7%; 90% confidence limits $\pm 2.5\%$).

6.6 Discussion

The novel finding of this study was that caffeine increased the exercise-induced testosterone response to resistance exercise in a dose-dependent manner. Caffeine ingestion also increased the cortisol response to resistance exercise and, at the highest caffeine dose, cortisol continued to increase post exercise. Epidemiological studies have reported a positive association with caffeine intake and elevated bioavailable testosterone levels in adult males (Svartberg, et al., 2003). Despite the widespread use of caffeinated products, there is a lack of studies investigating the effect of caffeine on testosterone concentrations during and following resistance exercise. The anxiogenic properties of caffeine have been reported previously, and caffeine is reported to augment the increase in cortisol concentration in response to mental stress (al'Absi et al. 1998) and exercise (al'Absi et al., 1998; Lovallo, Farag, Vincent, Thomas, & Wilson, 2006).

There is growing evidence for the performance gains that can be achieved by athletes using caffeine for both endurance and sprint performance (MacIntosh & Wright, 1995; Stuart, et al., 2005; Bridge & Jones, 2006). A large number of reported physiological benefits of caffeine in-vivo, are now thought to be due to the pharmacological antagonistic actions of caffeine on adenosine receptors, including: potentiation of muscle contractions (Magkos & Kavouras, 2005); delayed fatigue via mechanisms in the central nervous system (Davis, et al., 2003); direct neuroendocrine activation (al'Absi, et al., 1998) and direct actions on skeletal muscle function (Tarnopolsky & Cupido, 2000). The physiological relevance of adenosine in exercise proposed by Biaggioni (Biaggioni, 2004) is that the increasing concentrations of adenosine produced during exercise act to inhibit sympathetic efferents and activate afferent nerves. The net effect of this inhibition is to protect the muscle tissue during ischemia or exhaustive exercise. Thus, the antagonistic actions of caffeine on adenosine

receptors have the potential to reduce the normal inhibitory effect of adenosine on motor efferents.

Testosterone and cortisol synthesis and release are classically described as having negative feedback control via the hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenal axes, respectively. However, in addition to these classical pathways, recent research *in vivo* has identified direct neural links between the paraventricular nucleus of the hypothalamus and testosterone (Selvage, Lee, Parsons, Seo, & Rivier, 2004) and cortisol secretion (Zaki & Barrett-Jolley, 2002). The implications of a direct neural link have particular relevance regarding a mechanism for caffeine-modulated effects if the neurons utilise adenosine as a neuro-modulator.

The current study demonstrates that caffeine has some potential to enhance the beneficial effects of training via an increase in bioavailable testosterone. Testosterone is a potent inducer of contractile protein synthesis and is necessary for muscular hypertrophy (Inoue, et al., 1994). Despite heterogeneity in hormonal responses to resistance training, it is accepted that testosterone increases in response to resistance exercise and that intensity and volume are important determinants of this response (Kraemer, et al., 1990).

The practical importance of acute testosterone responses to resistance training is evidenced by research showing that the magnitude of isometric strength increases are related to the magnitude of anabolic hormonal response (Hansen, et al., 2001). More recently Kvorning and colleagues (Kvorning, et al., 2006) demonstrated that suppression of endogenous testosterone attenuated strength gains achieved over an eight week training period. Such examples stress the importance of acute anabolic responses to training and, combined with the data presented in the current study, suggest some potential for caffeine to enhance training outcomes.

Our data shows also a moderate effect of caffeine in elevating cortisol concentrations during resistance exercise. The cortisol elevation observed is consistent with the results of Lane and colleagues (Lane, Pieper, Phillips-Bute, Bryant, & Kuhn, 2002), and this elevation may counter the anabolic effects of testosterone. These researchers also commented on the persistence of the caffeine effect, with the effects of a divided caffeine dose of 500 mg being apparent 10 or more hours after ingestion. Our experimental design only captured data for 150 min from the time of ingestion, but it was apparent that the 800-mg dose produced a substantial and long-lasting effect on cortisol concentration. Furthermore, our data indicate that caffeine potentiated the stress response to resistance exercise in a dose-dependent manner.

Recent research has reported that suppression of cortisol during resistance exercise by nutritional supplementation is associated with greater gains in muscle cross-sectional area (Bird, et al., 2006b). These observations suggest that the cortisol increase associated with caffeine ingestion may counteract to some degree any benefit derived from the increase in exercise-induced testosterone concentration. Indeed the anabolic/catabolic balance, as illustrated by the testosterone/cortisol ratio, showed an overall caffeine-induced decrease, with the decrease being most pronounced at the highest caffeine dose. These data suggest that catabolic processes were predominant in the recovery phase following resistance exercise when higher doses of caffeine were ingested.

Increases in testosterone at the 400- and 800-mg caffeine doses occurred within 15 min of commencing resistance exercise. Similar levels of testosterone were not seen in the placebo group until the 45-min time point. The immediacy of the testosterone rise suggests that caffeine may decrease in the latency of hormonal response to exercise stimuli. A caffeine-mediated potentiation would have important ramifications for training and training outcomes, however this observation requires further investigation as it is possible that this

observation is due to the greater magnitude of response rather than a difference in the time course of the response.

6.7 Conclusion

There are a growing number of studies showing performance benefits from caffeine. The consequences of routine use of high levels of caffeine in training are unknown. While this study reports a potentially important anabolic advantage from caffeine via the dose-dependent testosterone increase observed, it is tempered by a concurrent increase in cortisol raising questions about possible functional gains. A longer-term training study investigating the impact of caffeine on training outcomes is required.

CHAPTER SEVEN: GENERAL DISCUSSION

This discussion is divided into five sections, the first of which revisits the rationale for thesis. The second section summarises the conclusions and primary findings of the four studies that make up this thesis. The third section identifies the limitations of the current research including the relatively low subject numbers and the use in isolation of testosterone and cortisol as measures of the body's adaptive response to resistance exercise. The fourth section looks at future research areas identified as a result of the current investigations that have the potential to further enhance the functional gains obtained from resistance exercise. The final section distills the practical applications that can be drawn from this thesis that may be incorporated into resistance exercise protocols to quantifiably enhance training gains and ultimately functional performance within a competitive environment.

7.0 Thesis Rationale

This thesis set out to investigate the hypothesis that hormone-mediated strategies can enhance training and performance. This hypothesis was based on the premise that prior research had demonstrated a link between anabolic hormone environment and subsequent adaptation in well trained athletes. Thus, as stated in the thesis introduction, it was intended to investigate practical interventions that have the potential to maximise the functional gains of resistance exercise via hormone manipulation to assess practical strategies that modify hormones and investigate their influence on performance. Salivary hormones were monitored as saliva contains the free hormone that is available to interact with receptors and has been related to physiological and psychological measures.

Strategies by which hormonal responses, and subsequent functional outcomes, can be manipulated are becoming more common with protein and carbohydrate supplementation being obvious examples. Indeed, despite the hazards, the abuse of testosterone and its analogues to enhance muscle size and athletic performance is not uncommon due to their

proven efficacy. This thesis assessed four practical and morally-acceptable strategies may enhance adaptation and ultimately performance via modulation of steroid hormone responses. These strategies are: modulation of prescribed exercise variables; hormonal biorhythms and; supplementation.

The modulation of prescribed exercise variables was selected as they represent an integral determinant of subsequent adaptation and hormone responses. In that respect it follows on from earlier research I have been involved with investigating the hormone responses to distinct resistance exercise sessions in rugby players. In the context of this thesis, it was intended to identify how complex training prescription influenced steroid hormone responses.

The investigation into influence of the circadian biorhythm was based on observations in a monitoring study that was being conducted concurrently in association with the NZRU in which anecdotal evidence suggested that the time of day at which exercise was performed affected hormone responses. Indeed, steroid hormones can affect multiple aspects of the neuromuscular system and regulate performance factors such as maximal force and power output which likely contribute to strength and power adaptation. In the context of this thesis, it was intended to assess whether the time at which resistance exercise was performed within the circadian biorhythm could influence strength and power adaptive gains.

In assessing the diurnal biorhythms within endogenous hormone production, it was apparent that underlying ultradian rhythms played a key role in hormone regulation. Interestingly, research in a rodent model demonstrated that the pulsatile nature hormone production had the potential to modulate the physiological response to an applied stressor. Due to the comparable regulation of steroid hormones in humans, it was hypothesised that the responsiveness to stressors may dependent on the underlying dynamic state of the endocrine system. In the context of this thesis, it was intended to investigate steroid hormone pulsatility

and the potential of such a biorhythm to influence salivary hormone response to an exercise stimulus.

The premise for the use of caffeine as a supplement to modulate the hormonal response to resistance exercise was prior involvement with research investigating the effects of caffeine on performance during a simulated rugby match (Stuart et al., 2005). Although the hormonal data from this study was unpublished, they suggested that caffeine had the potential to elevate salivary testosterone in a placebo controlled study. Further, studies have shown a positive association between caffeine and acute and chronic increases in testosterone. In the context of this thesis, it was intended to assess the ability of caffeine to modulate salivary testosterone and cortisol responses to exercise.

The rationale behind investigating a range of strategies is two-fold. The strategies may influence hormone responses via distinct mechanisms raising the possibility of a cumulative effect. Additionally, from a practical perspective it may not be feasible to change the time of day at which you train or make use of ultradian rhythms. Therefore, investigating a range of possible strategies allows options that may be able to be utilised in a practical setting.

7.1 Conclusions and Primary Findings

Typically, the aim of resistance training is to improve neuromuscular function in order to increase the likelihood of successful performance outcomes. It has been previously demonstrated that the prescription of a resistance exercise protocol that maximises salivary testosterone response can enhance the primary goals of resistance training, namely, muscular adaptation (Beaven, et al., 2008a). The four studies that comprise this thesis provide information regarding strategies available to the resistance exercise practitioner that can modulate steroid hormone responses. Such information has the potential to enhance the adaptive gains associated with resistance training.

The study detailed in Chapter Three examined complex training and confirmed previously published research demonstrating that prescribed exercise variables such as volume,

intensity, and load are critical factors in effecting acute endocrine responses to resistance exercise (Kraemer, et al., 1990; Smilios, et al., 2003; J. P. Ahtiainen, et al., 2005; Crewther, et al., 2008). Furthermore, it is apparent from the results of this study that combining high velocity movements with a biomechanically similar high load exercise, results in an enhanced anabolic response that may be related to the observed adaptive benefits of complex training. Indeed, the order in which such exercise is performed is a further contributing factor to the hormonal response and may at least partially explain the effectiveness of complex training protocols in mediating the functional outcomes of resistance exercise.

It is recognised however, that monitoring dynamic changes in testosterone and cortisol levels is an overly simplistic measure of the effectiveness of a complex training stimulus given the large number of physiological processes activated by resistance exercise. Indeed, the enhanced neural drive associated with maximal effort, the rapid production of force over a large range of motion, and the eccentric loadings associated with the landing phase of the jump squat protocol also play critical roles in mediating the beneficial training adaptations of complex training.

The second study (Chapter Four) demonstrated that the time at which training is performed within a given phase of the circadian rhythm has the potential to modulate adaptive outcomes. While this study showed that strength gains were similar when semi-professional athletes performed a four week training block either in the morning or late afternoon, there was a trend for the power produced at a set relative load to improve at a greater rate when performed later in the day. Over a longer time period, and with a larger subject population, this difference may have become substantial. Given that elevated testosterone concentrations are capable of attenuating the HPA stress response, the ratio of testosterone to cortisol, which is clearly elevated in the afternoon compared to the morning, may play a role in the divergent pattern of adaptation. At the very least, it is apparent that the hormonal response to exercise stimuli is

dependent on the time of day, and that the phase of the circadian rhythm must be taken into account when reporting physiological variables that are regulated in this manner.

A further study (Chapter Five) demonstrated that the ultradian rhythm, or more specifically the dynamic state of the HPG axis immediately prior to exercise, appears to modulate the salivary testosterone response to strenuous exercise stimuli. Initially, it was possible to identify circadian secretory events in salivary testosterone using established and specialised endocrine pulse detection software. Based on stressor studies in rats, it was postulated that a similar cycle of active and refractory states may be present in human hormone response to a range of physiological and/or psychological stress. Indeed, it was apparent that the testosterone response to cycle sprints was associated with the dynamic change in testosterone levels prior to exercise. Notably, there was also a relationship between testosterone response and the approximate entropy of baseline secretion. These novel observations, in conjunction with described interactions between the glucocorticoids and HPG negative feedback mechanisms, could help to explain the variability inherent in hormonal response to stressors. Indeed, the knowledge that the ultradian rhythm can affect hormone responses suggests that it is possible to utilise the body's natural biorhythm in order to accentuate positive anabolic responses to resistance exercise.

The fourth study (Chapter Six) demonstrated for the first time a dose-response relationship between caffeine and both salivary testosterone and cortisol responses to resistance exercise. The pronounced and rapid onset of a testosterone response to exercise at caffeine doses above 400mg was tempered by a concomitant increase in cortisol. That is not to say that the use of caffeine is entirely unwarranted. Additional strategies to minimise the cortisol response, such as incorporating carbohydrate and protein supplementation into pre- and post-workout drinks, have the potential to diminish this undesirable catabolic response (Tarpenning, et al., 2001; Paddon-Jones, et al., 2004; Bird, et al., 2006b). The potent effect of caffeine, and indeed

carbohydrate/protein supplementation, highlight the key roles that supplements can play in modulating the hormonal responses to exercise and potentiate functional outcomes.

In fact one of the key outcomes from undertaking the thesis process has been that a number of distinct physiological processes are coordinated to ultimately manifest changes in muscular function. Complex molecular signaling cascades integrate the variety of inputs that arise from performing resistance exercise. Indeed, distilling resistance exercise down into three core stimulatory processes and addressing the transmutative pathways from the perspective of muscular adaption is relatively novel. An improved understanding of the intricate pathways involved in adaptive responses allows exercise practitioners to coordinate training, nutrition, and recovery protocols to modulate and enhance functional outcomes.

7.2 Limitations

As stated above, the signaling pathways involved in modulating the adaptive outcomes of resistance exercise are highly complex and ongoing research will likely provide deeper insight into the vital cellular and molecular interactions underpinning skeletal muscle adaptation. Elevations in testosterone and cortisol in response to exercise, while important, are components of a wider temporal signaling cascade that actualises skeletal muscle adaptation. Thus, it is acknowledged that testosterone and cortisol are only aspects of the vast and intricate signaling system, and a limitation of this thesis is that resources were not available to assess other factors that contribute to the mTOR signaling pathway, or indeed changes occurring within the muscle fascicles. However, putative sites of action within the mTOR signaling pathway have been demonstrated for testosterone (Altamirano, et al., 2009) and cortisol (Shah, et al., 2000c) and have been reported to mediate alterations in muscle function (Bird, et al., 2006b; Kvorning, et al., 2006; Beaven, et al., 2008a). These findings provide a clear mechanistic linkage between testosterone and cortisol responses and muscular adaptation.

The common practice of using relatively invasive blood sampling techniques (e.g. venepuncture, cannulation) often limits sampling, and especially serial sampling, in athletic

populations. In addition, the stress associated with invasive techniques may perturb the activity of the HPA axis being monitored (Suay, et al., 1999; Gerra, et al., 2001). Salivary sampling was selected as a convenient and relatively stress-free method of hormone collection. Further, salivary estimation of free testosterone levels has been reported to be superior to other sampling methods in terms of assessing physiological relevant hormone levels (Wilke & Utley, 1987; Gozansky, et al., 2005). It is accepted though, that the inability to collect blood data in the practical setting precludes us from being able to draw direct inferences regarding the peripheral hormonal milieu experienced by the skeletal muscle tissue. The same limitation ultimately applies to any sampling that occurs proximal to the interstitial fluid in which an exercised muscle is encapsulated. Indeed, limitations currently exist for all approaches in their ability to predict the hormonal milieu experienced by the skeletal muscle tissue, and more specifically, the receptors present within that tissue.

By definition elite athletes are a select population with characteristics that set them apart from the general population. As such, it is a challenge to collect sufficient data in order to constitute a large sample size necessary for statistical rigour. Indeed, convincing coaches, trainers, and athletes to superimpose experimental interventions over established training and competition schedules is one of the primary reasons for the general paucity of literature on such individuals. Furthermore, due to ethical issues associated with including professional or semi-professional athletes as subjects, no non-training control group could be assigned in the training study performed. However, the data collected does contribute to the body of work that has assessed the endocrine responses of professional and semi-professional athletes within strenuous training phases.

The study that involved healthy male subjects (Chapter Five) was also limited by relatively low subject numbers. The time commitment required of the subjects, the strict experimental controls, and the intense nature of the sampling regime likely contributed to the difficulty in recruiting volunteers. In addition, the large number of samples to be collected per subject meant that the cost of analysis was substantially increased for each additional subject. The intensive nature of the sampling regime however, revealed for the first time that non-invasive salivary monitoring can distinguish ultradian

pulses in steroid hormones in adult males that have the potential to modulate subsequent stressor response.

Three out of the four studies that comprise this thesis were conducted entirely in gymnasiums where the athletes involved were accustomed to training. All experimental protocols were incorporated into the standard training schedule. As such these can be considered field based experiments and suffer from limitations associated with such trials. For example, it was not possible to standardise sleep and diet prior to every session as would be possible in a more controlled environment. Despite being a limitation, the field-based nature of the experimental data collected can also be viewed as adding validity to the trials, as these data more closely reflect the conditions under which athletes train.

7.3 Future Research Areas

As a result of the literature review and studies conducted in this thesis it is apparent that modulation of the mTOR signaling pathway can have substantial ramifications on adaptive outcomes. Future research will need to employ sophisticated molecular techniques to assess the effects of resistance exercise on specific aspects of this pathway. Exercise practitioners should be aware that some knowledge of mTOR function will allow them to prescribe effective exercise regimes with a solid scientific foundation.

It is accepted that the hormonal responses to resistance exercise are further mediated by variables such as load intensity, rest periods, and volume (Kraemer, et al., 1990; J. P. Ahtiainen, et al., 2003b; Smilios, et al., 2003; J. P. Ahtiainen, et al., 2005; Kraemer & Ratamess, 2005). Indeed, increases in muscular characteristics in men have been linked to increases in exercise-induced testosterone levels (Alen, Pakarinen, Häkkinen, & Komi, 1988; Staron, et al., 1994; Beaven, et al., 2008a). Individual variability in hormone response has been proposed to affect hypertrophic responses (Kraemer et al., 1999) and individual adaptation to training (Jensen, et al., 1991).

The existence of response thresholds that are dependent on protocol variables has been suggested for testosterone (Viru, 1992; J. P. Ahtiainen, et al., 2003b; Beaven, et al., 2008a) and cortisol (Kraemer, et al., 1991; Schwab, Johnson, Housh, Kinder, & Weir, 1993). If there were individual variations in threshold levels amongst the population, it would make sense that individuals respond to resistance training differently. For example, individuals with low thresholds may have an enhanced hormonal response to a low-load, explosive training that would be inappropriate for individuals with a higher hormonal threshold level. Work to establish a non-invasive and rapid method for determining an individual's likely responsiveness to an exercise protocol would prove worthwhile. One possible method could be to investigate whether hormonal responsiveness is related to muscle fibre type characteristics or to specific aspects of mTOR regulation. Alternatively, performing an incremental resistance exercise test to establish individual thresholds of a range of biomarkers, may give exercise practitioners an insight into protocols that are more suited for an individual to attain strength, power, and muscle mass gains.

The circadian rhythm of cortisol and testosterone is well established (Lévi, et al., 1988; Cooke, et al., 1993; Kraemer, et al., 2001; Diver, et al., 2003). Furthermore, it has been demonstrated that cortisol can have a direct inhibitory effect on testosterone production (Bambino & Hsueh, 1981), feedback (Waite, et al., 2009), and response to endurance exercise (W. Daly, et al., 2005). Despite these observations, the effect of elevated cortisol levels in the early morning on resistance exercise outcomes is seldom considered. Although it was not possible to make a conclusive statement as a result of the study that looked at the training effects in athletes training within different phases of the circadian rhythm (Chapter Four), a trend was evident for improved power production in the afternoon when cortisol levels were lower. Further research effort may demonstrate that the hormonal environment in the late afternoon is more conducive to power adaptation.

The study conducted to examine the effect of ultradian pulsatility in healthy adult males (Chapter Five) suggested for the first time that this characteristic of hormone regulation has the

potential to modulate physiological exercise outcomes. Clear results have previously demonstrated that both the behavioural and physiological stress response in rats is affected in a similar manner, with secretory phases associated with amplified stress responses and refractory periods associated with a lack of response to an audio stressor (Windle, et al., 1998). The demonstration in this thesis that the salivary testosterone response to exercise behaves in a similar manner has the potential to modulate training outcomes and change the way variability in hormonal exercise response is perceived. Indeed, unpublished work from the study suggested a refractory period of approximately 30 minutes during which the sprint cycle intervention failed to evoke a salivary testosterone response. Research into the intra-individual reliability of the pulse characteristics and ability to modulate training outcomes are warranted. The ability to elicit or at least reliably predict anabolic responses is a further area of research that could prove invaluable.

The novel finding in this study of ultradian pulsatility of a relationship between the approximate entropy of basal testosterone secretion on non-intervention days and the testosterone response to the cycle sprint exercise, demonstrated that this statistic may provide data regarding the sensitivity of the HPG axis to physiological stimuli. As such, approximate entropy may be capable of distinguishing individuals who are susceptible to respond in a positive anabolic manner to a specific combination of exercise variables and thus provide insight into differential inter-individual responses to an identical stimulus. Further research to confirm an enhanced susceptibility of individuals with high approximate entropy values to hypertrophy may prove worthwhile and could assist talent identification in some athletic endeavours.

Interestingly, Pound *et alia* (2009) found a relationship between ‘masculine’ facial structure and the salivary testosterone response to a competitive task that involved predicting the outcome of a Sumo wrestling bout ($r = 0.36$; $p = 0.013$). The second-to-fourth digit ratio, which is a putative measure of prenatal testosterone exposure, has also been proposed to be positively associated with aggression (Bailey & Hurd 2005; Millet & Dewitte 2007). Therefore,

anthropometric measures of masculinity such as a high facial masculinity index or a low second-to-fourth digit ratio may further elucidate inter-individual differences in the magnitude of testosterone responses to various stimuli.

The physiological outcomes of cortisol production are diverse and have evolved to prepare the body for a wide range of physical and mental stressors. Many of these outcomes, such as the increase in the available amino acids, gluconeogenesis, and alanine synthesis, could theoretically be made redundant through the supplementation of exogenous macronutrients. As muscle accretion is an energy intensive process, it requires sufficient fuels for optimal functioning of the molecular and cellular systems to actualise muscular adaptation. The observation that branched chain amino acids have the ability to directly influence aspects of protein synthesis independently of mTORC1 (Deldicque, et al., 2005), represents an area of research that has substantial potential for muscular adaptation. The ability of hydration status and supplementation to modulate glucocorticoid responses and mediate resistance exercise outcomes have been demonstrated (Bird, et al., 2006b; Judelson, et al., 2008). The reported ability of cortisol to modulate testosterone responses to exercise (Brownlee et al., 2005; Daly et al. 2005) also demonstrates that acute cortisol responses may be undesirable from the perspective of strength and power athletes. Further work could specifically target suppression of cortisol response outcomes such as alanine synthesis and free fatty acid liberation to enhance prospective training gains.

This thesis demonstrated that caffeine can potentiate the salivary testosterone and cortisol response to exercise. The substantial and long lasting elevation of cortisol suggests that it would be unwise to recommend caffeine supplementation alone as an intervention to improve resistance exercise outcomes. The combination of caffeine with supplements that are known to attenuate cortisol responses, such as carbohydrate and protein solutions, may have some merit however and deserves research attention. Indeed, further mechanisms notable for reducing stress responses such as massage (Field, Henandez-Reif, & Diego, 2005), or even music (Miluk-

Kolasa, Obminski, Stupnicki, & Golec, 1994; Khalfa, Bella, Roy, Peretz, & Lupien, 2003), may have a role to play in ‘best practice’ exercise prescription. The use of caffeine as a training aid should also be tempered by the knowledge that caffeine dosage has been reported to attenuate growth hormone response to resistance exercise via an increase in free fatty acids (Wu & Lin 2010).

The ability of further dietary factors to influence key elements of hormone regulation and bioavailability has also been demonstrated. Research has demonstrated that cholesterol-free olive oil was capable of increasing testosterone levels in rats by modulating the function of key enzymes involved in testosterone biosynthesis (Hurtado de Catalfo, et al., 2009). These authors suggested that the monounsaturated, oleic fatty acid was important for normal androgenic function. Increased magnesium ion concentrations within the biological range have also been shown to increase biologically available testosterone *in vitro* via the disruption of the binding of testosterone to SHBG (Excoffon, et al., 2009). This observation suggests that magnesium has the potential to modulate exercise outcomes, as appropriately timed administration may increase the concentration of testosterone available for mediating adaptive outcomes. Thus, it is apparent that a range of supplements such as caffeine, carbohydrates, specific amino acids, oleic acid, and magnesium are capable of modulating aspects of hormone regulation, availability, and response via distinct mechanisms.

Skeletal muscle is a mechanosensitive cell type and is capable of distinguishing between various types of mechanical deformation by activating distinct cellular and molecular signaling pathways. Knowledge of the evolved mechanotransduction mechanisms highlights the fact that multi-axial stretch is unique in its ability to induce skeletal muscle adaptation via p70^{S6k} phosphorylation (Figure 3). As such, exercises that incorporate loaded multi-axial motions are more likely to activate the signaling processes associated with muscular adaptation than those that only evoke uni-axial deformation. This premise however requires investigation in terms of the impact on functional benefits. The observation of a strong linear relationship between the

activation of mitogen-activated protein kinases and peak tension (Martineau 2001) also suggests that exercises that generate high levels of muscular tension, such as eccentric actions, are likely to be more efficient at initiating skeletal muscle adaptations than exercises that produce lesser tension levels.

The role of phospholipase D in mechanotransduction may also be worth further investigation. It is known that *ex vivo* muscle mechanical stimulation induces mTOR signaling via a phospholipase D-dependent increase in phosphatidic acid (Hornberger et al. 2006). Indeed, these authors demonstrated that an elevation in phosphatidic acid was sufficient to activate mTOR signaling possibly through competitive binding of phosphatidic acid to the FKBP-rapamycin-binding domain on mTOR. Thus, phosphatidic acid or analogues that compete with rapamycin for essential mTOR binding sites have the potential to manipulate muscular adaptation.

The incorporation of localised blood flow restriction into training has previously been shown to elevate levels of growth factors and increase measures of muscle size and function (Abe, et al., 2005a; Abe, et al., 2006), as well as muscle protein synthesis and aspects of the mTOR pathway (Fujita, et al., 2007a). These observations suggest that athletes do not necessarily have to lift high volumes or at high intensities to achieve the functional gains associated with resistance training. These observations may prove useful for the rehabilitation of athletes who are unable to load the skeletal musculature. Indeed, further research may investigate the benefits of performing resistance exercise in a hypoxic chamber as opposed to the occlusive cuffs that have been used previously to elicit hypoxia in specific muscle groups. Also possibly of interest in terms of low impact interventions to assist in muscle growth and rehabilitation, is the observation that blue monochromatic light elevated testosterone levels and muscular growth in chickens (Cao et al. 2008), although whether such results would translate into quantifiable gains in humans is not known. It is known however, that monochromatic light can affect physiological and psychological functions in humans (Huang et al. 2009).

7.4 Practical Applications

From the perspective of strength and conditioning coaches, there are always novel training methods being introduced that claim to be superior to current ‘best practice’. Gaining some understanding of the core stimulatory processes that activate the cellular and molecular processes, and ultimately mediate muscular adaptation, will hopefully allow practitioners to continue to improve training methods and assess the effectiveness of both current and novel training stimuli.

The work in this thesis demonstrates that specific areas of the signaling pathway that lead to functional adaptation are susceptible to interventions that are practical for strength and conditioning coaches to implement.

- Complex training, or more specifically performing a power-type exercise after a biomechanically similar heavy resistance training stimulus, was effective at elevating salivary testosterone. Therefore, the order in which exercise is performed is important as this complex exercise sequence provided an enhanced anabolic response for adaptation, even when compared to an exercise bout with an identical physiological load and volume.
- The strength and conditioning practitioner may be unaware of the circadian biorhythms that occur in many physiological systems. The demonstration in this thesis that the biorhythms of testosterone and cortisol may modulate training outcomes, or more specifically improvements in lower-body power, may suggest that sessions designed to improve power should be performed in the late afternoon.
- The learnings from the study that identified an interaction between the dynamic state of the salivary testosterone concentration and acute response to exercise (Chapter Five) are more difficult to apply in a practical setting at this time. Indeed, near real-time, non-invasive technology will be required to take advantage of such knowledge

unless it is possible to reliably predict or elicit ultradian pulsatility. However, strength and conditioning practitioners should be cognizant of the influence of such biorhythms on systems that mediate adaptive outcomes. The ability to conveniently assess the approximate entropy statistic of athletes may also prove useful in the future for predicting subsequent anabolic trainability.

- Caffeine is a practical intervention that can be readily administered and has the potential to enhance aspects of testosterone regulation and production. This thesis demonstrated though, that a concomitant increase in cortisol should discourage the use of caffeine in isolation in a resistance training setting. Although conjecture, the combination of caffeine with additional supplementation known to attenuate acute cortisol responses, such as carbohydrate/protein ingestion, has the potential to enhance resistance training outcomes.

It is intended that the research contained in this thesis will give exercise practitioners a new perspective on ‘best practice’ training techniques and provide an understanding of the processes that mediate skeletal muscle adaptation. This thesis concentrates on the steroids testosterone and cortisol, but also endeavours to illustrate how these hormones are integrated into the byzantine signaling cascade associated with resistance training and its outcomes. An updated diagram based on Figure One presented in the introduction to this thesis is provided to demonstrate the scope and future research available in the field of hormone-mediated strategies to enhance training and performance (Figure 20). In providing practitioners with knowledge regarding the mechanisms underpinning adaptation it is hoped that individual exercise sessions will be more effective, cumulative training outcomes will be quantifiably enhanced, and that improvements will ultimately be observed in functional performance within a competitive environment.

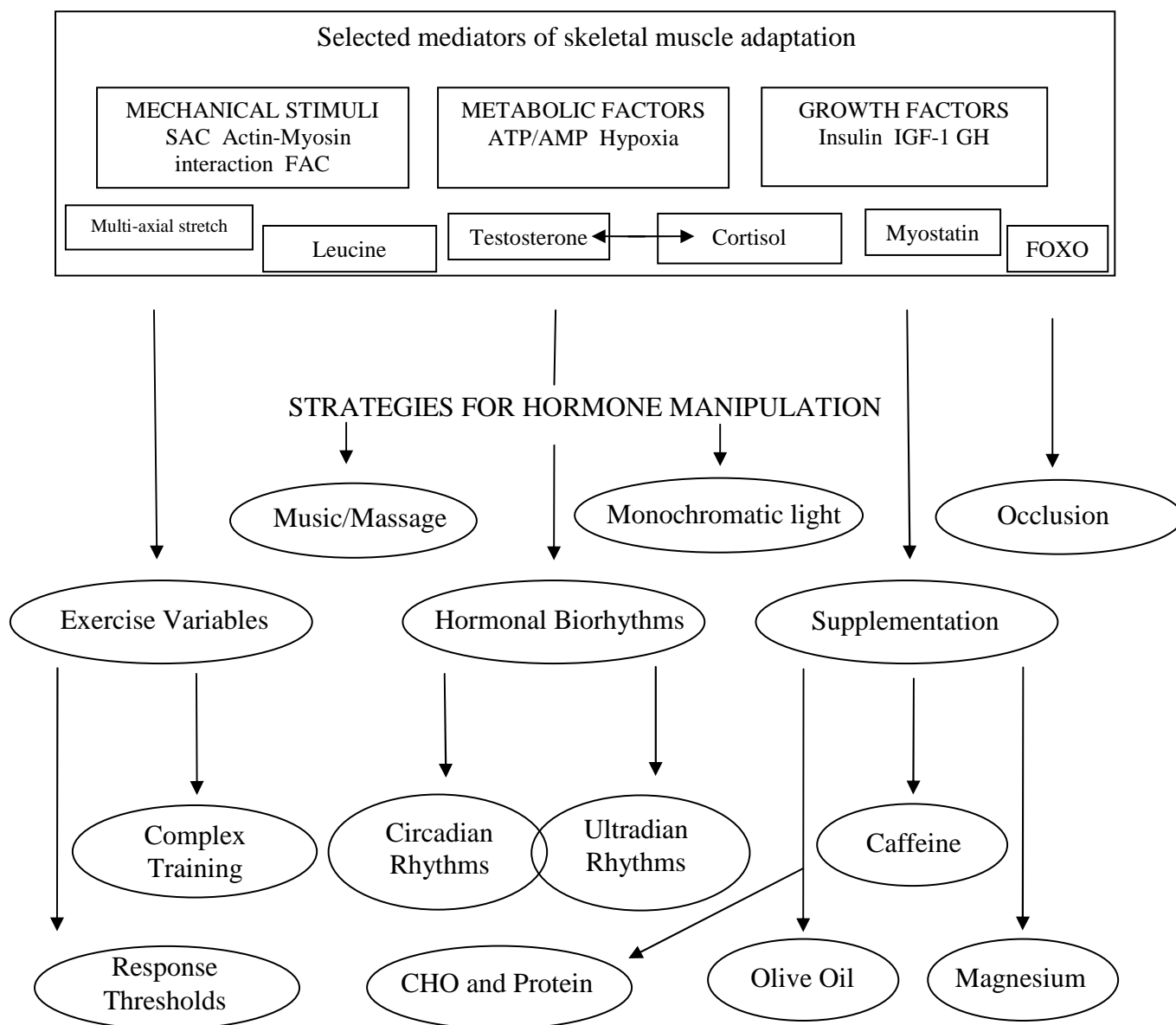


Figure 20. Updated diagram of strategies for modulating hormonal response to exercise

SAC = stretch-activated ion channels; FAC = focal adhesion complexes; ATP = adenosine triphosphate; AMP = adenosine monophosphate; IGF = Insulin-like growth factor; GH = Growth hormone; FOXO = Forkhead box O (transcription factor); CHO = Carbohydrate.

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APPENDICES

Appendix A: Salivary Testosterone Assay

The procedure follows the basic principle of radioimmunoassay (RIA) where there is competition between a radioactive and a non-radioactive antigen for a fixed number of antibody binding sites. The amount of [I-125]-labelled testosterone bound to the antibody is inversely proportional to the concentration of unlabelled testosterone present. The separation of free and bound antigen is achieved by use of a double antibody system.

Reconstitute lyophilised standards as provided by Diagnostic Systems Laboratories (DSL) from Testosterone RIA DSL-4100 kit with deionised water.

Dilute with Phosphate Buffered Saline (PBS) to provide standards of 0, 1, 5, 25, 100, 250, and 500pg·ml⁻¹.

Add 100µl of standards and unknown samples to labelled 5-ml test tubes (Labserv, LBS514, East Tamaki, Auckland, N. Z.).

Assay buffer comprises 0.05% Bovine serum albumin (BSA [ICP-BIO, ABGE-100G, Henderson, Auckland, N.Z.]) in PBS. PBS is made using tablets (P4417-100TAB, Sigma-Aldrich, St. Louis, U.S.A). Make up this buffer fresh each week as well as the 6% polyethylene glycol (PEG) solution in deionised water (BDH Prolabo, Geldenaaksebaan, Leuven, Belgium).

Use a 5-ml pipette tip and add the 340µl of a 13:1 rabbit anti-testosterone serum solution diluted in assay buffer to unknown samples and controls. This solution is **NOT to be added** to the total counts or NSB.

Non-specific binding (NSB) tubes have no antibody added but have 440µl of PBS solution instead.

Total count tubes only have 50µl of the provided [I-125]-labelled testosterone added.

VORTEX

Incubate at 4°C overnight

Add 50µl of [I-125]-labelled testosterone using 2.5-ml multi tip.

VORTEX

Incubate in a 37°C water bath for three hours.

Take the precipitating agent provided by DSL (containing goat anti-rabbit gamma globulin serum with polyethylene glycol as a precipitating aid) from the fridge. This reagent should be mixed thoroughly before use.

Dilute the precipitating agent with the 6% PEG solution in a 1:2 ratio. Refrigerate.

Add 500µl of the resulting solution to standards and unknown samples using a 10-ml pipette tip.

VORTEX

Incubate at room temperature for 20 min.

Cool in ice water slurry for at least five minutes. Centrifuge bucket inners should be cooled as well.

Centrifuge at 3700 rpm at 4°C for 15 min. All tubes yet to be spun should be left in the ice slurry.

Decant by simultaneous inversion, holding an inversion with a sponge rack into a radioactive waste receptacle.

Allow them to drain on paper towels for 15-30 sec. Gently blot the tubes to remove any droplets adhering to the rim before returning them to the upright position. Failure to blot tubes adequately may result in poor replication and spurious results.

Measure counts using gamma counter for 120 sec.

The mean counts per minute (CPM) for each standard, control, and unknown sample is used to calculate the %B/Bo as follows:

$$\% \text{ B/Bo} = (\text{Mean Sample Counts} / \text{Mean Counts of 0 pg}\cdot\text{ml}) * 100$$

Plot a curve of % B/Bo for the standards (y-axis) against the concentration (x-axis) in a log-linear (semi-log) manner. Draw a standard curve through the mean of the duplicate points.

Determine the concentration of the means of the controls and unknown sample from the triplicate counts on the standard curve.

Appendix B: Salivary Cortisol Assay

The procedure follows the basic principle of radioimmunoassay as described for testosterone.

Use the prepared stripped 51.2 ng·ml⁻¹ saliva standard and dilute in assay buffer to provide standards of 0, 0.05, 0.2, 0.8, 3.2, 12.8, and 51.2 ng·ml⁻¹.

Assay buffer comprises 0.05% Bovine serum albumin (BSA [ICP-BIO, ABGE-100G, Henderson, Auckland, N.Z.]) in PBS. PBS is made using tablets (P4417-100TAB, Sigma-Aldrich, St. Louis, U.S.A). Make up this buffer fresh each week

Add 50µl of standards and unknown samples to labelled 5-ml test tubes (Labserv, LBS514, East Tamaki, Auckland, N. Z.).

Use a 5-ml pipette tip to add 300µl of the rabbit anti-cortisol serum provided in the DSL-2000 Cortisol kit to all tubes except NSB and total counts.

NSB tubes have no antibody added but have 350µl of PBS solution instead.

Total count tubes only have 100µl of the provided [I-125]-labelled cortisol added.

VORTEX

Incubate at 4°C overnight

Add 100µl of [I-125]-labelled cortisol to all tubes using 5-ml pipette tip.

VORTEX

Incubate in a 37°C water bath for two hours.

Add 500µl of cold 6% PEG 6000 to all tubes except total counts.

VORTEX

Incubate at room temperature for one hour.

Cool in ice water slurry for at least five minutes. Centrifuge bucket inners should be cooled as well.

Centrifuge at 4°C 3700 rpm for 15 min. All tubes yet to be spun should be left in the ice slurry.

Decant by simultaneous inversion, holding an inversion with a sponge rack into a radioactive waste receptacle.

Allow them to drain on paper towels for 15-30 sec. Gently blot the tubes to remove any droplets adhering to the rim before returning them to the upright position. Failure to blot tubes adequately may result in poor replication and spurious results.

Measure counts using gamma counter for 60 sec.

The mean counts per minute (CPM) for each standard, control, and unknown sample is used to calculate the %B/Bo as follows:

$$\% \text{ B/Bo} = (\text{Mean Sample Counts} / \text{Mean Counts of 0 pg·ml}) * 100$$

Plot a curve of % B/B₀ for the standards (y-axis) against the concentration (x-axis) in a log-linear (semi-log) manner. Draw a standard curve through the mean of the duplicate points.

Determine the concentration of the means of the controls and unknown sample from the triplicate counts on the standard curve.

Appendix C: Deconvolution Process

The information provided in this section is available via the Pulse_XP homepage <http://mljohnson.pharm.virginia.edu/home.html>.

Hormone Sampling

Endocrine gland signaling has a unique, virtually universal feature of secreting hormone in a pulsatile manner rather than in a time-invariant or in a constant pattern of release. For many hormones, these temporal variations in serum hormone concentrations are thought to be a major part of the signaling pathway through which endocrine glands communicate with remote target organs. Hormone concentration time-series data has a number of unique features, e.g. a small number of data points and large experimental uncertainties. The small number of data points is primarily a consequence of the high cost of the clinical laboratory assays, the cost of the data collection staff, and the limitation imposed by Human Investigation Committees of total blood volume sampled. The experimental uncertainties (i.e. measurement errors) are usually Gaussian distributed with a magnitude that varies with the hormone concentration. These data will typically have missing values and contain outliers. An outlier is a value that appears to be inconsistent with its neighboring values. Since in most cases it is impossible to “get more data”, we need to be able to analyze the data which we have. Thus, it is important that the analysis methods be capable of a statistically correct treatment of data sets which contain large variable Gaussian distributed experimental uncertainties in the presence of missing values and outliers.

The standard procedure for the measurement of hormone concentrations is to repeat the assay a small number of times to create replicate values. These are then averaged to obtain a mean concentration at each time point. The average value for each time point, however, does not provide sufficient information for a hormone pulsatility analysis. Information about the precision of the measured hormone concentration is also required for this analysis.

This software requires four numbers at each data point for each hormone concentration time series:

- 1) the mean hormone concentration;
- 2) the time that the hormone was sampled;
- 3) the number of replicates that were averaged to determine the mean hormone concentration; and
- 4) information about the precision of the mean hormone concentration (i.e., the variance model for the data).

Variance Model

For a typical clinical laboratory hormone analysis, the coefficient of variation (Standard Error of the Mean / Mean) for the assay is nearly a constant in the middle region of the standard curve and goes to infinity at both very low and very high concentrations. Although the high end of the scale is usually not a problem because a sample can be diluted to bring it into the “linear region” of the assay, the analysis procedure must be capable of correctly treating this variable uncertainty of the data particularly for the very low concentration values.

In the limit of a very large number of replicates the information about the precision of the mean hormone concentration can be obtained by calculating the Standard Error of the Mean (SEM) of the replicates. However, for the small number of replicates commonly used for these data, the SEM of the replicates cannot be utilised.

This filter assumes that the variance model is of the form:

$$\text{Variance} = SD^2 = [MDC/2]^2 + ((CV/100)*Y_i)^2$$

where *MDC* is the minimal detectable concentration for the assay, the *CV* is the assay coefficient of variation (as a percent) near the middle of the calibration curve, and Y_i is the

average concentration at the i th data point. The definition of *MDC* is twice the standard of a large number of replicates with a zero hormone concentration.

Outliers

The algorithms used within this software assume that outliers do not exist within the data. Basically an outlier is an unusual data value that is not consistent with the flanking values. There are many possible causes of outliers, such as data entry errors and/or a problem with the assay. Keep in mind that what appears to be an outlier could be an actual concentration spike and thus should not be removed from the analysis.

Potential outlier locations can be identified with the Cluster8 algorithm. This algorithm does not always identify the exact location of the potential outlier. Occasionally, the actual outlier might be the data point preceding or following the marked location. If the Outlier T-Score in the Cluster8 algorithm is set to 3.0 and the data set contains 144 data points it is expected that the outlier routine will mark one or two outliers that are not actually outliers. These potential outliers are simply on the tail of the distribution of normal values. Possible outliers should not simply remove it from the data before ascertaining the cause. If the outlier is due to a data entry error then the error should be corrected. If the blood samples have been archived then the user should have the sample re-assayed to verify whether or not the value is actually correct. If the outlier is not too far out of line perhaps it is simply on the tail of the distribution and should not be removed from the data. This software does not automatically remove the outliers. Since the statistical justification for the removal of outliers is always questionable, the removal of outliers is the responsibility of the user.

Minimal Detectable Concentration

The minimal detectable concentration of each assay is usually determined as part of the quality controls performed by most clinical laboratories. This concentration is the lowest concentration that can be determined to be not equal to zero with a probability of 0.95. The

MDC is evaluated as twice the standard deviation of the values determined by a large number of repeated assays of a sample with a zero concentration of the hormone.

Deconvolution Algorithms

The ‘Deconv’ program is a multi-parameter deconvolution model which was introduced to aid in the estimation of the constituent processes which contribute to the observed hormone concentration time series. While it is relatively easy to calculate the total hormone concentration from a data series if the secretion and elimination are known, it is much more difficult to calculate the inverse - that is, to calculate both the secretion and elimination that generated the concentration by starting with just the concentration as a function of time. This inverse process is known as deconvolution. The approach taken by the software utilised to the deconvolution of hormone time series is to employ an algorithm to separate an observed hormone concentration time series into its component parts, namely the secretion and the elimination as a function of time. Thus, deconvolution techniques provide estimates of hormone secretion and clearance rates based on serial hormone concentration measurements. Both waveform-defined and waveform-independent algorithms have been designed as complementary tools to aide in the unraveling of hormone secretion.

The ‘Deconv’ algorithm has been further developed into the *AutoDecon* program which adds and removes presumed secretory events. These algorithms provide parameter estimates for temporal peak positions, amplitudes of the secretory peaks, a calculated standard deviation, and a probable elimination half-life by making use of the actual hormone concentrations and their variances. The actual fitting for these algorithms is done by using a damped, Gauss-Newton weighted nonlinear least-squares method.

The techniques used here are iterative approximations i.e. the algorithm begins with initial estimates of the model parameters and then attempts to provide a better estimate of those model parameters. These newest results are then used as the latest initial estimates of

the parameters and the cycle begins again. This process is repeated until the latest initial estimates and the improved calculated results are virtually indistinguishable.

These algorithms provide numerical values for all of the model parameters. A realistic estimate of the statistical uncertainties of the calculated parameters is also provided along with the secretion time series and the elimination time series. Only positive values for the secretory event amplitudes are provided in order to give meaningful values for these events.

AutoDecon consists of three separate modules:

- 1) a parameter *fitting* module;
- 2) an *insertion* module; and
- 3) a *triage* module.

The *fitting* module employs weighted nonlinear least-squares parameter estimation by the Nelder-Mead Simplex algorithm providing a result with the highest probability of being correct. The *insertion* module inserts the next presumed secretion event at the location of the maximum of the Probable Peak Index (PPI). The *triage* module performs the statistical test to determine whether or not a presumed peak is or is not statistically significant. This test requires two weighted nonlinear least-squares parameter estimations: one for the presumed peak present and one for the peak removed. The ratio of the variance of the fit resulting from these two parameter estimations is related to the probability that the presumed secretion event does not exist, P , by an F statistic.

Every cycle of the *triage* module performs this statistical test for each secretion event in order of smallest to largest. If a peak is found to be non-significant, it is removed and the *triage* module restarted. The *triage* module cycles continue until there are only statistically significant peaks remaining. Each cycle of the *triage* module performs $m+1$ weighted nonlinear least-squares parameter estimations where m is the current number of secretion events

in the current cycle; one where all of the secretion events are present and one where each of the events has been individually removed (tested).

As its first step, the *fitting* module calculates basal secretion and the concentration at time zero. This module then estimates any other parameter which may be included in the current fit apart from the half-life and standard deviation. If there are no events associated with the data series, the *insertion* module is called. This module adds presumed secretion events which are then fit. This step is then immediately followed by the *trriage* module which tests the significance of all presumed events now included in the fit.

If the *trriage* module does not remove any secretion events at this time, the *insertion* module is repeated inserting new presumed secretion events. This step is repeated until no additional secretory events have been added in the *insertion* cycle followed by the *trriage* cycle. The half-life and secretory burst SD are still not fitted during these steps. It is during the final step that the *fitting* module within the *trriage* module is repeated - this time estimating all of the current model parameters, *including* the elimination half-life and the standard deviation of the secretion events. This final step is repeated until no additional secretion events have been added in the *insertion* followed by *trriage* cycle.

AutoDecon does not add presumptive peaks automatically. Additional presumptive peaks may be inserted manually at positions indicted by PPI markers. This individual addition of peaks should continue until no additional significant peaks can be added. To do this, using the mouse to line up the on-screen cross-hairs with the highest PPI marker located in the secretion panel with the approximate height in the concentration curve panel. Clicking will add a new presumptive peak. The user should then re-fit the data to determine whether or not to accept or reject this manually inserted peak is statistically significant. Additional fits should be carried out until any remaining PPI markers clearly indicate positions in the data that, when adding a peak, does not enhance the fit and is not statistically significant.

Approximate Entropy (ApEn)

Approximate Entropy (ApEn) is a statistic which was developed to quantify the orderliness of serial data. ApEn provides a statistical measure for the determination of this regularity. Approximate Entropy is a model-independent regularity measure which monitors sample-by-sample pattern irregularity. ApEn assigns a non-negative number to a time series, with larger values corresponding to greater apparent serial irregularity in the hormone release patterns over time.

Approximate Entropy is complementary to the pulse-detection algorithms in that it evaluates both dominant and subordinate patterns in the data. ApEn can detect changes in underlying episodic behaviour which may not be reflected in peak occurrences or amplitudes and provides an explicit barometer of feedback system changes in coupled systems. ApEn is scale-dependent meaning that “getting more data” does not necessarily enhance the results.

ApEn is from a two parameter family of statistics (m and r) with m being the length of compared runs and r the filter width. These two parameters (m and r) must be specified to compute approximate entropy, which then measures the logarithmic likelihood that runs in the patterns that are close (within r) for m contiguous observations remain close (within the same tolerance with r) on the next incremental comparisons. The r value used for the various endocrine hormone profiles is generally 20%. ApEn is performed on individual hormone concentration time-series. Normalizing r to each time-series SD gives approximate entropy a translation in scale and variance.

Software Validation Methods and Data Simulation Techniques

Validating any method of data analysis is of the utmost importance. If the “answers” or results of a series of data sets is known before any analysis is undertaken, the ability to determine how well the software functions is fairly straightforward. For example, one early study in the field of endocrinology allowed for the determination of the mass of hormone secreted. Serial injections of exogenous LH were administered to a hypogonadotropic man

who was LH-deficient. Thus, the amount of hormone secreted was known. Recovery of this known mass of LH was determined through multi-parameter deconvolution analysis. This method, though, of obtaining reliable “known” answers from a biological system is very difficult to achieve. Hence, a complementary method for validating software has been the mathematical approach of simulating large numbers of data series which mimic “real” experimental data. With this method, the user can apply the software to the synthetic files and determine how well the calculated values match the known results.

The simulation of hormone concentration time series is done by generating waveforms that quantitatively as well as qualitatively resemble observed experimental data. These synthetic data sets have known values for all of the model parameters and also contain realistic amounts of experimental variance or noise. The goal is to make any set of simulations as close to “real” data as possible. Thus, making conclusions about the operating behavior of the software is much more accurate. For every experimental protocol a new set of simulations should be created. Thus, the operating features can be known for each specific group or set of subjects. The parameters of a given group of experimental data must be known before any simulations can be undertaken.

In creating the synthetic data, the first step the simulator performs is that of locating the positions in time of the secretion events. In a 24-hr data series, the simulation should begin well before the 24-hr period of interest and continue on for several additional hours after the period of interest since in experimental data the initial sampling may take place at any point during a secretory event. Based upon the desired characteristics of the simulation, inter-pulse intervals are then generated with a pseudo-random Gaussian distribution with negative inter-pulse intervals being discarded. The first event is given a value of -24-hr plus the first interval in time. The next secretory event is assigned the first event time plus the second interval, etc until the entire series has been completed, plus several additional hours.

The simulator then must generate the matching series of secretion event amplitudes as pseudo-random numbers based upon the desired parameter characteristics. Since negative event heights are not realistic, any negative value is replaced with a subsequent positive value. After the locations with their corresponding amplitudes are generated, these values are then used with the basal secretion, half-life, and standard deviation to calculate perfect noise-free sets of simulated data. Adding Gaussian distributed random numbers which correspond with the minimal detectable concentration and coefficient of variation is the last step. This process provides the simulated measurement uncertainties to the data.

A large number of data sets should be generated. In conjunction with these synthetic files will be the same number of “answer” files containing the results. The algorithm should then be applied to each of these files and best results stored. The expected results of the software can then be compared with the known results. When determining how well a peak-detection software is working, there are several operating characteristics of interest. The *true positive rate* is the percent of actual known secretion events that were correctly identified by the program as being secretion events. The *false positive rate* is the number of peaks identified by the program as statistically significant secretory events which are not, in fact, secretion events. The *false negative rate* represents the “real” known secretory peaks which the program failed to detect as secretory events.

The goal is for the true positive rate to be close to 100% with the false positive and false negative rates hovering near zero. Other parameters to examine include the location and size of the true positive events as well as the calculated half-life and basal secretion rate. Therefore, the overall operating characteristics of the software can be ascertained with thoughtfully and carefully generated simulated data.

Simulation Data from Ultradian Study

Sim. #	Actual	AutoDecon	User Defined (UD)	AutoDecon/Actual	UD/Actual	OVERESTIMATION		IDENTICAL		UNDERESTIMATION	
						AutoDecon	UD	AutoDecon	UD	AutoDecon	UD
1	9	9	9	1.00	1.00			1	1		
2	8	8	8	1.00	1.00			1	1		
3	7	7	8	1.00	1.14		1	1			
4	12	12	12	1.00	1.00			1	1		
5	10	10	10	1.00	1.00			1	1		
6	8	8	8	1.00	1.00			1	1		
7	6	7	8	1.17	1.33	1	1				
8	10	10	10	1.00	1.00			1	1		
9	11	10	11	0.91	1.00				1	1	
10	8	8	8	1.00	1.00			1	1		
11	8	8	9	1.00	1.13		1	1			
12	6	7	6	1.17	1.00	1			1		
13	10	8	11	0.80	1.10		1			1	
14	9	9	9	1.00	1.00			1	1		
15	7	7	7	1.00	1.00			1	1		
16	12	10	12	0.83	1.00				1	1	
17	11	3	11	0.27	1.00				1	1	
18	10	9	10	0.90	1.00				1	1	
19	8	8	8	1.00	1.00			1	1		
20	9	10	9	1.11	1.00	1			1		
21	10	10	10	1.00	1.00			1	1		
22	9	8	9	0.89	1.00				1	1	
23	8	9	8	1.13	1.00	1			1		
24	9	9	9	1.00	1.00			1	1		
25	10	6	10	0.60	1.00				1	1	
26	10	9	9	0.90	0.90					1	1
27	6	6	6	1.00	1.00			1	1		
28	7	7	7	1.00	1.00			1	1		
29	10	9	9	0.90	0.90					1	1
30	10	7	10	0.70	1.00				1	1	
31	10	11	11	1.10	1.10	1	1				
32	11	10	10	0.91	0.91					1	1
33	10	9	9	0.90	0.90					1	1
34	11	5	11	0.45	0.91				1	1	
35	8	8	8	1.00	1.00			1	1		
36	9	12	10	1.33	1.11	1	1				
37	9	10	11	1.11	1.22	1	1				
38	10	9	10	0.90	1.00				1	1	
39	10	7	10	0.70	1.00				1	1	
40	11	8	10	0.73	0.91					1	1
Average	9.18	8.43	9.25	0.94	1.01	7	7	17	27	16	6
		91.8%	100.8%			17.5%	17.5%	42.5%	67.5%	40.0%	15.0%

One hundred data sets were generated by the deconvolution software using the variability parameters established from actual data sets and assay characteristics (%CV and MDC of 4.32 and 1.38, respectively). Of the 100 data sets, 40 were analysed at random and actual results were compared with the results obtained by either the automatic AutoDecon or standard user defined (UD) parameters established previously (Ingram, Lo, Atkinson, Martinsen, & Beaven, 2008). It is evident that the user defined parameters for the deconvolution analysis generally out-performed the automatic deconvolution as the UD correctly identified 100% of peaks 25% more often than the AutoDecon program. The UD parameters overestimated the number of peaks in a hormone profile to a similar extent to the AutoDecon program, but were superior in terms of underestimation by 25%. Overall the ratio of actual peaks to identified peaks was within 1% for the UD parameters whereas the AutoDecon program underestimated by approximately 8%.

Appendix D: Notices of Ethical Approval



Wintec Research Office

Waikato Institute of Technology
W-Block cnr Tristram Street & Ward Street
Private Bag 3036
Hamilton 3204
e-mail research@wintec.ac.nz

Telephone 07 834 8800 Extn 8460
Fax 07 834 8884

Wednesday 13 June 2007

Martyn Beaven
Sports & Exercise Science

Dear Martyn

Re: Human Ethics in Research Committee Meeting 17 May 2007
Project Title: Physiological monitoring of elite rugby players (Application #210605)

Thank you for your above application and for the supplementary information provided.

It is with pleasure I am able to advise ethical approval for your above project is confirmed.

Please accept my apologies for any inconvenience the delay in responding may have caused. The Committee wishes you every success with the project.

Yours sincerely

Faye Hope

Faye Hope
Executive Officer
Wintec Human Ethics in Research Committee

Copied to: Katherine O'Regan



MEMORANDUM

To: Will Hopkins
 From: **Madeline Banda** Executive Secretary, AUTECH
 Date: 2 November 2005
 Subject: Ethics Application Number 04/95 **The effect of caffeine supplementation on hormonal response to weight training.**

Dear Will

Thank you for providing written evidence as requested. I am pleased to advise that it satisfies the points raised by the Auckland University of Technology Ethics Committee (AUTECH) at their meeting on 10 May 2004. Your ethics application is now approved for a period of three years until 2 November 2008.

I advise that as part of the ethics approval process, you are required to submit to AUTECH the following:

- A brief annual progress report indicating compliance with the ethical approval given using form EA2, which is available online through <http://www.aut.ac.nz/research/ethics>, including a request for extension of the approval if the project will not be completed by the above expiry date;
- A brief report on the status of the project using form EA3, which is available online through <http://www.aut.ac.nz/research/ethics>. This report is to be submitted either when the approval expires on 2 November 2008 or on completion of the project, whichever comes sooner;

You are reminded that, as applicant, you are responsible for ensuring that any research undertaken under this approval is carried out within the parameters approved for your application. Any change to the research outside the parameters of this approval must be submitted to AUTECH for approval before that change is implemented.

Please note that AUTECH grants ethical approval only. If you require management approval from an institution or organisation for your research, then you will need to make the arrangements necessary to obtain this.

To enable us to provide you with efficient service, we ask that you use the application number and study title in all written and verbal correspondence with us. Should you have any further enquiries regarding this matter, you are welcome to contact Charles Grinter, Ethics Coordinator, by email at charles.grinter@aut.ac.nz or by telephone on 921 9999 at extension 8860.

On behalf of the Committee and myself, I wish you success with your research and look forward to reading about it in your reports.

Yours sincerely

Madeline Banda
 Executive Secretary
 Auckland University of Technology Ethics Committee

Appendix E: Informed Consent Forms and Information Sheets

PHYSIOLOGICAL MONITORING OF ELITE RUGBY PLAYERS

INFORMATION SHEET FOR PARTICIPANTS

Thank you for considering taking part in this project. Please read this information sheet carefully before deciding whether or not to participate. If you decide to participate we thank you. If you decide not to take part there will be no disadvantage to you of any kind and we thank you for considering our request.

What is the aim of the project?

The aim of the project is to examine the hormonal state of individual players in order to guide conditioning programs. The anabolic (muscle building) status of the players will be assessed and this information delivered to the trainers. By adjusting training loads based on this information it is intended that individual gains will be maximised. The ability of the body to respond to specific resistance training stimuli will also be assessed to provide the trainers with information to assist with periodisation and keeping players fresh and get maximal benefits out of their time in the gym.

What will participants be asked to do?

Subjects will be asked to provide a saliva sample before, during and after specified training sessions.

Can participants change their mind and withdraw from the project?

You may withdraw from participation in the project at any time, without needing to give a reason, and without any disadvantage to yourself of any kind.

What data or information will be collected and what use will be made of it?

Saliva samples will be taken and analysed. This information is stored confidentially, and may not be released to anyone for any other purpose than this study without your permission. The only exception to this would be if a court ordered that the information were to be obtained (subpoenaed) for a particular purpose. However, you may choose to have your samples destroyed immediately following analysis. Results of this project may be published but any data included will in no way be linked to any specific participant, so your confidentiality will be maintained. You are most welcome to request a copy of your individual results, or the general summary of results of the project should you wish. The data collected will be securely stored in such a way that only the researchers, your management and you will be provided with information that allows identification of individuals.

Is the process of collecting samples safe?

Yes, the process of collecting a saliva sample is as simple as spitting into a tube.

What if Participants have any Questions?

If you have any questions about our project, either now or in the future, please feel free to contact either:

Martyn Beaven
Research Coordinator
Ph 07 858 4756 or
Ph 021 259 6480
Email: cmbeaven@hotmail.com

Dr. Nicholas Gill
Principal Lecturer
Centre for Sport and Exercise Science
Waikato Institute of Technology
Ph 07 834 8800 x8407
Ph 0274 899 447
Email: nicholas.gill@wintec.ac.nz



PHYSIOLOGICAL MONITORING OF ELITE RUGBY PLAYERS

I have read the Information Sheet concerning this project and understand what it is about. All of my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:

1. My participation in the project is entirely voluntary;
2. I am free to withdraw from the project at any time, without reason, and without any disadvantage;

3. I would like my samples destroyed within 12 months of collection

☐

OR

I would like my samples destroyed immediately after initial analysis.

☐

4. The data will not be released to third parties for any purpose without my express consent. The only exception to this would be in a case where the researchers were required to release the data by a court order and this would only be possible if you select the first option in point 3;
5. The results of the project may be published but my anonymity will be preserved.

I agree to take part in this project.

.....

Print name

.....

.....

(Signature of participant)

(Date)

This project has been assessed and approved by Wintec Ethics Committee.



PHYSIOLOGICAL MONITORING OF ELITE RUGBY PLAYERS SERIAL MONITORING OF SALIVA IN HEALTHY SUBJECTS

INFORMATION SHEET FOR PARTICIPANTS

Thank you for considering taking part in this project. Please read this information sheet carefully before deciding whether or not to participate. If you decide to participate we thank you. If you decide not to take part there will be no disadvantage to you of any kind and we thank you for considering our request.

What is the aim of the project?

The aim of the project is to examine the hormonal pulses that have been observed in pilot work. Identification and replication of this pulsatility would be a novel discovery and understanding the nature of these pulses could have important implications for training and training adaptation. Physical (e.g. exercise) and physiological (e.g. audio/visual) stimuli will be presented in an attempt to modify hormonal responses and investigate their effect on the normal daily rhythms.

What will participants be asked to do?

Subjects will be asked to provide a saliva sample every ten minutes of the course of an eight hour day.

Can participants change their mind and withdraw from the project?

You may withdraw from participation in the project at any time, without needing to give a reason, and without any disadvantage to yourself of any kind.

What data or information will be collected and what use will be made of it?

Saliva samples will be taken and analysed. This information is stored confidentially, and may not be released to anyone for any other purpose than this study without your permission. The only exception to this would be if a court ordered that the information were to be obtained (subpoenaed) for a particular purpose. However you may choose to have your samples destroyed immediately following analysis. Results of this project may be published but any data included will in no way be linked to any specific participant, so your confidentiality will be maintained. You are most welcome to request a copy of your individual results, or the general summary of results of the project should you wish. The data collected will be securely stored in such a way that only the researchers, your management and you will be provided with information that allows identification of individuals.

Is the process of collecting samples safe?

Yes, the process of collecting a saliva sample is as simple as spitting into a tube.

What if Participants have any Questions?

If you have any questions about our project, either now or in the future, please feel free to contact either:

Martyn Beaven

Research Coordinator

Ph 07 858 4756 or

Ph 021 259 6480

Email: cmbeaven@hotmail.com

Dr. Nicholas Gill

Principal Lecturer

Centre for Sport and Exercise Science

Waikato Institute of Technology

Ph 07 834 8800 x8407

Ph 0274 899 447

Email: nicholas.gill@wintec.ac.nz

This project has been assessed and approved by Wintec Ethics Committee.

SERIAL MONITORING OF SALIVA IN HEALTHY SUBJECTS

I have read the Information Sheet concerning this project and understand what it is about. All of my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:

1. My participation in the project is entirely voluntary;
4. I am free to withdraw from the project at any time, without reason, and without any disadvantage;

5. I would like my samples destroyed within 12 months of collection

☐

OR

I would like my samples destroyed immediately after initial analysis.

☐

4. The data will not be released to third parties for any purpose without my express consent. The only exception to this would be in a case where the researchers were required to release the data by a court order and this would only be possible if you select the first option in point 3;
5. The results of the project may be published but my anonymity will be preserved.

I agree to take part in this project.

.....

(Signature of participant)

.....

(Date)

This project has been assessed and approved by Wintec Ethics Committee.





Participant Information Sheet

Project Title

The Effect of Caffeine Supplementation on Hormonal Response to Training

Invitation

As members of the Auckland Warriors Development Squad you are invited to participate in this research.

What is the purpose of the study?

To determine the effect of caffeine supplementation on hormonal responses to weight training and field training.

What happens in the study?

One hour before each of four weight-training sessions over a 3-week period in late November through mid December, you will take capsules containing one of four doses of caffeine. The highest dose will be 9 mg of caffeine per kg of body mass, which corresponds to the caffeine contained in about 6 cups of coffee. This dose is at the high end of doses used in this kind of research. The moderate dose will be 6 mg per kg, which is the usual amount. The low dose will be 3 mg per kg, and one of the doses will have no caffeine at all.

The order of the doses will be randomised, and you will not be told which dose you are receiving before each session. You will receive all four doses over the 3-week period. You will be required to give a saliva sample at the time you take the capsules, then immediately before and immediately after the training session. The sample will be analysed to determine the concentration of the hormones testosterone and cortisol.

You will also give saliva samples on Monday and Friday mornings in each week of the study, to monitor any overall effects of training on testosterone and cortisol over the study period.

In January or February next year, we will go through the same procedures, but we will replace the four sessions of strength training with four of your usual field-training sessions involving game practice.

You will be asked to keep a diet history for the day prior to the first training session so that you may replicate that diet prior to the other sessions. You will also be asked not to eat food and drink containing large amounts of caffeine (coffee, Red Bull, "V", chocolate) during this time.

How much time is involved?

The study is being organised as part of your usual training sessions, so there should be no additional demands on your time.

What are the discomforts and risks?

The higher doses of caffeine capsules can cause sleep disturbance and feelings of anxiety in some individuals. On average these effects are small in magnitude, but they could be moderate in some individuals and trivial in others. If you experience these effects and you find them unpleasant, you should withdraw from the study. In a very small percentage of individuals, caffeine can disturb the

electrical activity in the heart, giving you a fluttering sensation in the chest. If you have ever experienced this sensation after drinking coffee, you should not take part in the study.

What are the benefits?

There are no immediate benefits from this study. If we find that caffeine raises testosterone more than cortisol, there is a good chance that taking caffeine before training sessions will increase the effectiveness of the sessions. We would need to do more research to find out.

Access to results?

You will receive a report of the average effects of caffeine on the squad and the effects on you personally.

How is my privacy protected?

At all stages throughout the research project your name and results will remain confidential to the researchers and yourself.

Withdrawal?

At any stage during this study you are free to withdraw with no explanation required.

Participant Concerns

Any concerns regarding the nature of this project should be notified in the first instance to the project supervisor, Will Hopkins 921 9793, or to John Cronin 921 9999 ext 7353, or to your coach, Keir Hansen 526 8837. These are all daytime phone numbers.

Concerns regarding the conduct of the research should be notified to the Executive Secretary, AUTECH, Madeline Banda, madeline.banda@aut.ac.nz, 921 9999 ext 8044.

Approved by the Auckland University of Technology Ethics Committee on ...*2nd November*.....

AUTECH Reference number*04/95*.....

Appendix F: Abstracts of experimental chapters formatted for submission

Acute Salivary Hormone Responses to Complex Exercise Bouts (Chapter Three)

C. Martyn Beaven, Nicholas D. Gill, John R. Ingram, & Will G. Hopkins. *Journal of Strength and Conditioning Research, In Press*

The combination of resistance and plyometric training, or complex training, may yield greater functional gains than either method alone. As steroid hormones respond to exercise stimuli and modulate the functional outcomes, it is possible that complex training creates an enhanced anabolic physiological milieu for adaptation. **Purpose:** To investigate acute responses of salivary testosterone and cortisol to complex exercise protocols. **Methods:** After a standardised warm-up, 16 semi-professional rugby players performed one of four exercise protocols in a cross-over manner: power-power; power-strength; strength-power; or strength-strength. The power block consisted of three sets of three repetitions of jump squat exercise at 50% of one-repetition maximum (RM) load. The strength block consisted of three sets of three repetitions of box squat exercise at a 3-RM load. There were 3-min rest periods between sets and 4-min rest periods between exercise blocks. Saliva was sampled before and after the warm-up, at the mid-point of the protocol, and immediately after the protocol. Data were log-transformed to estimate percent effects with mixed modeling, and effects were standardised to assess magnitudes. **Results:** The greatest overall hormonal responses were a small testosterone increase (13%; 90% confidence limits $\pm 7\%$) and a trivial increase in cortisol (27%; $\pm 30\%$) following the strength-power protocol. A clear difference was observed between the strength-power and the power-power protocols immediately after exercise for both testosterone (10%; $\pm 8\%$) and cortisol (29%; $\pm 17\%$). The preceding exercise block had little effect on subsequent strength and power performance. **Conclusion:** The greatest testosterone response was observed when a power-type training stimulus was applied after a heavy resistance training stimulus suggesting that this exercise sequence provides an enhanced anabolic milieu for adaptation compared to the other training methods examined.

Key Words: STRENGTH, POWER, COMBINATION TRAINING, TESTOSTERONE, CORTISOL.

Lower-body Strength and Power Development during Different Phases of the Circadian Rhythm (Chapter Four)

C. Martyn Beaven, John R. Ingram, Nicholas D. Gill, & Will G. Hopkins.

Aim: To discern a circadian rhythm in salivary steroid hormones and investigate any effect of circadian rhythmicity on hormonal responsiveness to resistance training protocols and training outcomes. **Methods:** Eight semi-professional male rugby players from a single team performed identical back squat exercise bouts within two consecutive 4-wk pre-season training blocks. The first block was performed in the morning (0800-0900) and the following pre-season the bouts were performed in the afternoon (1500-1600). Saliva samples were collected before and after exercise for the determination of testosterone and cortisol. **Results:** Pre-exercise testosterone and cortisol concentrations were clearly higher in the morning compared to the afternoon with morning values being greater by a factor of 1.8 ($\times/\div 1.1$; 90% confidence limits) and 5.7 ($\times/\div 2.3$), respectively. The ratio of pre-exercise testosterone to cortisol however, was clearly lower in the morning by a factor of 0.3 ($\times/\div 2.4$). The afternoon training resulted in a greater increase in peak power produced at load equivalent to 50% of 1-RM compared to morning training (PM, $8.5 \pm 11.2\%$; AM $3.8 \pm 6.7\%$; mean $\pm 90\%$ confidence limits); however similar increases in box squat strength were observed during each four week block regardless of the training time (PM, $17.4 \pm 4.1\%$; AM, $16.6 \pm 2.5\%$). **Discussion:** These data corroborate a circadian rhythm in salivary steroid hormones. The greater gain in peak power during a countermovement jump-squat when training was performed in the late afternoon suggests that the phase of the circadian rhythm at which training is performed can modulate specific adaptations to resistance exercise. **Conclusion:** The testosterone to cortisol ratio, rather than absolute testosterone and cortisol concentrations, may modulate power performance gains.

Key Words: TESTOSTERONE, CORTISOL, SALIVA, RESISTANCE TRAINING.

Ultradian Rhythmicity and Induced Changes in Salivary Testosterone (Chapter Five)

C. Martyn Beaven, John R. Ingram, Nicholas D. Gill, & Will G. Hopkins. *European Journal of Applied Physiology, In Review*

Aim: To investigate the effect of the circadian cycle on hormonal responsiveness to resistance training protocols. **Methods:** Eight semi-professional male rugby players from a single team performed identical back squat exercise sessions within two 4-wk pre-season training blocks either in the morning (0800-0900 hrs) or in the afternoon (1500-1600 hrs). Saliva samples were collected before and after exercise for the determination of testosterone and cortisol. **Results:** Clear differences were observed between pre- and post-exercise hormone concentrations. The ratio of testosterone to cortisol was clearly superior in the afternoon. Similar increases in box squat strength during each four week block were observed regardless of training time; however peak power increased to a greater degree when training was performed in the afternoon. **Discussion:** The obvious differences between the hormonal environments experienced by an individual exercising at different times within the circadian rhythm have the potential to modulate adaptation to exercise. We observed greater gains in peak power produced at 50% of 1-RM load during a countermovement jump-squat when training was performed in the late afternoon. The importance of accounting for circadian variation when prescribing training and reporting endocrine responses was evident.

Key Words: TESTOSTERONE, CORTISOL, SALIVA, RESISTANCE TRAINING.

Dose Effect of Caffeine on Testosterone and Cortisol Responses to Resistance Exercise
(Chapter Six)

C. Martyn Beaven, Will G. Hopkins, Kier T. Hansen, Matthew R. Wood, John B. Cronin, & Timothy E. Lowe. *International Journal of Sport Nutrition and Exercise Metabolism*, Vol. 18, No. 2, April 2008, pp. 131-141.

Introduction: Interest in the use of caffeine as an ergogenic aid has increased since the International Olympic Committee lifted the partial ban on its use. Caffeine has beneficial effects on various aspects of athletic performance, but its effects on training response have been neglected. Purpose: To investigate the acute effect of caffeine on the exercise-associated increases in testosterone and cortisol in a double-blind crossover study. **Methods:** 24 professional rugby-league players ingested caffeine doses of 0, 200, 400 and 800 mg in random order 1 h before a resistance-exercise session. Saliva was sampled at the time of caffeine ingestion, at 15-min intervals throughout each session, and at 15 and 30 min after the session. Data were log transformed to estimate percent effects with mixed modeling, and effects were standardised to assess magnitudes. **Results:** Testosterone concentration showed a small increase of 15% (90% confidence limits, $\pm 19\%$) during exercise. Caffeine raised this concentration in a dose-dependent manner by a further small 21% ($\pm 24\%$) at the highest dose. The 800-mg dose also produced a moderate 52% ($\pm 44\%$) increase in cortisol. The effect of caffeine on the testosterone/cortisol ratio was a small decline (14; $\pm 21\%$). **Conclusion:** Caffeine has some potential to benefit training outcomes via the anabolic effects of the increase in testosterone concentration, but this benefit may be counteracted by the opposing catabolic effects of the increase in cortisol and resultant decline in the testosterone/cortisol ratio.

Key Words: PERFORMANCE, ANABOLIC, ATHLETE, CATABOLIC, STRENGTH TRAINING, TESTOSTERONE/CORTISOL RATIO.

Appendix G: Poster Presented at International Conference on Strength and Conditioning



C. Martyn Beaven^{1,2}
Nicholas D. Gill¹, Christine K. Angus¹,
John R. Ingram¹, Will G. Haydon¹,
Martyn Beaven@plantandfood.co.nz



**INSTITUTE OF
SPORT + RECREATION
RESEARCH NZ**

Plant & Food RESEARCH
RANGAHAU AHIMĀRA KAI



Acute Salivary Hormone Responses to Complex Exercise Bouts

Unit of Sport and Recreation Research, AUT University, Auckland, NZ
Tea and Food Group, Plant and Food Research, Auckland, NZ

www.plantandfood.com

THE NEW ZEALAND INSTITUTE FOR PLANT & FOOD RESEARCH LIMITED

Introduction

Testosterone and cortisol respond to resistance exercise and mediate subsequent neuromuscular adaptation^{1,12}. Exercise designed to improve maximal strength is primarily morphological adaptation generally produces larger increases in testosterone than those designed to enhance strength through neural adaptation^{6,70}. Dynamic power bouts designed to increase power, in which relatively light weights are lifted with explosive intent, also produce significant androgen responses⁷¹ similar to those seen in hypertrophy-type bouts¹⁸. Recent data suggest that prescribing resistance exercise based on salivary testosterone responsiveness produces superior gains in both upper and lower body strength⁷². Training that combines both power and strength stimulus has been reported to be superior to more conventional weight training bouts in actualizing strength and power gains^{1,3,8}. Indeed, the use of a combination of high force and high power appears to be superior to classical exercise prescriptions in terms of functional benefits^{1,3,8}. In view of the association between acute testosterone responses to resistance exercise stimuli and enhanced functional training benefits, we decided to investigate a potential role of hormones in actualizing the observed benefits of complex training.

Methods

Seventeen semi-professional male rugby union players (Mean ± SD Age: 20.6 ± 1.4 y; Height: 187 ± 6 cm; Body mass: 100 ± 12 kg) were recruited to complete each of four exercise bouts randomly twice a week over a four-week period, which incorporated box squats and/or jump squats. After a standard warm-up, players performed one of two exercise blocks (1) power: jump squat exercises, 3 sets of 3 repetitions at 50% 1-RM with 3-min rest between sets; or (2) strength: box squat exercises, 3 sets of 3 repetitions at a 3-RM load with 3-min rest between sets. Each player then performed a second exercise block (either power or strength) after a further 4-min rest. Thus, there were four exercise bout combinations: power followed by power (power-power); power followed by strength (power-strength); strength followed by power (strength-power); and strength followed by strength (strength-strength). Figure 1 shows an example power-strength bout. Saliva samples were collected before, during and after exercise to determine free testosterone and cortisol concentrations using radioimmunoassay. Power was assessed using a GymAwareTM optical encoder.



Figure 1. An example power-strength bout illustrating rest periods, exercise blocks and exercise intensity. Raps represent the number of repetitions. Int: Intensity; Ex: Exercise; Rest: Rest. Type of exercise, RM: Repetition maximum.

Discussion

The salivary hormone data presented are consistent with a favourable anabolic milieu following the strength-power bout compared with the power-power bout. To our knowledge, no other studies have reported that acute testosterone responses are enhanced following a complex training bout. Dodd and Alvar⁷³ have previously found that, when heavy resistance sets were immediately followed by a biomechanically similar exercise at <30% of 1-RM, sprint times and broad jump improved to a greater extent. In other acute studies, Dero and colleagues have shown that an additional set at 50% of 1-RM following a strength session stimulated growth hormone secretion and the addition of these low-intensity sets increased 1-RM strength gains over time⁷⁴.

The current study, in combination with earlier work, is suggestive of a hormonal mechanism that mediates superior functional gains when heavy resistance exercise is combined with subsequent low-intensity power-type exercises. Such findings reinforce the importance of understanding the training responses and the resulting hormonal milieu to enhance training outcomes.

Summary

The greatest testosterone response was observed when a power-type exercise was applied after a heavy resistance training stimulus. This complex exercise sequence provided an enhanced anabolic milieu for adaptation compared with other training methods assessed. As recent research has linked salivary testosterone responses with enhanced strength and body weight gain⁷², it is suggested that the superior functional gains associated with complex training are related to the improvements in the hormonal environment.

Results

Thirteen players completed every exercise bout over the four-week period. Performance and body composition changes observed over the four-week training period are reported in Table 1.

Table 1. Performance and body composition percentage changes in 13 rugby players over four weeks

	Mean ± s.d.	Effect Size
Max squat 1-RM (kg)	32 ± 45	Large
Peak Power (W)	7.6 ± 5.8	Moderate
Maximum vertical jump (cm)	5.0 ± 2.0	Small
Body fat (%)	-2.9 ± 3.3	Small
Body mass (kg)	0.9 ± 1.1	Trivial
Soft lean mass (kg)	-0.6 ± 1.1	Trivial
Cardiac leg volume (L)	0.3 ± 0.6	Trivial

RM, Repetition maximum; s.d. = SD; Confidence limits for post-hoc comparisons of magnitude effects were standardized by dividing by the between-subjects SD in the relevant condition. Effect sizes were categorized using thresholds of 0.2, 0.6, and 1.2 for small, moderate and large, respectively.

Figure 2 shows that the largest exercise-induced testosterone change was a small increase (10%; 90% confidence limits ±7%) in response to the strength-power bout. The strength-strength, power-power, and power-strength bouts induced clear but trivial increases in testosterone. A small increase in testosterone concentration in the strength relative to the power exercise blocks was seen at the mid-point of the exercise (9% ± 6%).

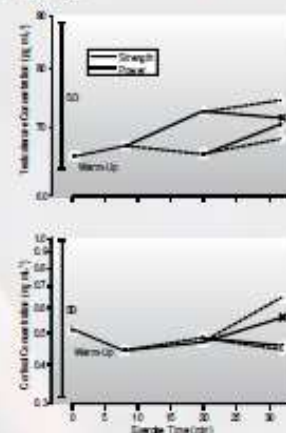


Figure 2. Mean salivary testosterone and cortisol concentrations in response to four exercise bouts. SDs = Standard deviation in the resting condition. Warm-Up = Four sets of five Squats at increasing load consisting of 15, 12, 10 and 8 repetitions with a 30-second rest period. Strength = Three sets of three repetitions box squats at 3 Repetition Maximum load with a three-minute rest period. Power = Three sets of three repetitions jump squats at a load equivalent to 50% of 1 Repetition Maximum with a three-minute rest period. Int = 12.

In contrast to testosterone, cortisol showed little difference between the strength and power exercise bouts at the mid-point of the exercise. The overall exercise-induced cortisol response to each bout was also trivial.

Acknowledgements

The authors wish to thank the contributions of Wally O'Leary, James Field, Simon Fother, Sam Higgins, Rick Ellis and Gary Ziemer to the smooth running of the experimental protocol. Funding for the project was supplied by the New Zealand Rugby Union, the Tertiary Education Commission and the New Zealand Plant and Food Research Institute of Ltd.

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1. Beaven C, Gill ND, Haydon W. Testosterone and cortisol responses to resistance exercise. *Appl Physiol*. 2012;112:1212-1218.
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3. Beaven C, Gill ND, Haydon W. Testosterone and cortisol responses to resistance exercise. *Appl Physiol*. 2012;112:1212-1218.
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LIST OF ABBREVIATIONS AND TERMS

1-RM	1-repetition maximum
3 β HSD	3- β -hydroxysteroid dehydrogenase
17 β HSD	17- β -hydroxysteroid dehydrogenase
4E-BP1	Eukaryotic initiation factor 4E binding protein-1
ACTH	Adrenocorticotrophic hormone
AICAR	5-aminoimidazole-4-carboxamide ribonucleotide (an AMPK agonist)
Akt/PKB	Protein kinase B
AMP	Adenosine monophosphate
AMPK	Adenosine monophosphate-activated protein kinase
ApEn	Approximate entropy
ATP	Adenosine triphosphate
BCAA	Branched-chain amino acid
[Ca ²⁺]	Calcium ion concentration
CBG	Cortisol-binding globulin
CV	Coefficient of variation
COX-2	Cyclooxygenase 2
CRH	Corticotropin releasing hormone
DHEA	Dehydroepiandrosterone
DNA	Deoxyribonucleic acid
eEF2	Eukaryotic elongation factor 2
eIF4E	Eukaryotic initiation factor 4E
eIF4F	Eukaryotic initiation factor 4F
ERK	Extracellular signal-regulated kinase
FAC	Focal adhesion complexes
FOXO	Forkhead box O (transcription factor)
FSH	Follicle stimulating hormone
G-protein	Guanine nucleotide-binding protein
GH	Growth hormone
GnRH	Gonadotropin-releasing hormone
GSK-3	Glycogen synthase kinase 3
HMB	β -hydroxy- β -methylbutyrate (supplement)
HPA	Hypothalamic-pituitary-adrenal (axis)
HPG	Hypothalamic-pituitary-gonadal (axis)
IGF-1	Insulin-like growth factor-1

IL-6	Interleukin 6 (an inflammatory cytokine)
JNK	c-Jun-N-terminal kinase
Kaatsu	Training involving artificial reduction of muscle blood flow
LH	Luteinizing hormone
MAPK	Mitogen-activated protein kinases
mTOR	Mammalian target of rapamycin
mTORC1	Mammalian target of rapamycin complex 1
mTORC2	Mammalian target of rapamycin complex 2
mRNA	Messenger ribonucleic acid
NFAT	Nuclear factor of activated T-cell (transcription factor)
NO	Nitric oxide
NRL	National Rugby League (Australian professional rugby league competition)
p70 ^{S6k}	Ribosomal S6 kinase
PA	Phosphatidic acid
PGF _{2α}	Prostaglandin F _{2α}
PI3-K	Phosphoinositide 3-kinase
Pulse_XP	Deconvolution software
PVN	Paraventricular nucleus
Raptor	Regulatory associated protein of mTOR
REDD1	A gene induced by hypoxia
RIA	Radioimmunoassay
RNA	Ribonucleic acid
RT	Resistance training
SAC	Stretch-activated ion channels
SHBG	Sex hormone-binding globulin
TNF- α	Tumour necrosis factor- α (an inflammatory cytokine)
tRNA	Transfer ribonucleic acid
TSC	Tuberous sclerosis complex
VJ	Vertical jump
Vps34	Vacuolar protein sorting mutant 34
ZMA	Zinc-magnesium aspartate (supplement)