ANDROGEN AND GLUCOCORTICOID RECEPTOR PHOSPHORYLATION FOLLOWING AN ACUTE RESISTANCE EXERCISE BOUT IN TRAINED AND UNTRAINED MEN

By

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ABSTRACT

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INTRODUCTION: Optimizing the concentration of hormones through variation of load, repetitions, and rest periods is suggested for building muscle mass in current resistance exercise prescription guidelines. Physiological actions of testosterone and cortisol occur when bound to their respective intracellular receptor. Muscle growth is initiated when testosterone binds to its androgen receptor (AR); conversely, muscle breakdown is initiated when cortisol binds to its glucocorticoid receptor (GR) in muscle cells. The secretion of both testosterone and cortisol increase following a single bout of resistance exercise (RE). While both *in vitro* and *in vivo* models indicate the significant contribution to muscle growth, the importance of the acute hormonal response in humans has been justified and refuted. However, there is recent evidence showing phosphorylation and regulation of ARs and GRs can occur in the absence of testosterone and cortisol. The equivocal results of prior studies on hormonal responses and muscle adaptation could be clarified by understanding how AR and GR are regulated.

PURPOSE: The purpose of this study is to examine the differences in the androgen and glucocorticoid receptor phosphorylation between resistance trained and untrained men following an acute bout of moderate intensity resistance training. It will also look at the biopsy time point post resistance exercise for AR and GR phosphorylation following resistance training.

METHODS: Ten resistance trained (RT) and ten untrained (UT) healthy, college aged (18-30) men volunteered for this study. One UT subject was unable to complete the protocol resulting in an N of 9. Subjects performed 1RM tests for back squat and leg extension 4-7 days prior to the RE protocol. There were no differences in the protocol between RT and UT groups. Subjects arrived at the lab at least 6 hours fasted and euhydrated between 10:00am-2:00pm to control for diurnal variations of hormones. Prior to training, baseline blood and muscle biopsy were collected for baseline levels. After a warm-up, subjects performed 6 sets of 10 repetitions at 75% of their 1RM with 1.5 minutes rest following each set followed by 4 set of 10 repetitions at 75% of their 1RM for leg extension with 1.5 minutes rest after each set. After completing the leg extension exercise,

blood samples were collected at 5 min, 15 min, and 45 min post exercise, and biopsies were taken at 10 min, 30 min, 60 min, and 180 min post exercise. Hormonal data were analyzed using parametric methods. 2 x 4 (group x time) RMANOVAs were used to determine potential differences in testosterone and cortisol between groups (group) at PRE, 5P, 15P and 45P (time). Total receptor data were not normally distributed, thus total receptor data were analyzed using Mann-Whitney U test, Friedman test, and Wilcoxon signed-rank test. Performance data were analyzed using RMANOVAs and independent t tests.

RESULTS: 1RM was significantly different between groups ($p < .05$). There were no significant differences between groups for any set for RPE ($p>0.05$), although there were RPE significant differences across sets. There were significant differences for mean force (N) between RT and UT groups for all sets, and the RT group began to significantly decrease by set 6. There was a significant difference in work (J) between groups for sets 1 and 2, with the RT group significantly decreasing by sets 5 and 6. In both the RT and UT groups testosterone significantly increased from PRE values at 5min and 15min post exercise ($p < .05$), then began to return to baseline by 45min post exercise. There was a moderate effect at 15P and 45P (Cohen's D) between groups with the RT group being higher. Cortisol significantly increased in the RT group at 5min and 15min from PRE values ($p < .05$); whereas, the UT group was elevated from PRE at all three time points (5min, 15min, 45min) ($p < .05$). For total AR expression, there were no differences between time points within the RT group ($p > .05$); however, there were differences between time points within the UT group ($p = .016$). In the UT group, total AR expression significantly decreased at 30P (-19.33%Δ, $z = -2.192$, $p = .027$) and 60P (-10.89%Δ, $z = -2.192$, $p = .027$) post exercise, but returned to baseline values by 180P (3%Δ, $z = -.178$, $p > .05$). There were significant decreases at 10P in p-AR Ser^{213} in both the RT (-28.73%Δ, $z = -2.293$, $p = .020$) and UT (-32.25%Δ, $z = -2.073$, $p = .039$) groups. There were no differences between or within groups ($p > .05$) for p-AR Ser^{81}, p-AR Ser^{515}, or p-AR Ser^{650}. For Total GR content, there were no differences between time points within the RT or UT groups ($p > .05$). Total GR content was significantly greater in the RT group compared to the UT group at 10P (Mann-Whitney $U = 19$, $z = -2.123$, $p = .035$). For p-GR Ser^{134} the RT group was significantly higher than the UT group at PRE (Mann-Whitney $U = 15$, $z = 2.449$, $p = .014$), but the UT group was significantly higher than the RT group at 30P (Mann-Whitney $U = 9$, $z = -2.939$, $p = .003$). Within the RT group for p-GR Ser^{134} there were decreases compared to PRE at 10P (-33.13%Δ, $z = -2.395$, $p = .017$), 30P (-32.89%Δ, $z = -2.803$, $p = .005$), and 60P (-22.71%Δ, $z = -2.803$, $p = .005$), but returned to baseline by 180P (-6.63%Δ, $z = -1.274$, $p = .203$); conversely, in the UT group, increases were shown compared to PRE values at 30P (85.84%Δ, $z = -2.666$, $p = .008$), 60P (111.24%Δ, $z = -2.666$, $p = .008$), and 180P (68.30%Δ, $z = -2.666$, $p = .008$). There were significant decreases in p-GR Ser^{211} from PRE to 60P (-30.76%Δ, $z = -2.701$, $p = .007$) and 180P (-30.33%Δ, $z = -2.599$, $p = .009$) in the RT group and from PRE to 180P (-42.98%Δ, $z = -2.666$, $p = .008$) in the UT group. In p-GR Ser^{226}, the UT group had a higher expression at 10P (Mann-Whitney $U = 21$, $z = -1.960$, $p = .050$) and 180P (Mann-Whitney $U = 20$, $z = -2.041$, $p = .041$) compared to the RT group. There were significant increases from PRE in

both the RT group at 10P (311.5%Δ, z = -2.803, p = .005), 30P (33.97%Δ, z = -2.803, p = .005), 60P (387.42%Δ, z = -2.803, p = .005), and 180P (240.16%Δ, z = -2.803, p = .005) as well as the UT group at 10P (615.52%Δ, z = -2.666, p = .008), 30P (568.66%Δ, z = -2.666, p = .008), 60P (441.12%Δ, z = -2.666, p = .008), and 180P (395.26%Δ, z = -2.666, p = .008).

CONCLUSION: When analyzing androgen and glucocorticoid receptors, training status needs to be accounted for as there are training status dependent differences. Although the importance of the acute hormonal response on muscle hypertrophy is still controversial, it appears to have some effect at the receptor level in preserving content and in phosphorylation of various receptor sites that cannot be ignored. The RT group was able to maintain their total AR content up to 180 min post RE; whereas, the UT group saw decreases at 30 min and 60 min post exercise. In addition, the ligand dependent GR Ser^{211} site did not show a phosphorylation decrease in the UT group, who had a prolonged elevation in cortisol compared to the RT group, until 180 min post RE, whereas the RT group decreased at 60 min post RE. Also, at the ligand dependent and independent GR Ser^{134} site we saw almost opposite effects in training group where the RT group decreased phosphorylation at 10P, 30P, and 60P, but the UT group saw and increase in phosphorylation at 30P, 60P, and 180P. Phosphorylation of GR Ser^{226} increased at all post time points in both groups, but was higher in the UT group at 10P and 180P. AR Ser^{213} decreased in both groups at 10P, and no differences were seen at AR Ser^{81}, Ser^{515}, or Ser^{650} sites in our moderate intensity protocol. Further research could elucidate the hormone-receptor and receptor phosphorylation responses to RE by looking at a variety of training protocols, later muscle collection time points, MAPK responses, chronic training, and responses in a fed state.

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Chapter I

Introduction

Optimizing the concentration of hormones through variation of load, repetitions, and rest periods is suggested for building muscle mass in current resistance exercise prescription guidelines (1). Physiological actions of testosterone and cortisol occur when bound to their respective intracellular receptor. Muscle growth is initiated when testosterone binds to its androgen receptor (AR); conversely, muscle breakdown is initiated when cortisol binds to its glucocorticoid receptor (GR) in muscle cells. The secretion of both testosterone and cortisol increase following a single bout of resistance exercise (RE) (8).

While both *in vitro* and *in vivo* models indicate the significant contribution to muscle growth (2, 19), the importance of the acute hormonal response in humans has been justified (23, 15, 16, 32) and refuted (24, 25, 37). Thus, the importance of the acute hormonal response is unclear in current exercise guidelines for optimally increasing muscle mass. Likewise, although cortisol's role following endurance exercise has been well described, its role following RE remains unclear. However, there is recent evidence showing phosphorylation and regulation of ARs and GRs can occur in the absence of testosterone and cortisol (9, 10, 19). The equivocal results of prior studies on hormonal responses and muscle adaptation could be clarified by understanding how AR and GR are regulated.

MAPK proteins are inside muscle cells and are able to activate AR and GR by phosphorylation independently of hormones (10, 19). ERK, JNK, and p38 are the three most studied MAPKs. Of these MAPKs, ERK is responsible for growth, JNK is sensitive to the amount of weight lifted by the muscle, and p38 responds to inflammation and metabolic stress. In addition,

they are all associated with different phosphorylation sites on the AR and GRs. Data indicates that ERK activity is altered following long term exercise (11), but following intense stressful training, all three are regulated differently (26). MAPK activation preferably occurs under high intensity loads (>90% maximum strength) in trained subjects (21).

Because AR and GR responses can be modulated by MAPKs, it is probable that MAPKs play a role in the activity and regulation of these receptor following exercise. The amount of these receptors in muscles is altered with RE (24, 26, 38). Spiering et al (2009) reported that the RE induced testosterone increase in the high hormone condition potentiated and sustained AR content while the low hormone condition saw a decrease in AR content. The controversy over hormonal responses and adaptation after exercise could be due to a lack of understanding of how MAPKs and hormones *simultaneously* modulate receptor function in human muscle.

It has been shown there are differences in testosterone and cortisol secretion (8), MAPK activation (11, 26), AR and GR content (24, 37), and protein synthesis responses (35) between trained and untrained subjects. Further investigation of the responses to RE between different training statuses is needed to clarify how MAPKs and hormones concurrently modulate receptor activity following resistance exercise and influences muscle growth.

Significance

There is much controversy regarding the importance of the role of the acute hormonal response following resistance exercise on muscle hypertrophy. This study will obtain data to support or refute the supposed physiological mechanisms for muscle growth and hypertrophy as

well as exercise prescription guidelines currently set forth by the American College of Sports Medicine (ACSM) and National Strength and Conditioning Association (NSCA). Therefore, data from this study will aid in the guidance of exercise prescription for medical, health, fitness, and sport practitioners. Moreover, muscle-wasting diseases such as sarcopenia and cancer cachexia could be combated by the development of drugs that modulate AR and GRs. Understanding how AR and GRs are activated and regulated in living human muscle tissue could lay the foundation for the development of tissue specific and receptor selective drugs. This study will also set up future studies for further understanding AR and GR receptor activation and optimal timing for tissue collection after exercise and for training status.

Purpose

The purpose of this study is to examine the differences in the androgen and glucocorticoid receptor phosphorylation between resistances trained and untrained men following an acute bout of moderate intensity resistance training. It will also look at the biopsy time point post resistance exercise for AR and GR phosphorylation following resistance training.

Hypothesis

Resistance training will increase the phosphorylation of all measured sites on the AR and GRs in both Resistance Trained and Untrained subjects, but there will be a greater response in Untrained subjects.

Dependent Variables

1. Phosphorylated Androgen and Glucocorticoid Receptors

   p-AR (Ser^{81}, Ser^{213}, Ser^{515}, Ser^{650})

   p-GR (Ser^{134}, Ser^{211}, Ser^{226})

2. Total AR and GR expression

3. Testosterone and Cortisol

4. Myosin Heavy Chain (MHC) Isoform Percentage

5. Muscular Performance

   Barbell Back Squat One Repetition Maximum Strength

   Barbell Back Squat Rating of Perceived Exertion (RPE)

   Barbell Back Squat Concentric Work

   Barbell Back Squat Force production

Delimitations

1. Subjects were men ages 18-30.
2. This study is only looking are resistance exercise.
3. This is not a full body protocol. Only back squat and leg extension exercises will be used.
4. Subjects will be fasted for their exercise visit.

Assumptions

1. Subjects accurately answer health history questionnaire.
2. Subjects refrain from physical activity other than what is required during their participation in the study
3. Subjects will arrive at least 6 hours fasted for the experimental visit.
4. Subjects have not used steroids during or prior to this study

Chapter II

Review of Literature

While there is an abundance of research on steroid hormones and how they are affected and regulated in response to resistance exercise (RE), little research has been done on RE and the regulation of steroid hormone receptors (SHRs) although the hormones have little to no effect on muscle until they bind to their receptor. Understanding how SHRs are regulated and how exercise affects regulation could clarify how muscle adapts to exercise, the importance or insignificance of acute hormonal responses to exercise, and aid in developing optimal prescription for RE programs. This review will discuss the androgen receptor (AR) and glucocorticoid receptor (GR), their regulators at specific phosphorylation sites, and how RE could potentially activate AR and GR regulators.

Steroid Hormone Receptors

The androgen and glucocorticoid receptors are steroid hormone receptors (SHR). SHRs belong to a superfamily of transcription factors called nuclear receptors. To regulate transcription, SHRs can be either ligand dependent or ligand independent (36, 17) through signal transduction pathways (3).

Ligand Dependent Regulation

In the classic model of SHR activation a ligand, or protein bound hormone (i.e. testosterone, cortisol), diffuses across the cell membrane to bind to its SHR (i.e. AR, GR). Prior to ligand binding in the absence of the hormone, SHRs are bound to a chaperone protein (36, 20) called heat shock proteins (hsp) that are responsible for facilitating the folding and stabilization of the receptor to put it in a high affinity conformation for ligand binding (29). Due to conformational

changes induced by a hormone binding to its receptor, the chaperone protein dissociates from the SHR. The ligand bound SHRs then form homodimers and translocate from the cytoplasm to the nucleus where they bind DNA (20) and to hormone response elements (HREs) (36). Binding to HREs causes the recruitment of coactivator complexes facilitating target gene transcription. Coactivator recruitment and target gene expression can also be induced by SHRs binding to transcription factors (TF) sitting on transcription factor response elements; in addition, SHRs can also inhibit gene transcription (36). Kinases (i.e. Akt, MAPKs) can be activated by and interact with dimerized SHRs resulting in the phosphorylation and activation of other TFs bound to their response elements (36).

SHR Structure

SHRs consists of an N-terminal domain (NTD), a DNA-binding domain (DBD), a hinge region, and a C-terminal ligand-binding domain (LBD). The LBD is where AR and GR activity as a transcription factor is controlled by androgens and glucocorticoids respectively (6), and where ligand binding in the previous section occurs. The LBD is associated with a transcriptional regulatory function (AF-2) (36, 20, 9). The LBD is connected to the DBD by the hinge region (36, 20, 6, 9) which contains a nuclear localization signal (6, 9). The NTD, next to the DBD, also has a transcriptional regulatory domain (AF-1) and can function in the absence of a ligand (20, 9). GRs are phosphorylated on Serine and Threonine residues, and ARs are phosphorylated on Serine, Threonine, and Tyrosine residues. AR phosphorylation is believed to alter the activity of the AR through modification of protein interactions (20). Specific AR and GR phosphorylations are kinase dependent (36, 9). Four phosphorylation sites on the AR will be discussed: Ser^{81}, Ser^{213}, and Ser^{515}

located on the NTD as well as Ser^{650} located in the hinge region of the AR. Three phosphorylation sites will be discussed on the GR: Ser^{134}, Ser^{211}, and Ser^{226} located on the NTD.

Phosphorylation of the Androgen Receptor

p-AR Ser^{81}

Ser^{81} is the most highly phosphorylated site in response to androgen stimulation and continues to accumulate ~8hrs post androgen stimulation (14). Phosphorylation of AR Ser^{81} is required for induction of endogenous AR target genes and AR nuclear retention (3). A family of kinases called cyclin dependent kinases (CDKs) have been shown to phosphorylate the AR, specifically CDK1, CDK5, and CDK9 on the Ser^{81} site (3, 5, 17). CDK1, which is activated in mitosis, phosphorylates Ser^{81} and sensitizes, or upregulates, the AR to low levels of androgens (5). Phosphorylation by CDK5 has been shown to be associated with nuclear localization and stabilization of the AR (17). It has been proposed that the phosphorylation of AR Ser^{81} by CDK9 in nonmitotic cells (i.e. skeletal muscle) stabilizes AR-chromatin binding and is required for chromatin remodeling and transcriptional activation as it is associated with a complex that stimulates transcriptional elongation (3). CDK9 is also responsible for the cellular distribution of the AR (3).

Kim et al. (19) suggests that AR induction and regulation in skeletal muscle is regulated by insulin-like growth factor-1 (IGF-1) with coactivation by mitogen activated protein kinase (MAPK) pathways. IGF-1 alters AR function by altering AR phosphorylation resulting in AR activation (19). MAPK pathways are downstream signaling pathways of IGF-1 and have been

shown to modulate ARs in the absence of ligands; therefore, AR activation in the absence of a ligand would still require the presence of IGF-1 (19).

p-AR Ser^{213}

Akt and PIM-1 have been known to phosphorylate Ser^{213}, but AR activity by Akt seems to be pathway dependent while AR activity by PIM-1 seems to be isoform dependent (36, 20, 22, 34). PIM-1 isoforms recruit E3 ligases resulting in either AR degradation or AR activation and stabilization (36, 20). Akt directly binds to Ser^{213} (22, 34). IGF-1 activates the PI3/Akt pathway (22, 19) which could lead to the activation of Ser^{213}. The effects of the activation of Ser^{213} are AR stability and AR translocation (20).

p-AR Ser^{515}

Phosphorylated AR Ser^{515} is coregulated with p-AR Ser^{578} (located on the DBD), with the phosphorylation of Ser^{515} being maximal when Ser^{578} is not phosphorylated (28). Ser^{515} plays a role in increased transcriptional activity, AR transactivation, and nuclear localization (20, 28). Ser^{515} is phosphorylated by CDK7 (20) and MAPKs (20, 40). Studies continue to suggest that in the absence of androgens, AR transcriptional activity involves growth factor signaling and its downstream effects on MAPKs (28, 19). Epidermal growth factor (EGF) has been shown to increase transcriptional activity through both Ser^{515} and Ser^{578} (28). Extracellular-signal regulated kinase (ERK) responds to growth factors (3) and is most likely the downstream MAPK pathway being signaled in response to EGF.

p-AR Ser^{650} and the Hinge Region

Ser^{650} is located on the hinge region of the AR. The hinge region is more than a flexible linker between the DBD and LBD as it also plays a role in nuclear import and export, DNA selectivity and affinity, and AR transactivation potential (6). Reports have shown that phosphorylation of AR Ser^{650} is stimulated by a stress induced kinase pathway and enhances nuclear export (13, 4). Activation of MAPKs p38 and c-Jun N-terminal Kinase (JNK) primarily phosphorylate AR Ser^{650} while ERK may play a slight role (13). Phosphorylation of AR Ser^{650} can negatively regulate AR transcriptional activity, but it seems to be specific to stress kinase pathways (13). Inhibition of stress kinases reduced AR nuclear-cytoplasmic shuttling, while changes in nuclear-cytoplasmic shuttling regulated by Ser^{650} may result in inhibition of AR transcription (13).

Phosphorylation of the Glucocorticoid Receptor

Glucocorticoids, specifically cortisol, are typically thought of as stress hormones as they respond and react to various stresses on the body. While excessive and prolonged releases of these hormones can be problematic, the glucocorticoid response is essential for adapting to the stresses by allowing the body to utilize essential building blocks needed to remodel and adapt. Cortisol is often described as catabolic while testosterone is described as anabolic; although, testosterone is anabolic and commonly thought to play a role in muscle hypertrophy, it is only part of the picture. How both of these hormones and their receptors respond to exercise will give a greater understanding in the role of hormones and their receptors in muscle adaptations.

p-GR Ser^{134}

GR transcriptional activity seems to be primarily regulated by ligand binding as it allows GR translocation from the cytoplasm to the nucleus (9); however, there is now evidence that the GR can be phosphorylated independently of ligands at certain sites.

A variety of stressful conditions including glucose starvation, oxidative stress, UV irradiation, and osmotic shock have been shown to phosphorylate the Ser^{134} site on the GR independently of hormones (10). Energy starvation in cells induces stress activating MAPKs p38 and JNK. Galliher-Beckley et al. (2011) reported results showing starvation-induced phosphorylation of Ser^{134} was blocked by a p38 MAPK inhibitor concluding Ser^{134} on the GR is phosphorylated responding to cellular stress signals that activate p38 MAPK.

p-GR Ser^{211} and p-GR Ser^{226}

Unlike Ser^{134}, Ser^{211} on the GR is phosphorylated in a hormone-dependent manner (9, 10) by directly binding with glucocorticoids, specifically cortisol. Increased Ser^{211} phosphorylation within the GR results in higher transcriptional activity; consequently, reducing Ser^{211} phosphorylation within the GR resulted in decreased nuclear localization and decreased transcriptional activity of the GR (9). Transcriptional activity of the GR is greatest when Ser^{211} phosphorylation is greater than Ser^{226} phosphorylation and is enhanced when Ser^{226} phosphorylation is blocked (9). This is similar to the coregulation of Ser^{515} and Ser^{578} on the AR with the effects of Ser^{515} phosphorylation being maximal when Ser^{578} is not phosphorylated suggesting the possibility of similar regulatory mechanisms between SHRs. Phosphorylated GR

Ser^{226} seems to be phosphorylated by JNK and CDK5 and has been found to enhance nuclear export of the GR, thus blunting hormonal signaling (9, 18). However the exact role of p-GR Ser^{226} is still unclear due to research suggesting the GR receptor to still be transcriptionally active when phosphorylated (9).

Resistance Exercise and Steroid Hormone Receptors

Testosterone and Cortisol

Muscle growth is initiated when testosterone binds to its androgen receptor; conversely, muscle breakdown is initiated when cortisol binds to its glucocorticoid receptor. However, the importance of the acute hormonal response in humans has been justified (23, 15, 16, 32) and refuted (24, 25, 37). Just a single session of RE increases secretion of testosterone (8), but it is controversial if the acute hormonal response is important in muscle fiber hypertrophy.

AR Content, Testosterone, and Resistance Exercise

Spiering et al. (2009) reported in untrained subjects, elevated endogenous testosterone concentrations following acute RE not only potentiated the AR responses but sustained AR content; whereas, the low testosterone condition saw a decrease in AR content post exercise. Acute RE-induced elevations in testosterone seemed to prevent catabolism of the ARs following RE indicating that the combination of both muscle contraction and elevated testosterone concentrations enhance AR content in overloaded muscles (32). It is hypothesized that potentiation of AR content was due to enhanced mRNA translation and/or increased AR half-life (32). In the absence of androgens, the AR half-life is only 3.1 hours compared to a more than doubled 6.6 hour half-life when androgens are present in rats (33).

Willoughby and Taylor (2004) reported sequential bouts of RE with 48hrs between sessions leading to increases testosterone post exercise resulting in increased AR mRNA expression 48 hours post exercise as well as myofibrillar protein expression in untrained subjects. AR mRNA and protein expression were significantly correlated with serum testosterone levels and upregulated after three sequential RE bouts (38). Although studies have reported increases in AR expression in untrained individuals, Ratamess et al. (2005) reported a decrease in AR expression 1 hour following a multi-set resistance training bout in resistance trained men. Thus, training status could play a role in how the muscle adapts (30).

GR Content, Cortisol, and Resistance Exercise

While testosterone increases in response to RE appear to lead to increased AR activation, expression, and content resulting in increased protein synthesis and thus hypertrophy, the increase in cortisol in response to RE results in increased GR activation resulting in myofibrillar proteolysis. Although the glucocorticoid response is needed to enable remodeling and grow, excessive catabolism will result in atrophy. Willoughby et al. (2003) reported significantly increased cortisol levels following an eccentric RE bout up to 48 hours post exercise. They also reported significantly increased GR protein content at 6 and 24 hours post eccentric RE bout supporting that cortisol upregulates GR in skeletal muscle. It has been shown that protein synthesis can be increased up to 48 hours post RE (39). The extended increase of cortisol levels after an eccentric exercise bout could assist in making amino acids available during protein synthesis; however, GR activation in response to RE is still not completely understood.

MAPKs

Previous sections mention how the AR and GR could be regulated in the absence of testosterone or cortisol respectively (10, 32) primarily through MAPKs ERK, JNK, and p38 found inside muscle fibers and Akt signaling. ERK is activated by growth factors and muscle contraction (11). JNK is tension sensitive so activation is related to stress placed on the muscle, the intensity, and duration of the RE load (12). p38 MAPK responds to metabolic stress and inflammation. IGF-1 activates the PI3/Akt pathway which is stimulated by RE and growth hormone (GH), but the role of RE on Akt and AR/GR activation is not yet clear. The activation of these stress induced kinase pathways could potentially be altered by different RE protocols.

RE Protocols and Responses

The five acute RE program variables are: Choice of Exercise, Order of Exercise, Intensity of Exercise, Volume of Exercise, and Rest Interval duration during exercise (7). Altering the RE program design by modifying these variables could specify the signaling response (7, 31). JNK and p38 MAPK are stress induced kinases, and consequently activation is related to RE intensity, volume, and rest. Higher intensities and higher volumes put more stress on the muscles, as well as short rest periods, possibly resulting in the increase in JNK and p38 MAPK activation, therefore, the phosphorylation of Ser^{650} of the AR and Ser^{134} of the GR. Phosphorylation of Ser^{650} inhibits AR transcription (13), and phosphorylation of Ser^{134} alters GR transcriptional response (10). There is a possibility Ser^{515} of the AR could be regulated by RE intensity and volume because both intensity and volume affect muscle contraction—a stimulus for ERK; however, EGF directly stimulates Ser^{515}, yet the relationship between this, RE, and AR activation is still unclear. RE guidelines to promote anabolic hormones associated with muscle hypertrophy such as testosterone,

GH, and IGF-1 suggest moderate intensity, high volume, and short rest periods during training (1). IGF-1 stimulates the Akt pathway. Since Akt can directly phosphorylate Ser^{213} of the AR (22, 34), Ser^{213} could be activated indirectly through IGF-1 resulting in AR stabilization and AR translocation due to a moderate intensity and high volume RE bout. It has also been suggested that IGF-1 activates the AR with MAPKs as coactivators (19). This activation may take place on the AR Ser^{81} site responsible for AR transcription and nuclear retention (3). If IGF-1 results in the phosphorylation of both AR Ser^{81} and AR Ser^{213}, then both sites would be activated by the same RE stimulus and would have the potential to work together to complete and optimize the transcription process. These possible relations are not yet understood, and future research will need to be done.

Summary

Steroid hormone receptors can be activated in the presence and the absence of a steroid hormone. MAPK and Akt signaling are able to phosphorylate the androgen and glucocorticoid receptors independently of their respective hormones. While the exact mechanism of ligand independent AR and GR phosphorylation is equivocal, it appears to be linked to nuclear-cytoplasmic shuttling. Since many sites have specific roles (i.e. translocation, nuclear retention, receptor transcription) it may be possible that the sites work together or in series to achieve their goal, and coregulation has already been shown with Ser^{515} and Ser^{578} of the AR and possibly Ser^{211} and Ser^{226} of the GR.

AR Ser^{81} is the most highly phosphorylated site, and it can continue to accumulate for over 8 hours after androgen stimulation. AR Ser^{81} might be the most associated with muscle

hypertrophy as it is directly related to AR transcription and responds in a similar way to an androgen stimulus as muscle does to a training stimulus. Skeletal muscle shows an increase in protein synthesis for an extended period of time post training stimulus. Spiering et al. (2009) reported that elevated endogenous testosterone concentrations following acute RE potentiated the AR responses. Acute RE-induced elevations in testosterone prevented catabolism of the ARs following RE indicating that the combination of both muscle contraction and elevated testosterone concentrations enhance AR content in overloaded muscles (32). The importance of the acute testosterone response on muscle hypertrophy is controversial; however, it clearly plays some role and would be interesting if it played a role in Ser^{81}'s continued accumulation post androgen stimulus.

Ser^{650} negatively regulates the AR when activated. JNK and p38 MAPK—both associated with stress—activate Ser^{650}, and it is possible that this site is associated with the responses of overtraining and overreaching if it were strongly activated for excessive amounts of time without proper recovery. In addition, Ser^{134} of the GR has been recently shown to be activated in a hormone independent manner, and it has been reported that it is phosphorylated by the same stresses that activate p38 MAPK. While it is unclear if these receptors interact and respond to each other, their responses to stress, specifically stress that activates p38 MAPK, result in similar responses leading to muscle adaptation. Stresses activate the GR increasing GR activity, but those stresses that phosphorylate the AR result in decreased AR activity.

Although not much is known about the relationship between resistance exercise and SHR regulation, further research could explain how SHRs are regulated, how RE affects regulation,

provide further clarifications on RE protocols for optimal adaptations, and explain relations with SHRs like the AR and GR. Future studies will look at AR and GR activation between different RE protocols. Studies comparing both AR and GR activation may receive a better insight to how SHRs work and if there are relationships between them.

References Chapter 1 & 2

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Chapter III

Introduction

Optimizing the concentration of hormones through variation of load, repetitions, and rest periods is suggested for building muscle mass in current resistance exercise prescription guidelines (1). Physiological actions of testosterone and cortisol occur when bound to their respective intracellular receptor. Muscle growth is initiated when testosterone binds to its androgen receptor (AR); conversely, muscle breakdown is initiated when cortisol binds to its glucocorticoid receptor (GR) in muscle cells. The secretion of both testosterone and cortisol increase following a single bout of resistance exercise (RE) (7). While both *in vitro* and *in vivo* models indicate the significant contribution to muscle growth (3, 17), the importance of the acute hormonal response in humans has been justified (13, 14, 22, 28) and refuted (23, 24, 32). However, there is recent evidence showing phosphorylation and regulation of ARs and GRs can occur in the absence of testosterone and cortisol (5, 9, 10, 11, 15, 17, 18, 21, 30, 31, 35). The equivocal results of prior studies on hormonal responses and muscle adaptation could be clarified by understanding how AR and GR are regulated.

The androgen and glucocorticoid receptors are steroid hormone receptors (SHR). SHRs consists of an N-terminal domain (NTD), a DNA-binding domain (DBD), a hinge region, and a C-terminal ligand-binding domain (LBD). Four phosphorylation sites on the AR will be discussed: Ser^{81}, Ser^{213}, and Ser^{515} located on the NTD as well as Ser^{650} located in the hinge region of the AR. Three phosphorylation sites will be discussed on the GR: Ser^{134}, Ser^{211}, and Ser^{226} located on the NTD.

Ser^{81} is the most highly phosphorylated site in response to androgen stimulation and continues to accumulate ~8hrs post androgen stimulation (12). Phosphorylation of AR Ser^{81} is required for induction of endogenous AR target genes and AR nuclear retention (4). It has been proposed that the phosphorylation of AR Ser^{81} by CDK9 in nonmitotic cells (i.e. skeletal muscle) stabilizes AR-chromatin binding and is required for chromatin remodeling and transcriptional activation as it is associated with a complex that stimulates transcriptional elongation (4). CDK9 is also responsible for the cellular distribution of the AR (4). Kim et al. (17) suggests that AR induction and regulation in skeletal muscle is regulated by insulin-like growth factor-1 (IGF-1) with coactivation by mitogen activated protein kinase (MAPK) pathways. IGF-1 alters AR function by altering AR phosphorylation resulting in AR activation (17). MAPK pathways are downstream signaling pathways of IGF-1 and have been shown to modulate ARs in the absence of ligands; therefore, AR activation in the absence of a ligand would still require the presence of IGF-1 (17).

Akt and PIM-1 have been known to phosphorylate Ser^{213} (18, 21, 30, 31). PIM-1 isoforms recruit E3 ligases resulting in either AR degradation or AR activation and stabilization (18, 31). Akt directly binds to Ser^{213} (21, 30). IGF-1 activates the PI3/Akt pathway (17, 21) which could lead to the activation of Ser^{213}. The effects of the activation of Ser^{213} are AR stability and AR translocation (18).

Ser^{515} plays a role in increased transcriptional activity, AR transactivation, and nuclear localization (18, 26). Ser^{515} is phosphorylated by CDK7 (18) and MAPKs (18, 35). Studies

continue to suggest that in the absence of androgens, AR transcriptional activity involves growth factor signaling and its downstream effects on MAPKs (17, 26).

Ser^{650} is located on the hinge region of the AR. The hinge region plays a role in nuclear import and export, DNA selectivity and affinity, and AR transactivation potential (6). Reports have shown that phosphorylation of AR Ser^{650} is stimulated by a stress induced kinase pathway and enhances nuclear export (5, 11). Activation of MAPKs p38 and c-Jun N-terminal Kinase (JNK) primarily phosphorylate AR Ser^{650} while ERK may play a slight role (11). Phosphorylation of AR Ser^{650} can negatively regulate AR transcriptional activity, but it seems to be specific to stress kinase pathways (11).

Glucocorticoids, specifically cortisol, are typically thought of as stress hormones as they respond and react to various stresses on the body. While excessive and prolonged releases of these hormones can be problematic, the glucocorticoid response is essential for adapting to the stresses by allowing the body to utilize essential building blocks needed to remodel and adapt. Cortisol is often described as catabolic while testosterone is described as anabolic; although, testosterone is anabolic and commonly thought to play a role in muscle hypertrophy, it is only part of the picture. How both of these hormones and their receptors respond to exercise will give a greater understanding in the role of hormones and their receptors in muscle adaptations.

A variety of stressful conditions including glucose starvation, oxidative stress, UV irradiation, and osmotic shock have been shown to phosphorylate the Ser^{134} site on the GR independently of hormones (10). Energy starvation in cells induces stress activating MAPKs p38

and JNK. Galliher-Beckley et al. (2011) reported results showing starvation-induced phosphorylation of Ser^{134} was blocked by a p38 MAPK inhibitor concluding Ser^{134} on the GR is phosphorylated responding to cellular stress signals that activate p38 MAPK.

Unlike Ser^{134}, Ser^{211} on the GR is phosphorylated in a hormone-dependent manner (9, 10) by directly binding with glucocorticoids, specifically cortisol. Increased Ser^{211} phosphorylation within the GR results in higher transcriptional activity; consequently, reducing Ser^{211} phosphorylation within the GR resulted in decreased nuclear localization and decreased transcriptional activity of the GR (9). Transcriptional activity of the GR is greatest when Ser^{211} phosphorylation is greater than Ser^{226} phosphorylation and is enhanced when Ser^{226} phosphorylation is blocked (9). Phosphorylated GR Ser^{226} is phosphorylated by JNK and CDK5 and has been found to enhance nuclear export of the GR, thus blunting hormonal signaling (9, 16).

Spiering et al. (2009) reported in untrained subjects, elevated endogenous testosterone concentrations following acute RE not only potentiated the AR responses but sustained AR content; whereas, the low testosterone condition saw a decrease in AR content post exercise. Acute RE-induced elevations in testosterone seemed to prevent catabolism of the ARs following RE indicating that the combination of both muscle contraction and elevated testosterone concentrations enhance AR content in overloaded muscles (28). It is hypothesized that potentiation of AR content was due to enhanced mRNA translation and/or increased AR half-life (28). In the absence of androgens, the AR half-life is only 3.1 hours compared to a more than doubled 6.6 hour half-life when androgens are present in rats (29). Willoughby and Taylor (2004) reported sequential bouts of RE with 48hrs between sessions leading to increases testosterone post exercise

resulting in increased AR mRNA expression 48 hours post exercise in untrained subjects. AR mRNA and protein expression were significantly correlated with serum testosterone levels and upregulated after three sequential RE bouts (33). Although studies have reported increases in AR expression in untrained individuals, Ratamess et al. (2005) reported a decrease in AR expression 1 hour following a multi-set resistance training bout in resistance trained men. Thus, training status could play a role in muscle adaptation (27).

Willoughby et al. (2003) reported significantly increased cortisol levels following an eccentric RE bout up to 48 hours post exercise. They also reported significantly increased GR protein content at 6 and 24 hours post eccentric RE bout supporting that cortisol upregulates GR in skeletal muscle. It has been shown that protein synthesis can be increased up to 48 hours post RE (34). The extended increase of cortisol levels after an eccentric exercise bout could assist in making amino acids available during protein synthesis; however, GR activation in response to RE is still not completely understood.

Steroid hormone receptors can be activated in the presence and the absence of a steroid hormone. MAPK and Akt signaling are able to phosphorylate the androgen and glucocorticoid receptors independently of their respective hormones. While the exact mechanism of ligand independent AR and GR phosphorylation is equivocal, it appears to be linked to nuclear-cytoplasmic shuttling. Since many sites have specific roles (i.e. translocation, nuclear retention, receptor transcription) it may be possible that the sites work together or in series to achieve their goal.

Subjects

Twenty college-aged men (ages 18-30) volunteered for this study. Ten resistance trained men (n = 10; $\bar{X}$ ± SD, age = 21.3 ± 1.7 yrs, height = 175.8 ± 6.8 cm, body mass = 84.5 ± 13.5 kg, back squat 1RM = 154.3 ± 19.3 kg, training history = 5.4 ± 2.0 yrs) with at least 2 years resistance training experience, have performed resistance exercise at least three times per week with at least one per week dedicated to the lower body for the previous 6 months, and can squat >1.5 times their body weight were classified as Resistance Trained (RT). Ten untrained men (n = 9; $\bar{X}$ ± SD, age = 20.8 ± 3.1 yrs , height = 178.7 ± 8.9 cm, body mass = 81.0 ± 14.0 kg, squat 1RM = 108.1 ± 13.7 kg, training history = 0.7 ± 1.7 yrs) with no history of structured resistance training within 3 years, have not performed lower body resistance exercise in the previous 6 months, and squat <1.5 times their body weight were classified as Untrained (UT). One UT subject was unable to complete the protocol resulting in an N of 9. Subjects were healthy, non-obese, non-smoking, and free of metabolic, cardiovascular, and kidney diseases, as well as free of a history of seizures. Individuals who regularly use drugs to reduce inflammation were excluded from this study. All participants were screened for participation using a health-history questionnaire for contraindications to exercise by American College of Sports Medicine (ACSM) guidelines. Each subject signed an informed consent statement as approved by the University Institutional Review Board and in accordance with the Helsinki Declaration. Participants had the timeline and procedures verbally explained as well as all risks and benefits with a physical copy of the informed consent to take with them. Subjects refrained from physical activity other than that required for experimental trials during their participation in the study.

Procedures

Study Timeline

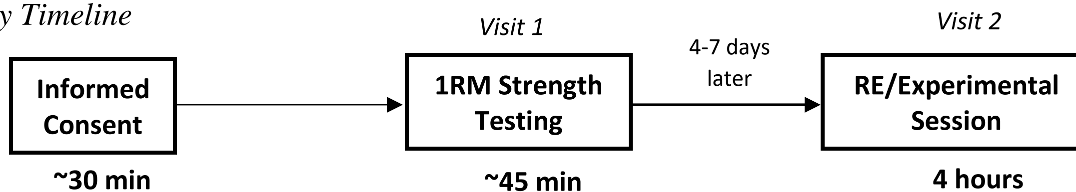


Figure 1. Study Timeline.

Anthropometrics, familiarization, one-repetition maximum (1RM) testing

Subjects arrived to the lab for Visit 1. Height and body mass were measured with a stadiometer and calibrated scale, respectively. Subjects were led to the Jayhawk Athletic Performance Lab where they performed warm-up for 5 minutes on a cycle ergometer with little resistance. Subjects were familiarized with proper back squat technique according to National Strength and Conditioning guidelines (Baechle and Earle 2008). They were instructed to stand with their feet approximately shoulder width apart and descend until the femur was parallel to the ground, then ascend to starting position once depth is achieved. A Certified Strength and Conditioning Specialist was present at each visit for each subject to evaluate safety and technique of the lift as well as assessing squat depth. The 1RM strength tests were completed for the barbell squat exercise according to previously described methods (Kraemer et al. 2006). Subjects performed a light warm-up of 5-10 repetitions at 40%-60% perceived maximum. After 1 minute rest, subjects performed 3-5 repetitions at 60%-80% perceived maximum. After 2-4 minutes rest, subjects performed 2-3 repetitions at 90% of perceived maximum. Conservative increases in weight were made, then the subject will attempt a 1RM lift. Two-4 minutes rest periods were given after each successful attempt, then the next 1RM lift was attempted. Following 1RM testing for

the back squat, 5-10 minutes rest was given to each subject, then subjects completed 1RM testing for leg extension.

Acute Resistance Exercise Bout

There were no differences in the protocol between Resistance Trained and Untrained groups. Subjects arrived at the lab at least 6 hours fasted and euhydrated between 10:00am-2:00pm to control for diurnal variations of hormones. Prior to training, subjects relaxed for 10 minutes, then blood and a muscle biopsy were collected for baseline levels. Subjects warmed-up on a cycle ergometer for 5 minutes at a light resistance. Then, subjects performed two warm-up sets of barbell back squat at 35% and 55% 1RM with 1 minute rest following each set. Subjects then performed 6 sets of 10 repetitions at 75% of their 1RM with 1.5 minutes rest following each set. Following the back squat exercise, subjects performed 4 set of 10 repetitions at 75% of their 1RM for leg extension with 1.5 minutes rest after each set. When a subject was unable to complete a set for either lift, the load was reduced prior to starting the next set. After completing the leg extension exercise, blood samples were collected at 5 min, 15 min, and 45 min post exercise, and biopsies were taken at 10 min, 30 min, 60 min, and 180 min post exercise. A TENDO unit was be attached to the barbell during the back squat exercise and the weight stack during the leg extension exercise to measure the mean and peak power, velocity, and force from each repetition.

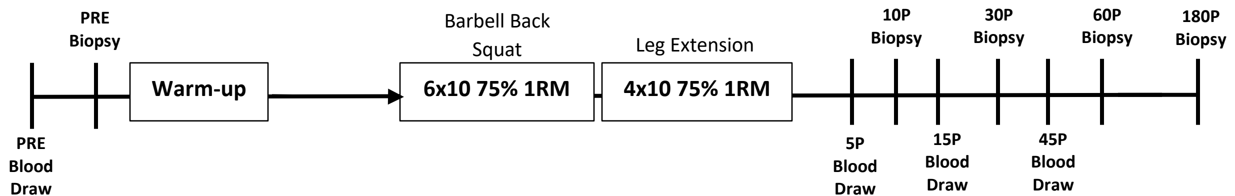


Figure 2. Visit 2 Timeline.

Muscle Biopsies

All biopsies were taken from the vastus lateralis at rest (PRE) and at 10 minutes, 30 minutes, 60 minutes, and 180 minutes post exercise. Sterile disposable instruments were used. The subjects' thighs were shaved and cleaned with a Betadine solution to prepare for the biopsies. Prior to the PRE biopsy, 3mL of 2% lidocaine solution was injected into the skin and surrounding tissues of the leg. Ten minutes was allowed to pass to ensure time for the agent to affect the area. A 14-guage spring-loaded breast biopsy needle was inserted into the middle of the muscle at a depth of approximately 3cm, with samples taken weighing 10-25 mg. Following the PRE biopsy, sterile and flexible pressure wraps were placed over the biopsy site. The subject was then led to begin their warm-up. Following the RE bout, at 10 min and 30 min post, biopsies were taken from the same leg 1cm away from the previous biopsy site. That leg was bandaged, then the opposite leg was prepped with the same methods previously mentioned to prep the first leg. The 60 min and 180 min biopsies will be taken from the opposite leg. Immediately after extraction, muscle samples were cooled with liquid nitrogen to -159°C and were stored at -60°C.

Blood Measures

Blood samples were collected from an antecubital vein using a needle and Vacutainer™ tube. Blood was collected before the first biopsy (PRE) and 5, 15, and 45 minutes post exercise. Samples were measured for hemoglobin and hematocrit to account for potential plasma volume shifts from exercise. Samples clotted for 20 minutes, then they were spun in a centrifuge at 2000 x g for 15 minutes at 4°C. The serum was pipetted into four aliquots, then stored at -60°C.

Enzyme-linked Immunosorbent Assay (ELISA)

Serum samples were analyzed for testosterone and cortisol via enzyme-linked immunosorbent assays (ALPCO; Salem, NH). All samples were ran in duplicate. All assays were performed by the same investigator.

Tissue Sample Preparation for Western Blots

The whole muscle sample was placed in 1mL of lysing buffer containing 10% (w/v) glycerol, 5% (v/v) β-mercaptoethanol, and 2.3% (w/v) SDS in 62.5mMTris·HCL buffer (pH 6.8), with a 1% solution of Halt Protease and Phosphatase Inhibitor Cocktail (Thermo Fischer Scientific Inc., Waltham, MA, USA). Samples were homogenized in a test tube for 30s, then, heated for 10min at 60°C. Samples were pipetted into 3 aliquots then stored at -60°C.

Western Blotting

Samples were assayed for total protein concentration using a micro Lowry method with Peterson's modification (Sigma Aldrich, Saint Louis, MO. No P5656). After protein determination, 20μg of protein was loaded into 4-15% precast gels (Bio-Rad, Irving, CA) and electrophoresed at a constant voltage of 200v for 30min. Proteins were then transferred from the gel to a hydrophobic polyvinylidene difluoride (PVDF) membrane (MilliporeSigma, Burlington, NH) at 15V for 90min using an Idea Scientific Co. wet transfer apparatus (Minneapolis, MN). Following the transfer, PVDF membranes were blocked in TBS Odyssey® blocking buffer for 1 hour at room temperature. After blocking, membranes were incubated with primary antibodies for p-AR [(ser^{515}) (1:1000, rabbit polyclonal, no. ab128250); ABCam (Cambridge, MA), p-AR (ser^{81}) (1:1000, rabbit polyclonal, no. PA5-64617), p-AR (ser^{213}) (1:1000, rabbit polyclonal, no. PA5-

37478), p-AR (ser^{650}) (1:1000, rabbit polyclonal, no. PA5-37479)], total AR (1:1000, mouse monoclonal, no. MA5-15598), p-GR [(ser^{134}) (1:1000, rabbit polyclonal, no. ABS1008); MilliporeSigma (Burlington, MA), p-GR (ser^{211}) (1:1000, rabbit polyclonal, no. 4161S); Cell Signaling Technologies Inc. (Danvers, MA), p-GR(ser^{226}) (1:1000, rabbit polyclonal, no. ABS994); MilliporeSigma (Burlington, MA)], and total GR (1:1000, mouse monoclonal, no. MA5-15801) overnight at 4°C with gentle agitation. After overnight incubation, membranes were washed with TBS plus Tween-20 (TBST) 3 times for 5 minutes (3 x 5min), then probed with infrared secondary antibodies (1:10,000) specific to the host animal (700nm anti-mouse, 800nm anti-rabbit) for 1 hour at room temperature. Following secondary antibody incubation, membranes were washed with TBST (3 x 5min) then rinsed with TBS. The membrane was scanned using an Odyssey Infrared Imaging System and accompanying software (v1.2, LI-COR Biosciences, Lincoln, NE) to quantify IR intensity for each labeled protein band. Total and phosphorylated bands were identified on the same membrane with IR markers scanned at two different wavelengths (700 nm; total-AR, total-GR, and 800 nm; p-AR, p-GR).

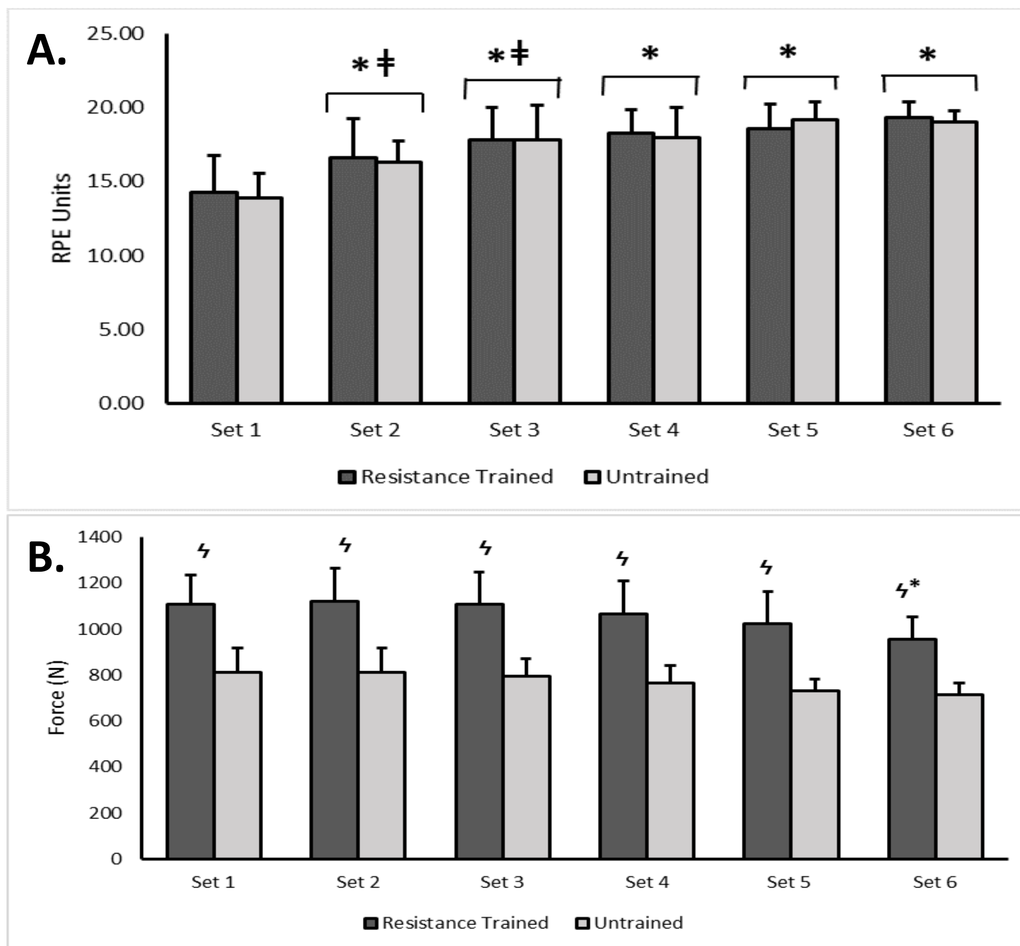
Statistical Analysis

Hormonal data were analyzed using parametric methods. 2 x 4 (group x time) RMANOVAs were used to determine potential differences in testosterone and cortisol between groups (group) at PRE, 5P, 15P and 45P (time). Total receptor data were not normally distributed, thus total receptor data were analyzed using Mann-Whitney U test, Friedman test, and Wilcoxon signed-rank test. Performance data were analyzed using RMANOVAs and independent t tests.

Results

Squat Performance

1RM was significantly different between groups ($p < .05$). There were no significant differences between groups for any set for RPE ($p>0.05$), although there were RPE significant differences across sets. There were significant differences for mean force (N) between RT and UT groups for all sets, and the RT group began to significantly decrease by set 6. There was a significant difference in work (J) between groups for sets 1 and 2, with the RT group significantly decreasing by sets 5 and 6.



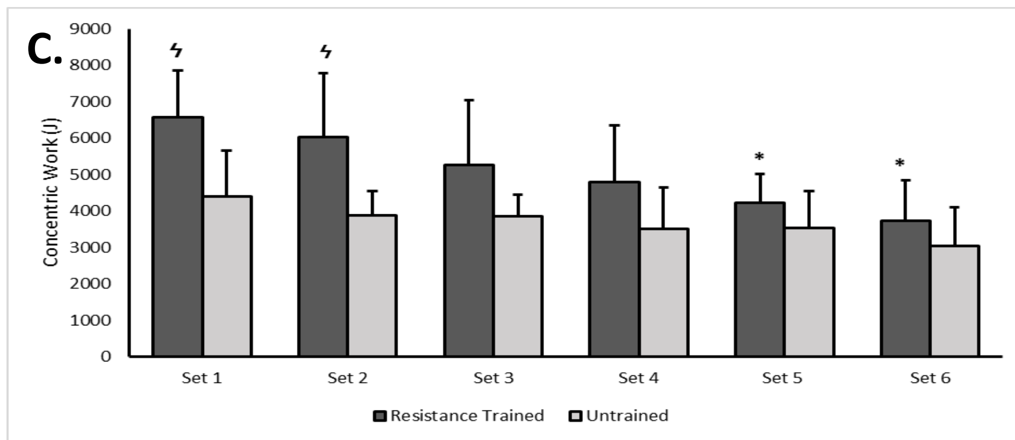


Figure 3. Performance data across the back squat in RT and UT subjects. A. Rating of perceived exertion (RPE) scores ($\bar{X} \pm$ SD) on a 6-20 scale immediately following each set. B. Mean Force (N) ($\bar{X} \pm$ SD) produced during each set. C. Concentric Work (J) ($\bar{X} \pm$ SD) produced during each set. * indicates significance ($p < .05$) from Set 1. ǂ indicates significance ($p < .05$) from previous set. ϟ indicates significance ($p < .05$) from the UT group at the same set.

Hormonal Data

In both the RT and UT groups testosterone significantly increased from PRE values at 5min and 15min post exercise ($p < .05$), then began to return to baseline by 45min post exercise. There was a moderate effect at 15P and 45P (Cohen's D) between groups with the RT group being higher. Cortisol significantly increased in the RT group at 5min and 15min from PRE values ($p < .05$); whereas, the UT group was elevated from PRE at all three time points (5min, 15min, 45min) ($p < .05$). Intra assay CV for testosterone and cortisol were 3.59 and 4.38 respectively and inter assay CV was 2.89 and 4.06 respectively. Plasma volume shift percent values from PRE for RT subjects were ($\bar{X} \pm$ SD) -3.3±4.0 at 5P, -0.9±5.6 at 15P, and 2.7±5.2 at 45P, and for UT subjects values were -1.2±10.6 at 5P, -1.8±12.9 at 15P, and 3.6±8.9 at 45P. Hormonal values were not adjusted for plasma volume shifts because the concentrations in the blood are the concentrations the receptors are exposed to.

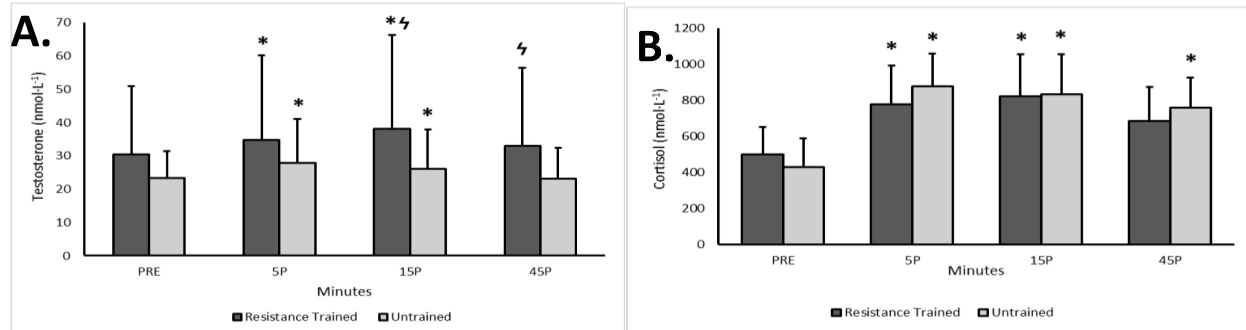


Figure 4. Acute hormonal response in RT and UT groups at baseline (PRE) and 5min (5P), 15min (15P), and 45min (45P) post RE bout. A. Serum Testosterone (nmol·L^{-1}) ($\bar{X}$ ± SD). B. Serum Cortisol (nmol·L^{-1}) ($\bar{X}$ ± SD). * indicates significance ($p < .05$) from PRE. ϟ indicates moderate main effect (Cohen's D).

Androgen Receptor Data

For total AR expression, there were no differences between time points within the RT group ($p > .05$); however, there were differences between time points within the UT group ($p = .016$). In the UT group, total AR expression significantly decreased at 30P (-19.33%Δ, $z = -2.192$, $p = .027$) and 60P (-10.89%Δ, $z = -2.192$, $p = .027$) post exercise, but returned to baseline values by 180P (3%Δ, $z = -.178$, $p > .05$). There were significant decreases at 10P in p-AR Ser^{213} in both the RT (-28.73%Δ, $z = -2.293$, $p = .020$) and UT (-32.25%Δ, $z = -2.073$, $p = .039$) groups. There were no differences between or within groups ($p > .05$) for p-AR Ser^{81}, p-AR Ser^{515}, or p-AR Ser^{650}.

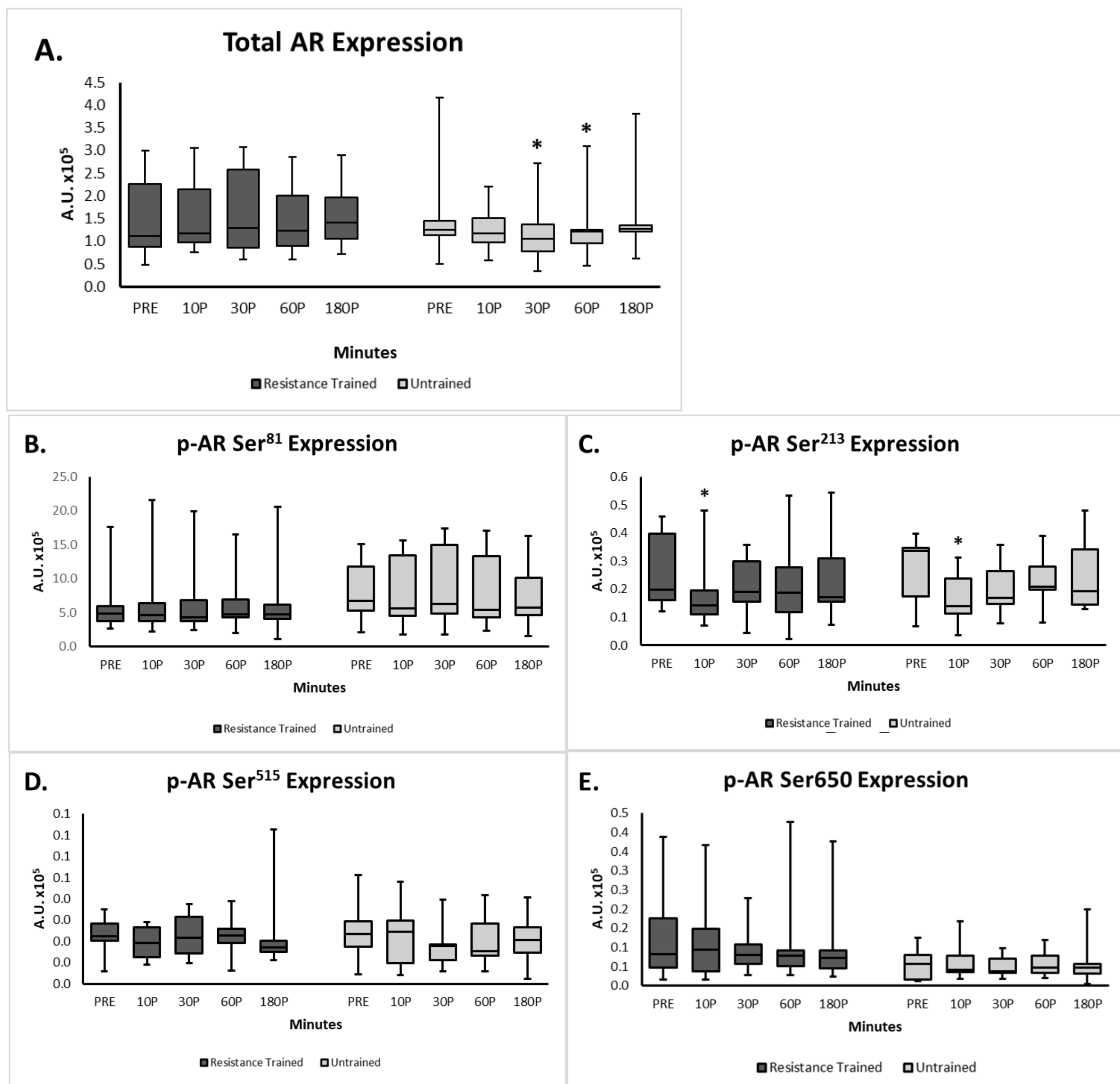


Figure 5. Total AR and p-AR responses (Arbitrary Units) in RT and UT groups at baseline (PRE) and 10 min (10P), 30min (30P), 60min (60P), and 180min (180P) post RE bout. A. Total AR Expression. B. p-AR Ser^{81} Expression – ligand binding, can be ligand independent, associated with nuclear retention. C. p-AR Ser^{213} Expression – ligand independent, associated with stability and translocation to the nucleus. D. p-AR Ser^{515} Expression – increased phosphorylation associated with increased transcriptional activity, transactivation, and nuclear localization. E. p-AR Ser^{650} Expression – negative regulator associated with nuclear export. * indicates significance ($p < .05$) from PRE.

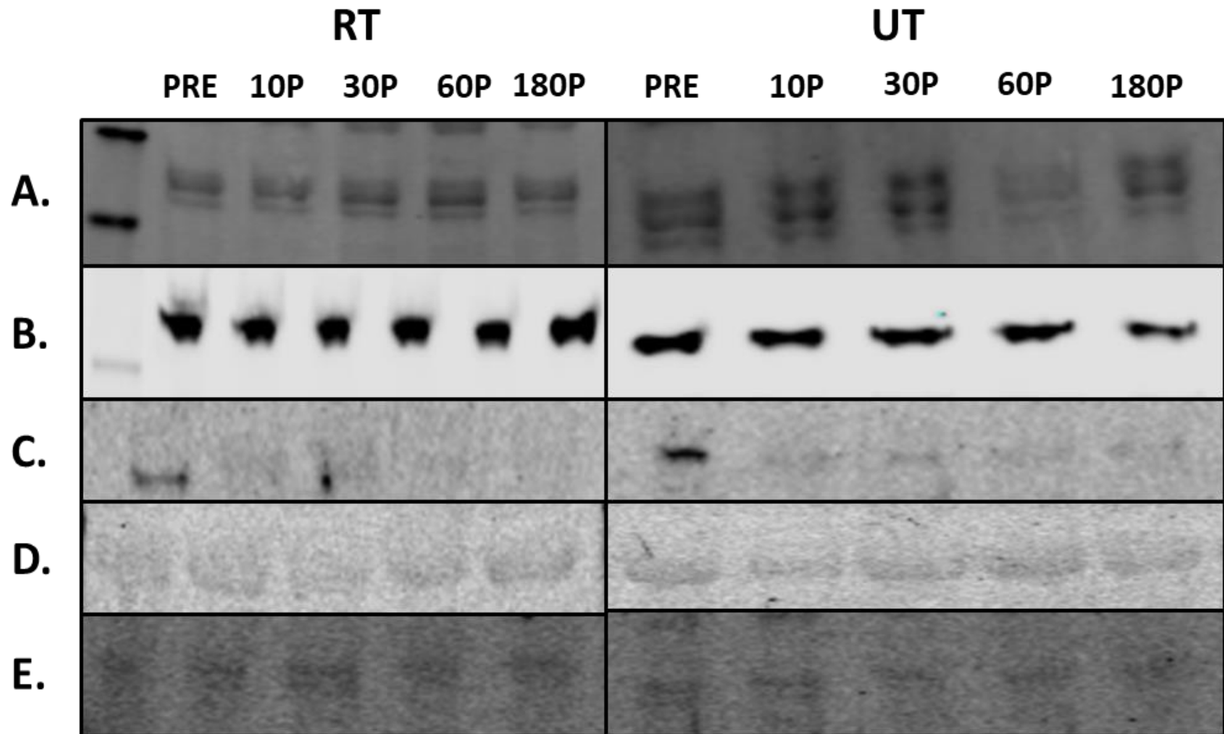


Figure 6. Representative blots for the androgen receptor in RT and UT groups at PRE, 10P, 30P, 60P, and 180P. A. Total AR expression. B. p-AR Ser^{81} expression. C. p-AR Ser^{213} expression. D. p-AR Ser^{515} expression. E. p-AR Ser^{650} expression.

Glucocorticoid Receptor Data

For Total GR content, there were no differences between time points within the RT or UT groups ($p > .05$). Total GR content was significantly greater in the RT group compared to the UT group at 10P (Mann-Whitney $U = 19$, $z = -2.123$, $p = .035$).

For p-GR Ser^{134} the RT group was significantly higher than the UT group at PRE (Mann-Whitney $U = 15$, $z = 2.449$, $p = .014$), but the UT group was significantly higher than the RT group at 30P (Mann-Whitney $U = 9$, $z = -2.939$, $p = .003$). Within the RT group for p-GR Ser^{134} there were decreases compared to PRE at 10P (-33.13%Δ, $z = -2.395$, $p = .017$), 30P (-32.89%Δ, $z = -2.803$, $p = .005$), and 60P (-22.71%Δ, $z = -2.803$, $p = .005$), but returned to baseline by 180P (-

6.63%Δ, z = -1.274, p = .203); conversely, in the UT group, increases were shown compared to PRE values at 30P (85.84%Δ, z = -2.666, p = .008), 60P (111.24%Δ, z = -2.666, p = .008), and 180P (68.30%Δ, z = -2.666, p = .008).

There were significant decreases in p-GR Ser^{211} from PRE to 60P (-30.76%Δ, z = -2.701, p = .007) and 180P (-30.33%Δ, z = -2.599, p = .009) in the RT group and from PRE to 180P (-42.98%Δ, z = -2.666, p = .008) in the UT group.

In p-GR Ser^{226}, the UT group had a higher expression at 10P (Mann-Whitney U = 21, z = -1.960, p = .050) and 180P (Mann-Whitney U = 20, z = -2.041, p = .041) compared to the RT group. There were significant increases from PRE in both the RT group at 10P (311.5%Δ, z = -2.803, p = .005), 30P (33.97%Δ, z = -2.803, p = .005), 60P (387.42%Δ, z = -2.803, p = .005), and 180P (240.16%Δ, z = -2.803, p = .005) as well as the UT group at 10P (615.52%Δ, z = -2.666, p = .008), 30P (568.66%Δ, z = -2.666, p = .008), 60P (441.12%Δ, z = -2.666, p = .008), and 180P (395.26%Δ, z = -2.666, p = .008).

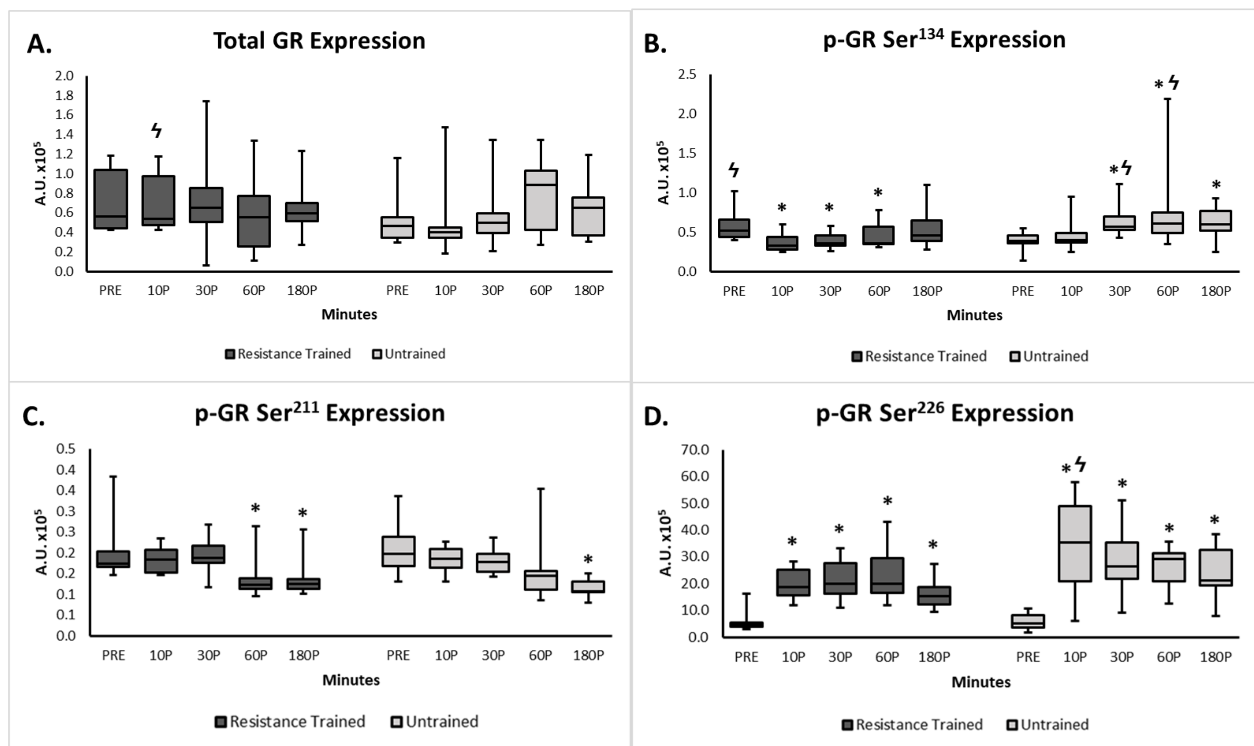


Figure 7. Total GR and p-GR responses (Arbitrary Units) in RT and UT groups at baseline (PRE) and 10 min (10P), 30min (30P), 60min (60P), and 180min (180P) post RE bout. A. Total GR Expression. B. p-GR Ser^{134} Expression – ligand binding associated with translocation to nucleus, ligand independent under stressful conditions. C. p-GR Ser^{211} Expression – ligand dependent associated with increased transcriptional activity, inverse relationship with Ser^{226}. D. p-GR Ser^{226} Expression – negative regulator associated with nuclear export. * indicates significance ($p < .05$) from PRE. ϟ indicates significantly greater than other group at same time point ($p < .05$).

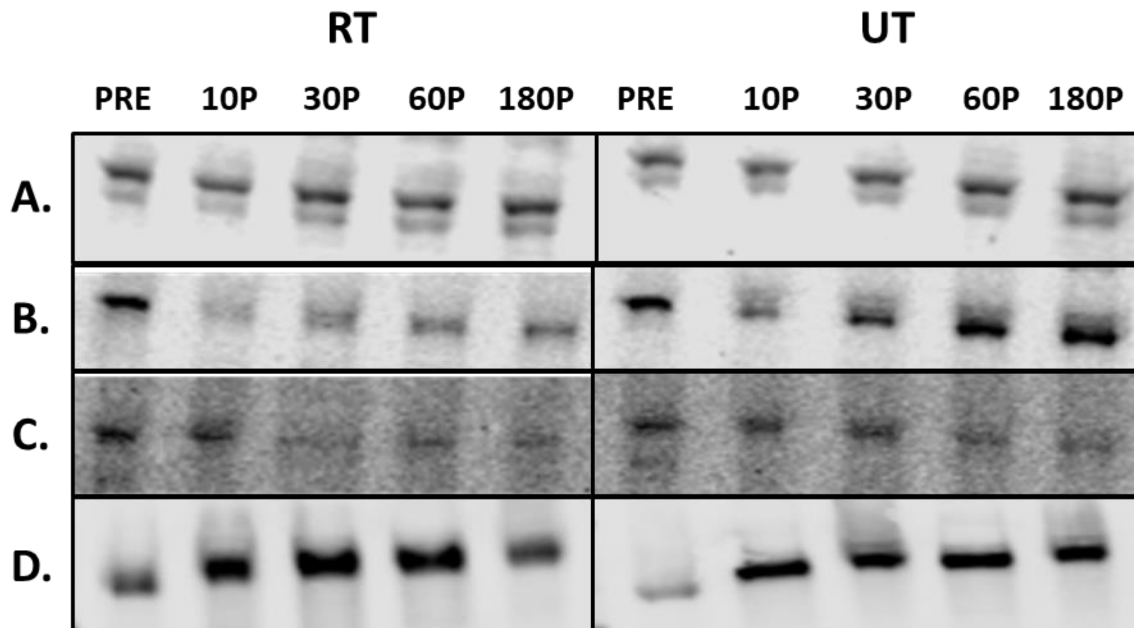


Figure 8. Representative blots for the glucocorticoid receptor in RT and UT groups at PRE, 10P, 30P, 60P, and 180P. A. Total GR expression. B. p-GR Ser^{134} expression. C. p-GR Ser^{211} expression. D. p-GR Ser^{226} expression.

Discussion

The results of this study show that there are differences depending on training status not only in the hormonal response but at the receptor level as well. As expected, the RT individuals were able to lift heavier loads resulting in performance differences, however, both groups were working at the same relative intensity. The RT group had higher testosterone levels while the UT group had prolonged elevated cortisol levels. These hormonal responses appear to influence total receptor content in addition to phosphorylation sites on the receptor. While the acute hormonal response may not be the only variable important to the adaptations to resistance exercise, such as muscle hypertrophy, they appear to play an important role at the receptor level.

Performance data allows us to analyze the stimulus and compare groups. As expected, back squat 1RM was higher in the RT compared to the UT group showing there were significant

differences in strength between groups; however, there were no differences in RPE between groups showing that the relative intensity was perceived the same in each group. This validates the difference in training status and the similarity in difficulty between groups. Due to the strength differences, the RT group produces more force at each of the 6 back squat sets compared to the UT group. But the RT group was only able to maintain force production for the first 5 sets as it decreased compared to set 1 by set 6; whereas, the UT group was able to maintain force for all 6 sets. The RT group was able to perform more concentric work for sets 1 and 2 compared to the UT group, most likely due to strength differences; however, there were no differences in work between groups for the remaining 4 sets. In addition, the RT group had a decrease in concentric work at sets 5 and 6 compared to set 1, but the UT group was able to maintain work. This data suggests that the RT group was fatiguing their higher threshold motor units that the UT group may have not been able to effectively recruit to begin with. Using EMG data in the future would help support this hypothesis.

In the hormonal data, we observed typical responses consistent with previous literature showing an adequate stimulus (2, 8, 19, 20, 27). For testosterone, we saw an increase from baseline at 5min and 15min post exercise in both RT and UT groups. On average, the RT group appeared to have larger testosterone values, and there was a moderate effect at 15 min and 45 min post RE bout. For the cortisol response both RT and UT groups increased at 5 min and 15 min post exercise, but the UT group remained elevated at 45 min post where the RT group returned to baseline showing a difference in the hormonal response depending on training status.

For total AR content we observed a decrease at 30 min and 60 min post exercise in the UT group but no change across time in the RT group. This suggests there is a difference between training status at the receptor level in response to RE. Although there were no statistical difference in the testosterone response between groups, it is probable that the higher values of testosterone in the RT group helped maintain AR content, but it is unclear if these testosterone values are due to a result of chronic training. These results differ from previous studies. Speiring et al. 2009 saw an elevated AR response in UT subjects 180 min post exercise where we saw no change at 180 min following a decrease at prior time points. This may be due to a difference in RE protocol as their subjects had larger testosterone values compared to our UT subjects. Ratamess et al. 2005 reported a decrease in AR content in RT subjects 60 min post exercise where we saw no change from baseline levels at 60 min post exercise. The testosterone response was similar in our subjects compared to the Ratamess et al. 2005 study, but there were slight differences in the protocol that may have led to different responses as they used a greater intensity and did not include leg extensions following the back squat. Though importance is supported through multiple studies including our data, it is still inconclusive if the acute hormonal response is playing a role in the total androgen receptor response and if biopsies are being obtained at optimal time points to measure responses accurately. Further research will need to be done to further clarification.

Phosphorylation of AR Ser^{213} decreased 10 minutes post exercise in both RT and UT groups. As this AR Ser^{213} site plays a role in AR stability and translocation, a decrease could explain the decrease in total AR content shown in the UT; however, it does not explain why the UT group might be more susceptible to this AR decrease than the RT group. It could be

hypothesized that the hormonal response played a role as stated in the previous paragraph or that the RT group became better at adapting overtime.

There were no differences across time within or between groups in the other measured p-AR sites (Ser^{81}, Ser^{515}, and Ser^{650}). It is possible that these sites require a different stimulus or RE protocol, chronic training, or that a different time point for collection needs to be used. Phosphorylation of AR Ser^{81} has been shown to accumulate ~8hrs post RE, and in other cells (i.e. prostate) it sensitizes the AR to low levels of androgens. Future research may want to look at this site at later time points as this could play a role in the increased protein synthesis following RE up to 48hrs post exercise. Nicoll et al. (2019) reported increases in p-AR Ser^{515} following a single speed squat RE bout, but after chronic training, a non-functional overreaching (NFOR) group had an even greater response compared to controls (25).

While no differences were observed across time in either group for total GR expression, the RT group was significantly greater than the UT group at 10P. It is equivocal if this was due to a difference in training status with the RT group being able to lift at a greater load or if a different mechanism was involved; however, in the UT group p-GR Ser^{226} was significantly higher than the RT group supporting its status as a negative regulator for the GR. GR Ser^{134} which can be ligand dependent or independent appears to be involved in translocation of the GR from the cytoplasm to the nucleus. We saw very different results depending on training status with p-GR Ser^{134} decreasing in the RT group from PRE values at 10P, 30P, and 60P, conversely, in the UT group we saw increases from PRE at 30P, 60P, and 180P. Although phosphorylation can be independent, it is probable the difference in the acute cortisol response has some responsibility. This shows a

clear difference in the phosphorylation response for GR Ser^{134} depending on training status. GR Ser^{211} is phosphorylated in a hormone dependent manner which increases GR transcriptional activity when phosphorylated. In the cortisol response, the RT group was elevated at 5P and 15P, but were able to recover and return to baseline by 45P whereas the UT group remained elevated to at least 45P. In the RT group, p-GR Ser^{211} had a significant decrease as early as 60P and 180P, but the UT group did not show a decrease until 180P. This supports that GR Ser^{211} is phosphorylated in a hormone dependent manner and remains phosphorylated with prolonged increases of cortisol. Transcriptional activity of GR Ser^{211} has been shown to be greatest when phosphorylation of GR Ser^{226} is less than that of Ser^{211}. GR Ser^{226} has been shown to enhance nuclear export and blunt hormonal signaling, like a negative regulator. We saw significant increases from PRE values at all post time points (10, 30, 60, and 180 min) in both the RT and UT groups with the UT group being higher at 10P and 180P compared to the RT group. This may give further insight as to why p-GR Ser^{211} did not increase despite the increase in cortisol following the resistance bout. It could also be hypothesized that UT individuals need a larger negative regulator response in the GR until they are able to adapt to stress responses more efficiently, which was potentially seen at the p-GR Ser^{134} site. However, blunting a ligand dependent response on a catabolic receptor could further support the hypertrophic response in the moderate intensity RE workout supporting the importance of the acute hormonal response.

It appears that differential patterns of receptor activity occur post-resistance exercise depending on training status. When given the same relative moderate intensity RE stimulus, the RT individuals tend to have a larger testosterone concentration and a quicker cortisol recovery compared to UT individuals. This hormonal difference seems to maintain AR receptor content,

thus potentially increasing protein synthesis resulting in muscle hypertrophy. However, hormones are not the only variable, as some receptor sites are able to function in a ligand-independent manner. Immediately following this RE bout there was a decrease in p-AR Ser^{213}, a ligand-independent site phosphorylated by Akt that increases receptor stability and translocation. Akt is elevated following RE, giving reason to believe that Akt preferentially fulfills its role in protein synthesis pathways prior to phosphorylation of receptor sites. The negative regulator, AR Ser^{650}, was unaffected to this RE stimulus. It is possible it needs to remain constant to fulfill its role, but may experience changes in an over-trained state. Meanwhile, the GR is simultaneously responding to the RE stimulus. When the negative regulator GR Ser^{226} is highly phosphorylated, there is a significantly less GR content seen 10 minutes post RE in our UT subjects compared to RT subjects. There is also a decrease in phosphorylation at the ligand dependent GR Ser^{211} site. This GR Ser^{226} site increases in response to RE in healthy individuals regardless of training status; however, it has been reported by Nicoll et al. (2019) that the positive response is lessened in individuals following a nonfunctional overreaching protocol (25). In addition, phosphorylation of ligand dependent GR Ser^{211}, associated with GR transcription, is maximal when p-GR Ser^{226} is low or blunted. This supports that the muscles are designed to adapt to physiological stresses and avoid excessive catabolism, but when over-worked (i.e. nonfunctional over-reaching, overtraining), it loses the ability to combat these stress hormones leading to potential performance and health problems. However, the increase of a negative regulator does not completely blunt the physiological actions of cortisol and the GR as catabolism is needed for muscle remodeling. GR Ser^{211} was still phosphorylated post RE, just no increase from baseline was observed. But, there was a decrease at 60P and 180P in the RT group who had a quicker cortisol recovery compared to the UT group, who did not experience the decreased p-GR Ser^{211} until 180P. These declines were most likely the

result of cortisol returning to baseline and possibly a shift towards a more anabolic state. Lastly, the GR Ser^{134} decreased in the RT group, but increased in the UT group. While this in part is likely due to the cortisol differences, other stresses are able to phosphorylate this site, and the RT group has had more experience in the ability to respond and adapt.

Conclusions

When analyzing androgen and glucocorticoid receptors, training status needs to be accounted for as there are training status dependent differences. Although the importance of the acute hormonal response on muscle hypertrophy is still controversial, it appears to have some effect at the receptor level in preserving content and in phosphorylation of various receptor sites that cannot be ignored. The RT group was able to maintain their total AR content up to 180 min post RE; whereas, the UT group saw decreases at 30 min and 60 min post exercise. In addition, the ligand dependent GR Ser^{211} site did not show a phosphorylation decrease in the UT group, who had a prolonged elevation in cortisol compared to the RT group, until 180 min post RE, whereas the RT group decreased at 60 min post RE. Also, at the ligand dependent and independent GR Ser^{134} site we saw almost opposite effects in training group where the RT group decreased phosphorylation at 10P, 30P, and 60P, but the UT group saw and increase in phosphorylation at 30P, 60P, and 180P. Phosphorylation of GR Ser^{226} increased at all post time points in both groups, but was higher in the UT group at 10P and 180P. AR Ser^{213} decreased in both groups at 10P, and no differences were seen at AR Ser^{81}, Ser^{515}, or Ser^{650} sites in our moderate intensity protocol. Further research could elucidate the hormone-receptor and receptor phosphorylation responses to RE by looking at a variety of training protocols, later muscle collection time points, MAPK responses, chronic training, and responses in a fed state.

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