EXERCISE-INDUCED HORMONES IN HUMAN SKELETAL MUSCLE ANABOLISM

THE IMPACT OF EXERCISE-INDUCED HORMONAL CHANGES ON HUMAN SKELETAL MUSCLE ANABOLIC RESPONSES TO RESISTANCE EXERCISE

By

DANIEL W.D. WEST, B.Sc.

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AUTHOR: Daniel W.D. West, B.Sc. (McMaster University)

SUPERVISOR: Dr. Stuart M. Phillips

SUPERVISORY COMMITTEE: Dr. Martin J. Gibala
Dr. Gianni Parise

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ABSTRACT

There is a prevalent belief in exercise-science that acute hormone responses to resistance exercise mediate adaptations in strength and hypertrophy; however, there is little direct supporting evidence. Therefore, for this thesis, we conducted three studies to examine the relationship between acute hormonal increases after resistance exercises and subsequent changes in skeletal muscle anabolism.

In our first two studies, we tested the hypothesis that exercise-induced responses of anabolic hormones—growth hormone (GH), insulin-like growth factor 1 (IGF-1) and testosterone—would elevate rates of myofibrillar protein synthesis (MPS) after an acute bout of resistance exercise, and would augment muscle hypertrophy after training. We concluded, however, that resistance exercise-induced increases in putative anabolic hormones do not enhance MPS or strength and hypertrophy adaptations.

Compared with men, women have low endogenous testosterone and do not experience acute post-exercise testosteronemia after resistance exercise. In the third study, we aimed to determine whether rates of MPS would be attenuated in women (compared with men) after resistance exercise and protein ingestion. We reported similar increases in MPS in men and women; post-exercise testosterone responses in women, which were 45-fold lower than men, did not attenuate elevations in MPS.

The sum of the works presented here lead to the conclusion that the acute rise in hormones such as testosterone, GH, and IGF-1, has very little bearing on post-exercise anabolic responses of MPS and hypertrophy. Instead, the rise in these hormones

appears to be a non-specific response to exercise stress rather than a response that is specific to resistance exercise or important for subsequent skeletal muscle anabolism. Contrary to principles that are currently widely used to create exercise programs, our data suggests that exercise programs should not be designed based on nuances in the acute post-exercise hormonal milieu. Alternatively, understanding local mechanotransduction, which is directly linked to muscle fibre loading, will reveal the processes that drive human exercise-mediated muscle hypertrophy.

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FORMAT AND ORGANIZATION OF THIS THESIS

This thesis was prepared in the "sandwich" thesis format as outlined in the McMaster University School of Graduate Studies Thesis Preparation Guide. This thesis is comprised of a general introduction, three original research papers (Chapters 2-4) and a general conclusion. All papers have been accepted for publication in peer reviewed journals with the candidate as first author.

CONTRIBUTION TO PAPERS WITH MULTIPLE AUTHORS

CHAPTER 2

Publication

West DW, Kujbida GW, Moore DR, Atherton P, Burd NA, Padzik JP, De Lisio M, Tang JE, Parise G, Rennie MJ, Baker SK, & Phillips SM (2009). Resistance exercise-induced increases in putative anabolic hormones do not enhance muscle protein synthesis or intracellular signalling in young men. *J Physiol* **587**, 5239-5247

Contributions

DWD West, GW Kujbida, DR. Moore, JE Tang and SM Phillips planned the study. DWD West and GW Kujbida obtained ethics board approval. DWD West, GW Kujbida, NA Burd, JP Padzik, SK Baker and SM Phillips collected the data. SM Phillips supervised the study and obtained muscle biopsies. DWD West, GW Kujbida, DR. DWD West, JP Padzik and T Rerecich performed blood metabolite analysis. DWD West, GW Kujbida, DR Moore and NA Burd performed analyses for measurement of myofibrillar protein synthesis using methods developed by DR Moore. Western blot analysis was performed by P Atherton and MJ Rennie and by M De Lisio and G Parise (JAK-STAT). DWD West drafted the manuscript. All authors contributed to revising intellectual content of the manuscript and approved the final version.

CHAPTER 3

Publication

West DW, Burd NA, Tang JE, Moore DR, Staples AW, Holwerda AM, Baker SK, & Phillips SM (2009). Elevations in ostensibly anabolic hormones with resistance exercise enhance neither training-induced muscle hypertrophy nor strength of the elbow flexors. *J Appl Physiol* **108**, 60-67.

Contributions

DWD West, JE Tang, DR. Moore and SM Phillips planned the study. DWD West obtained ethics board approval and conducted pre- and post- training assessments: strength testing and blood and biopsy trials (biopsies obtained by SM Phillips). SM Phillips supervised the study. DWD West supervised participant training with help from NA Burd and AM Holwerda. DWD West performed muscle fibre type analysis. DWD West and D. Inglis obtained MRI images which were analysed by DWD West. DWD West, JP Padzik and T Rerecich performed blood metabolite analysis. DWD West drafted the manuscript. All authors contributed to revising intellectual content of the manuscript and approved the final version.

CHAPTER 4

Publication

West DW, Burd NA, Churchward-Venne TA, Camera DM, Mitchell CJ, Baker SK, Hawley JA, Coffey VG, & Phillips SM (2012). Sex-based comparisons of myofibrillar protein synthesis after resistance exercise in the fed state. *J Appl Physiol* **112**, 1805-1813.

Contributions

DWDW, NAB and SMP designed the study. DWD West, TA Churchward-Venne, CJ Mitchell, SK Baker and SM Phillips collected the data. SM Phillips supervised the study and obtained muscle biopsies. DWD West performed signalling analysis by Western blotting with assistance by VG Coffey. DWD West, DM Camera and VG Coffey performed mRNA analysis. DWD West and NA Burd performed analyses for measurement of myofibrillar protein synthesis. DWD West drafted the manuscript. All authors contributed to revising intellectual content of the manuscript and approved the final version.

CHAPTER 1: INTRODUCTION

1.1 THESIS OUTLINE

This thesis begins with a general introduction, Chapter 1, which outlines the rationale and purpose of the studies reported herein. Next, the experimental models that we (the authors of Chapters 2-4) used are explained, with a brief mention of muscle protein synthesis which was the primary outcome variable in Chapters 2 and 4. [Note: technically, we measured the rate of *myofibrillar* protein synthesis, denoted herein as MPS; the myofibrillar fraction is comprised of contractile proteins and represents the majority fraction of mixed muscle]. The remainder of the introduction discusses four hormones—testosterone, growth hormone (GH), insulin-like growth factor 1 (IGF-1) and cortisol—that were measured in Chapters 2-4, and how they can affect protein metabolism. The middle of the thesis, Chapters 2-4, is comprised of the published manuscripts of the three studies that we conducted. Chapter 5 is a general discussion of topics and questions remaining in light of the work presented in Chapters 2-4, and includes a brief discussion of sex-based comparisons in muscle protein synthesis, and an examination of the time course and concentration of testosterone and how these two factors may affect protein metabolism. Chapter 5 concludes with a discussion of potential biological roles of the exercise-induced hormone response, areas of future research and an overall conclusion.

1.2 THESIS RATIONALE

Resistance exercise is an anabolic stimulus that promotes gains in skeletal muscle strength and hypertrophy over time. A prevalent viewpoint exists in exercise-science literature that acute exercise-induced changes in the concentrations of anabolic hormones such as testosterone and growth hormone regulate these adaptations. Direct quotations from a variety of publications are presented in Appendix 1 as evidence of this belief, and to give a sampling of the nature, variety and veracity of this viewpoint. These quotes convey a perspective that it is a foregone conclusion that acute hormone responses to resistance exercise mediate adaptation; however, upon closer examination, direct evidence to support this perspective is lacking.

1.3 STUDY OBJECTIVES

We conducted three studies to examine the effect of acute hormone responses after resistance exercises on subsequent changes in skeletal muscle anabolism. In the first study (Chapter 2), we manipulated the exercising muscle mass to create low and high systemic hormone concentrations to which the elbow flexor muscles, which performed identical resistance exercise in both conditions, were exposed post-exercise. We subsequently measured MPS using stable isotope methodology after the acute bouts of resistance exercise. Thus, the first study was designed to test the hypothesis that the exercise-induced response of anabolic hormones would enhance MPS and

protein signalling involved in translation initiation after an acute bout of resistance exercise.

In the next study (Chapter 3), we used the same experimental design as the previous study, but investigated whether there were differences in strength and hypertrophy between the low and high hormone conditions after fifteen weeks of training. Specifically, we aimed to determine whether repeated elevations in acute post-exercise concentrations of GH, IGF-1 and testosterone during resistance training would enhance muscle hypertrophy and strength gains. This training study allowed us to verify that our acute results were consistent with the training-induced phenotype. Additionally, measuring hypertrophy provided insight into potential hormonal effects on net protein balance (cf. in the first study we measured MPS, but not muscle protein breakdown).

The last study (Chapter 4) was designed to examine the effect of exercise-induced testosterone in a different manner than the first two studies: using divergent exercise-induced testosterone responses of men and women as a model. The purpose of this study was two-fold: i) to determine the impact of resistance exercise and protein feeding on rates of MPS and the molecular anabolic response during early (1–5 h) and late (24–28 h) recovery periods in men and women; and ii) to test the hypothesis that low post-exercise systemic testosterone in women would attenuate MPS after resistance exercise compared with men. Together with investiagting this fundamental comparison between men and women after resistance exercise with feeding, by

profiling the testosterone responses to resistance exercise, we were able to examine whether there was any association between the post-exercise testosterone response and subsequent rates of MPS.

1.4 EXPERIMENTAL MODELS OF EXERCISE-INDUCED HORMONE RESPONSES

It is clear that resistance exercise can elicit an increase in the systemic concentrations of testosterone, growth hormone and cortisol. This is especially true when the exercise bout has the following characteristics: moderate-high intensity (e.g., a load approximating 10 repetition maximum) (21, 48, 50), high volume (35, 37), short rest intervals (16, 50, 51), and engages a large muscle mass (40, 72). In the first two studies of this thesis, we employed a within-subject design in which we manipulated the quantity of exercising muscle mass in order to create a low and high post-exercise hormonal milieu to which the elbow flexors, a much smaller muscle mass, were exposed. In one condition, participants performed unilateral elbow flexion (i.e., a small exercising muscle mass alone) to induce little-to-no systemic post-exercise hormonal response such that hormone concentrations were similar to basal levels. In the opposite condition, elbow flexion exercise was performed using the contralateral arm, followed by a bout of lower-body exercise, in which we manipulated the variables noted above, to induce a high post-exercise hormone state.

Part of the reason that effects of exercise-induced hormones are not established, but instead largely surmised, is that it is difficult to find a suitable model that

manipulates or isolates potential effects. After using a model whereby we manipulated exercising muscle mass in Chapters 2 and 3, we were interested in studying physiological hormones in another model. We decided to study the effect of resistance exercise and dietary protein on MPS in men and women for two main reasons. First, it had never been done before and would provide fundamental information. Second, knowing that women do not exhibit hypertestosteronemia after exercise, this model allowed us to compare rates of MPS in a group that produced a robust exercise-induced testosterone response (men) with a group that did not have the 'benefit' of a post-exercise testosterone increase. Indeed our model was successful, as the increase in testosterone availability above baseline was 45-fold greater in men compared with women. Presumably, this divergence would be revealing in that it should stimulate differential rates of MPS if the post-exercise increment in testosterone was truly integral to elevating MPS after resistance exercise.

In summary, the present thesis contains studies that stemmed from both practical and basic sciences questions such as: Are exercise-induced hormones important regulators of skeletal muscle mass? Do exercise-induced hormones enhance muscle size and strength with training? Should small muscle groups be paired with large muscle groups in order for the small muscle groups to benefit from the hormonal milieu that is produced by exercising a large, but not a small, muscle mass? Is the anabolic response to feeding and resistance exercise in women attenuated by the lack of an exercise-induced testosterone response?

1.5 A BRIEF INTRODUCTION TO MUSCLE PROTEIN SYNTHESIS

In Chapters 2 and 4, we used a continuous infusion of an isotopically-labelled amino acid to make direct measures of the incorporation of amino acids into muscle proteins in order to determine changes in the rate of protein synthesis (70). In our method, isotopically-labelled phenylalanine is infused at a rate that has been determined to result in a steady-state enrichment of the plasma- and intracellular free-amino acid precursor pools (94). With the precursor pool enrichment now steady, the magnitude of the difference in the incorporated enrichment between two protein-bound biopsy samples will be directly proportional to the rate of muscle protein synthesis. The incorporation time, multiplied by the precursor pool enrichment, is the denominator to the difference in protein-bound enrichment, producing the final calculated fractional synthesis rate, often expressed in units of percent per hour.

A full discussion of effects of feeding on muscle protein synthesis is beyond the scope of this thesis (see references (67, 71) for a review). Briefly, muscle protein synthesis and breakdown are elevated after resistance exercise in the fasted state (19, 68, 99). Feeding protein post-exercise increases muscle protein synthesis (14, 63) and a feeding-induced insulin secretion (or aminoacidemia per se) attenuates muscle protein breakdown (33, 82), resulting in a net positive protein balance (14). Changes in the rate of muscle protein synthesis account for the majority (>70% (33)) of the increase in net protein balance that occurs after resistance exercise combined with feeding (14, 82). In

Chapters 2, 3 and 4, a whey protein supplement was given post-exercise to support net protein balance (42, 86, 87) and to represent a 'real-life' training scenario.

Processing a muscle biopsy sample for analysis of MPS generates a cytosolic homogenate that is suitable for Western blotting. Quantifying signalling events has the potential to provide mechanistic insight that is complementary to dynamic measures (e.g., muscle protein synthesis) and corroborate (or not) the regulation of translation initiation. A general caveat that should be noted is that there can be a dissociation between muscle protein synthesis and 'expected' (at least based on cell culture and/or animal experiments) signalling measures (36). In addition to assuming that phosphorylation of a given amino acid residue indicates activation (3, 66), other caveats of signalling analysis are mentioned in the discussion portion of Chapter 4. In Chapter 2, we quantified Janus kinase-Signal Transducer and Activator of Transcription (JAK-STAT) signalling which is thought to transduce growth hormone signalling (64). Additionally, in Chapters 2 and 4 we quantified changes in phosphorylation of the mTOR signalling pathway since this pathway has been shown to be a primary regulator of translation initiation (44), and is required for resistance exercise (25) and amino acids (23) to stimulate MPS. Notably, increases in phosphorylation of p70S6K^{Thr389} are thought to reflect increased p70S6K activation, which is a hallmark of mTOR activation (76, 81).

The primary hormones measured in our studies were testosterone, GH, IGF-1 and cortisol. The characteristics and impacts of these hormones on skeletal muscle protein metabolism form a foundation that we investigated in the context of resistance

exercise. An introduction of each these hormones and the role they have in relation to protein metabolism and exercise is given below.

1.6 TESTOSTERONE IN PROTEIN METABOLISM AND EXERCISE

Testosterone is an anabolic androgenic hormone that is released primarily from the testes in men and, in much smaller quantities, from the ovaries in women. Testosterone precursor hormones are also produced by the adrenal gland which is converted to testosterone in peripheral target tissues (53). Testosterone production in men leads to serum concentrations that are approximately 10-20 times the concentrations of women. Testosterone release is stimulated by luteinizing hormone which is secreted by the anterior pituitary gland in response to gonadotropin releasing hormone from the hypothalamus. The interest in testosterone in the present thesis is in its effects on processes that regulate skeletal muscle protein synthesis and accretion.

Testosterone acts through both classical genomic as well as non-genomic pathways. Much of what is known about the genomic pathway and androgen receptor signalling comes from work done in cell culture and prostate tissue. Accordingly, elucidation of signalling events in human skeletal muscle is necessary to have an accurate understanding of the effects of androgens on skeletal muscle biology.

In the genomic pathway, testosterone crosses the plasma membrane and binds to a cytosolic androgen receptor, inducing dimerization (17) and translocation to the nucleus (65). In the nucleus, the receptor complex binds to androgen-response

elements, enhancing or repressing target gene expression. Gene targets of testosterone in skeletal muscle are not fully elucidated but include genes whose proteins regulate DNA replication, transcription activation, nerve growth, differentiation and hypertrophy (18, 59). Exactly how steroids such as testosterone enter the cell is not clear. There appear to be two main mechanisms for how a given steroid hormone could exert intracellular biological action. First, the free (unbound) fraction of the circulating steroid crosses the plasma membrane by diffusion, exerting a cellular response that is dependent/proportional to the amount of this free fraction (61). Second, the carrier protein of the hormone—e.g., sex hormone binding globulin—is recognized by a cell-surface receptor which internalizes, by endocyctosis, the hormone-carrier protein complex, at which point the carrier protein is degraded (39), allowing the hormone to bind to an intracellular androgen receptor.

Testosterone has also been proposed to have rapid non-genomic action which can occur through a variety of mechanisms; some of these include: G-protein coupled receptor/androgen receptor binding at the membrane leading to mitogen activated protein kinase pathway activation, membrane intercalation and altered membrane fluidity, increased calcium permeability and secondary messenger activation (62). Non-genomic effects in skeletal muscle are currently poorly understood (62) and therefore the discussion here is minimal.

Briefly, any potential acute performance-enhancing effects (e.g., increased force production) that testosterone could have would presumably occur by activating the

aforementioned rapid non-genomic pathways in skeletal muscle or nervous tissue. However, the primary aim of our studies was to examine the impact of exercise-induced testosterone on adaptations to resistance exercise/training; our studies were not designed to investigate potential effects of testosterone on acute force/power output. Likewise, the studies presented herein were not designed to examine associations of chronic testosterone concentrations (or other hormone) with muscle protein synthesis (8), or with physical performance (38) that may be related to coincident associations with muscle mass (6) or as a result of aging (73).

Most of our knowledge of the effects testosterone in human skeletal muscle comes from studies that have supplemented exogenous testosterone or synthetic testosterone analogues. Testosterone can increase basal rates of muscle protein synthesis in the fasted (30, 77, 89) and fed (78) states and decrease muscle protein breakdown (27). Chronically, testosterone increases lean body mass and muscle size (and strength), both with (10) and without (10, 11, 77) resistance exercise. Testosterone-induced muscle hypertrophy is associated with type I and type II fibre hypertrophy (79), as well as an increased number of satellite cells (80) and centrally-located nuclei (26, 41), a hallmark of muscle fibre (re)generation.

As alluded to above, muscle fibre hypertrophy that results from combined resistance exercise and testosterone, or from testosterone alone, is presumably the chronic result of elevated muscle protein synthesis (30, 77, 78, 89) and satellite cell proliferation (26, 41, 80). Testosterone-stimulated increases in muscle protein synthesis

are primarily attributed to enhanced translational efficiency (30, 78) and intracellular amino acid reutilization (30); exactly how these changes occur requires further research. Decreases in muscle protein breakdown can also occur with testosterone administration, but this effect appears more pronounced after an extended period of supplementation (e.g., 6 months) (28). The mechanisms behind this delayed effect on muscle protein breakdown, and behind the effects of testosterone on protein breakdown in general, are not yet clear. In cell culture, testosterone suppresses atrogin-1 gene expression, a ubiquitin ligase that is associated with muscle wasting, but does not affect phosphorylation of its upstream regulator, Forkhead box O 3A (100). Taken together, pharmacological testosterone can: increase satellite cell number, increase muscle protein synthesis, decrease muscle protein breakdown, and result in a dose-response relationship of testosterone with change in muscle volume and fat free mass (11).

A dose-response relationship between exogenous testosterone administration with changes in muscle cross-sectional area, combined with articles entitled “Proof of the effect of testosterone on skeletal muscle” by foremost testosterone experts (12) convey a sense of imperativeness that *all* changes in testosterone concentration should be examined with the utmost scrutiny. In this thesis, we propose that this is a superficial view and one that tends to generate assumptions that lack evidence at the present time. The exercise-responsive nature of testosterone draws analysis and interpretation of

testosterone concentrations into the spotlight of a field based on hormone-induced adaptations, and is a primary focus of the present thesis.

1.7 GROWTH HORMONE AND INSULIN-LIKE GROWTH FACTOR 1 IN PROTEIN METABOLISM AND EXERCISE

Growth hormone (GH) and IGF-1 (insulin-like growth factor 1) are peptide hormones that are intrinsically tied to one another since GH directly regulates IGF-1, which negatively regulates GH secretion. Growth hormone releasing hormone from the hypothalamus stimulates the anterior pituitary gland to release growth hormone into systemic circulation where it is bound to growth hormone binding protein. In general, it is thought that a large proportion of the biological action of GH is exerted via IGF-1 (31). For this reason, GH and IGF-1 will be discussed jointly. IGF-1 is secreted by the liver and by extrahepatic tissues including skeletal muscle. Several splice variants of IGF-1 are produced in humans, with the variant IGF-1Ea being secreted primarily from the liver in response to GH (5) whereas IGF-1Ec (also known as mechano growth factor) is produced by skeletal muscle in response to mechanical stretch (22, 96), and independent of GH (5, 20, 22). While other tissues throughout the body produce IGF-1, systemic IGF-1 concentrations appear to be primarily reflective of changes in liver secretion where IGF-1 is highly expressed, produced and secreted, as opposed to other tissues including muscle, which hosts a local production/secretion system (83). The muscle IGF-1 system appears to be local, or bidirectionally contained, in the sense that systemic growth

hormone administration does not alter IGF-1 expression or GH or IGF-1 receptor expression (84), and overexpression of muscle IGF-1 does not alter systemic IGF-1 concentrations (9). Systemic IGF-1, but not local muscular IGF-1 was measured in the present thesis. Thus, it is important to note, since it will not be discussed further, that it is possible that mechanically regulated and locally synthesized IGF-1 contributed, via autocrine/paracrine action (1, 2), to some of the muscle-specific effects following resistance exercise (58) that we observed in our studies.

GH remains heavily studied in exercise science literature, and is proposed to mediate a variety of adaptations to resistance exercise, including effects on hypertrophy (46, 52). Indeed, the fact that GH concentrations increase dramatically in response to exercise (34, 49) makes it an enticing prospect that GH could be influencing training-induced gains after resistance exercise. However, the relevance and mechanics of GH (46, 69) (and IGF-1 (32, 83)) to skeletal muscle mass regulation is currently a matter of debate. With respect to GH, the argument against an anabolic effect on contractile protein stems primarily from two lines of evidence: i) acute GH administration does not stimulate muscle protein synthesis (97, 98) or myofibrillar protein synthesis (24), and ii) long-term GH supplementation does not enhance muscle strength (85) or hypertrophy (84).

1.8 CORTISOL IN PROTEIN METABOLISM AND EXERCISE

Cortisol is released from the adrenal cortex in response to stress or trauma (e.g., sepsis, head injury) (95), as well as exercise (47). Cortisol release is stimulated by adrenocorticotropic hormone which is secreted by the anterior pituitary gland in response to corticotropin releasing hormone from the hypothalamus. Cortisol, which was measured in the studies in the present thesis, belongs to the glucocorticoid family of steroid hormones. The term 'glucocorticoid' is a term that is often used in the literature, and is used in this thesis, to collectively refer to hormones such as cortisol, and synthetic derivatives of cortisol such as dexamethasone and prednisone, that bind to the glucorticorticoid receptor and which are thought to have similar effects in target tissues (60). Glucocorticoids: regulate blood osmolarity, blood pressure and immune function, can have diabetogenic and anti-inflammatory effects (4), and are elevated in various pathological conditions (88). While these effects of cortisol are beyond the scope of this thesis, cortisol will be discussed with respect to its potential effects on muscle protein balance, as well as other potential effects associated with an exercise-induced cortisol response (Chapter 5).

Glucocorticoids act through multiple pathways in skeletal muscle, exerting both genomic (92) and non-genomic (57, 75) effects on muscle protein synthesis (75) and breakdown (43, 55). The classically described genomic mechanism of action of cortisol is similar to the one described previously for testosterone: cortisol binds intracellular glucocorticoid receptors, which translocate to the nucleus and alter gene expression. In

the nucleus, gene expression of ubiquitin ligases muscle RING-finger protein-1 (MuRF-1) and atrogin-1 are upregulated stimulating myofibrillar proteolysis via the ubiquitin-proteosome pathway (15, 93). Recent evidence from human primary myotubes suggests that 11-beta-hydroxysteroid dehydrogenase, which converts inactive cortisone into the active cortisol, controls glucocorticoid-induced upregulation of MuRF-1, atrogin-1 and proteolysis (13). Additionally, upregulaton of MuRF-1 by the glucocorticoid receptor is synergistically enhanced by Forkhead box protein O1 (91). More recently, it was demonstrated using a muscle-specific glucocorticoid receptor knock-out mouse model that the glucocorticoid receptor is necessary to mediate atrophy from prolonged glucocorticoid administration (92).

Acutely, glucocorticoid treatment can downregulate translation initiation and muscle protein synthesis. Four hours after glucocorticoid injection in rats, decreases are observed in the phosphorylation of eukaryotic initiation factor 4E-BP1 and $p70^{S6K}$, and in the assembly of the eukaryotic initiation factor 4F translation initiation complex, decreases in all of which are concomitant with a 60% decrease in muscle protein synthesis (75). Glucocorticoids also suppress increases in mRNA translation that are stimulated by branched chain amino acids in human skeletal muscle (54).

As discussed above, glucocorticoids increase proteolysis in a process that involves the upregulating of MuRF-1. Interestingly, it was recently shown that knocking out MuRF-1 attenuates the glucocorticoid-induced decrease in muscle protein synthesis (7). This finding adds to the expanding roles of MuRF-1 as a regulator of muscle protein

breakdown (15), amino acid availability (45) and muscle protein synthesis (7, 45) during glucocorticoid administration. Glucorticorticoids may also counter the anti-proteolytic effects of insulin. Insulin infusion resulted in a 40% reduction in skeletal muscle proteolysis and positive net protein balance, but these effects were negated by glucocorticoid treatment, which resulted in a negative protein balance (56). Conversely, insulin and IGF-1 prevent dexamethasone-induced upregulation of MuRF-1, atrogin-1 and proteolysis in mouse myotubes (74). Taken together, glucorticoid exposure can upregulate muscle protein breakdown and/or inhibit protein synthesis through multiple mechanisms, promoting a negative net protein balance.

Much of the mechanistic information on cortisol's action in skeletal muscle comes from cell culture experiments, animal and animal knockout models, and has used indices (e.g., 3-methylhistidine release) for muscle protein breakdown. In normal adult humans, pharmacological doses of glucocorticoids decrease skeletal muscle net protein balance (29, 55). Three days of prednisolone administration doubled the rate of phenylalanine efflux from the leg, inducing a net negative phenylalanine balance while increasing intracellular free phenylalanine and essential amino acid concentrations (55). Prednisolone treatment did not affect the rate of phenylalanine disposal, indicating that the primary effects of glucocorticorticoids on muscle net protein balance may be to increase protein breakdown, with minor effects on protein synthesis (55). Further studies that use stable isotope-labeled amino acid tracer dilution/incorporation techniques will help establish the influence of cortisol muscle net protein balance.

Interestingly, and of particular relevance to the present thesis, are the findings of two studies (29, 55) that cortisol may play a permissive role to muscle proteolysis under already-catabolic conditions, but have minor effects alone in normal adults. Ferrando and colleagues (29) reported no effect of cortisol infusion on muscle net protein balance compared to an overnight fast; the study concluded that cortisol did reduce net protein balance, but only after 14 d bed rest, not after an overnight fast. These observations, which are drawn from studies inducing prolonged high glucocorticoid concentrations, raise questions regarding the catabolic effects of physiological fluctuations in cortisol, toward skeletal muscle under normal physiological conditions.

Nevertheless, cortisol concentrations are frequently examined (and in conjunction with testosterone in order to calculate a testosterone/cortisol ratio) with great interest based on the premise that they influence training adaptations. This sentiment is captured in the following statement from Viru and colleagues (90):

> "During the past 15-20 years several researchers in exercise physiology and sports medicine have had the opinion that the decreased ratio of testosterone/cortisol indicates a predominance of catabolism that is undesirable for adaptation and improvement of performance in athletes. In their opinion, an increased cortisol concentration is "guilty" of association with maladaptation."

A relevant question to ask, then, is whether exercise-induced cortisol is catabolic, or whether it is merely guilty by association. Cortisol was measured in each study in this thesis and is examined further in the concluding discussion.

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CHAPTER 2

TITLE: Resistance exercise-induced increases in putative anabolic hormones do not enhance muscle protein synthesis or intracellular signalling in young men

AUTHORS: West DW, Kujbida GW, Moore DR, Atherton P, Burd NA, Padzik JP, De Lisio M, Tang JE, Parise G, Rennie MJ, Baker SK, & Phillips SM

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RAPID REPORT

Resistance exercise-induced increases in putative anabolic hormones do not enhance muscle protein synthesis or intracellular signalling in young men

Daniel W. D. West[1], Gregory W. Kujbida[1], Daniel R. Moore[1], Philip Atherton[3], Nicholas A. Burd[1], Jan P. Padzik[1], Michael De Lisio[1], Jason E. Tang[1], Gianni Parise[1], Michael J. Rennie[3], Steven K. Baker[2] and Stuart M. Phillips[1]

[1]*Exercise Metabolism Research Group, Department of Kinesiology and* [2]*Michael G. DeGroote School of Medicine, Department of Neurology, McMaster University, Hamilton, ON, Canada*
[3]*School of Graduate Entry Medicine and Health, City Hospital, University of Nottingham, Derby, UK*

We aimed to determine whether exercise-induced elevations in systemic concentration of testosterone, growth hormone (GH) and insulin-like growth factor-1 (IGF-1) enhanced post-exercise myofibrillar protein synthesis (MPS) and phosphorylation of signalling proteins important in regulating mRNA translation. Eight young men (20 ± 1.1 years, BMI = 26 ± 3.5 kg m^{-2}) completed two exercise protocols designed to maintain basal hormone concentrations (low hormone, LH) or elicit increases in endogenous hormones (high hormone, HH). In the LH protocol, participants performed a bout of unilateral resistance exercise with the elbow flexors. The HH protocol consisted of the same elbow flexor exercise with the contralateral arm followed immediately by high-volume leg resistance exercise. Participants consumed 25 g of protein after arm exercise to maximize MPS. Muscle biopsies and blood samples were taken as appropriate. There were no changes in serum testosterone, GH or IGF-1 after the LH protocol, whereas there were marked elevations after HH (testosterone, $P < 0.001$; GH, $P < 0.001$; IGF-1, $P < 0.05$). Exercise stimulated a rise in MPS in the biceps brachii (rest = 0.040 ± 0.007, LH = 0.071 ± 0.008, HH = 0.064 ± 0.014% h^{-1}; $P < 0.05$) with no effect of elevated hormones ($P = 0.72$). Phosphorylation of the 70 kDa S6 protein kinase (p70S6K) also increased post-exercise ($P < 0.05$) with no differences between conditions. We conclude that the transient increases in endogenous purportedly anabolic hormones do not enhance fed-state anabolic signalling or MPS following resistance exercise. Local mechanisms are likely to be of predominant importance for the post-exercise increase in MPS.

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Corresponding author S. M. Phillips: Exercise Metabolism Research Group, Department of Kinesiology, McMaster University, 1280 Main Street West, Hamilton, ON L8S 4K1, Canada. Email: phillis@mcmaster.ca

Abbreviations 10RM, 10 repetition maximum; GH, growth hormone; IGF-1, insulin-like growth factor-1; MPS, myofibrillar protein synthesis.

Introduction

Resistance exercise can acutely increase serum concentrations of hormones such as testosterone, growth hormone (GH), and insulin-like growth factor-1 (IGF-1). The magnitude of this elevation is dependent on the intensity (Kraemer *et al.* 1990), volume (Gotshalk *et al.* 1997), rest interval (Kraemer *et al.* 1987) and exercising muscle mass (Kraemer & Ratamess, 2005) of the resistance exercise bout. Simply, exercising a large muscle mass at a moderate–high intensity and with high volume and short rest intervals leads to greater rises in GH, IGF-1 and testosterone. Given the known influence of anabolic hormones during development (Mauras, 2001), it has been suggested that the acute response of the neuroendocrine system to resistance exercise is of primary importance in remodelling skeletal muscle proteins and ultimately in regulating muscle hypertrophy (Kraemer & Ratamess, 2005). However, even though the hormonal responses to resistance exercise programme variables have been well characterized (Kraemer *et al.* 1990), the influence of exercise-induced hormone response

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to muscle protein synthesis has not yet been directly quantified.

Muscle protein synthesis, in particular the synthesis of the myofibrillar protein fraction (Moore *et al.* 2009*b*), is elevated in response to resistance exercise (Phillips *et al.* 1997). Repeated bouts of resistance exercise, especially when combined with high quality protein ingestion, result in small net accretions in muscle protein that summate over time to produce muscle hypertrophy (Phillips, 2004; Rennie *et al.* 2004). Importantly, the acute muscle protein synthetic response to resistance exercise is often predictive of adaptations to chronic resistance training (Hartman *et al.* 2007; Wilkinson *et al.* 2007). The activation (indicated by phosphorylation) of intracellular signalling proteins involved in the phases of mRNA translation initiation and elongation, while not necessarily linearly related to muscle protein synthesis (Greenhaff *et al.* 2008), is thought to be the primary site of regulation (Kimball *et al.* 2002); this appears to be especially relevant for the exercise-induced activation of p70S6K (Terzis *et al.* 2008). The Janus kinase (JAK)–signal transducers and activator of transcription (STAT) pathway mediates signal transduction of GH-regulated genes (Nielsen *et al.* 2008); it is not known whether these proteins are responsive to exercise-induced increases in GH.

Therefore, our aim was to test the hypothesis that a greater exercise-induced hormonal response would enhance MPS and the phosphorylation of key intracellular signalling proteins involved in translation initiation following an acute bout of resistance exercise. We used a unilateral protocol and manipulated the amount of active muscle mass to create both a low hormone (LH) condition using arm only exercise and a high hormone (HH) condition using arm and leg exercise within the same subject. This methodology probably represents the optimal design for assessing the physiological response to endogenous hormonal stimuli within a single individual. Thus we are measuring the stimulus posed by relatively transient resistance exercise-induced hormone elevations to MPS in contrast to the stimulus posed by persistant supraphysiological hormone levels that can be achieved through exogenous administration. We also performed the study in the fed state since this is the condition in which muscle protein balance is positive (Biolo *et al.* 1997) and when any elevated concentrations of endogenous hormones would be most likely to affect signalling and MPS.

Methods

Subjects

Eight healthy young men (20.0 ± 1.1 years, 1.79 ± 0.03 m, 84.1 ± 4.1 kg; means ± S.E.M.) volunteered to participate in the study after being informed of the procedures and potential risks involved in the investigation. Subjects were recreationally active with no formal weight-lifting experience or regular weight-lifting activity over the last year. The protocol was approved by the Research Ethics Board of Hamilton Health Sciences and McMaster University and was written in accordance with standards set by the *Declaration of Helsinki*.

Experimental design

Participants completed two infusion trials on separate days. In one trial, participants performed single arm (unilateral) cable 'preacher' curl exercises aimed exclusively at activating the biceps muscle. In the other trial, participants performed the same single arm exercise with their contralateral arm, followed immediately by a bout of high volume intense heavy leg exercise using short rest intervals designed to elicit a large increase in systemic hormones. Trial order and arm dominance were taken into account and trials were performed in a randomized counter-balanced fashion. Participants underwent strength testing at least 1 week in advance of the first infusion trial to determine an appropriate load for each exercise (e.g. 10 repetition maximum (10RM)). Exercise with arm alone consisted of four sets of 10 repetitions at a load that was ∼95% of their 10RM such that voluntary failure occurred during the final set. To elicit a large hormonal response, the same arm exercise was performed in the contralateral arm followed by five sets of 10 repetitions of leg press at ∼90% of 10RM and three sets of 12 repetitions of leg extension/leg curl 'supersets' (1 set of each back-to-back with no rest between sets). Between-set rest intervals for arm exercise and leg exercise were 120 s and 60 s, respectively. In pilot work from our lab we determined that the volume and intensity of the leg workout elicited a large increase in GH, IGF-1 and testosterone that was comparable to other studies

Infusion protocol

On the trial day, participants reported to the lab after an overnight fast and having refrained from any strenuous physical activity for at least 3 days. A 20-gauge plastic catheter was inserted into an antecubital vein of the non-exercising arm and a baseline blood sample was obtained. Following the start of a primed constant infusion of L-[*ring*-$^{13}C_6$]phenylalanine (prime: 2 μmol kg^{-1}; 0.05 μmol kg^{-1} min^{-1}), participants began their exercise protocol. Due to the differing durations of the two protocols (∼10 min for LH and ∼30 min for HH), participants completing the combined protocol began exercising immediately following the start of their isotope infusion whereas participants completing the arm only protocol sat quietly for 20 min while being infused

and then began their exercise. Immediately following the completion of the exercise protocol, participants had a second catheter inserted into an antecubital vein of the exercised arm that was used to sample blood for the remainder of the trial. Participants were weighed on the morning of each experiment trial and infusion rates were adjusted accordingly to maintain an infusion rate at 0.05 μmol L-[*ring*-$^{13}C_6$]phenylalanine kg^{-1} min^{-1}. Following exercise, the participants rested comfortably on a bed for the remainder of the infusion.

All participants ingested a bolus dose of 25 g of whey protein after arm exercise in both trials that was enriched to 6% with tracer to minimize disturbances in isotope equilibrium. The whey protein drink served as a typical post-workout protein supplement and provided essential amino acids as the substrate for the muscle protein synthetic response to exercise. Blood samples were processed as previously described (Moore *et al.* 2009*a*). A muscle biopsy (~80 mg) was taken from the biceps brachii of each arm 240 min post-exercise. Muscle biopsies were performed with a Bergström needle that was custom-modified for manual suction under local anaesthesia (2% xylocaine). Biopsy samples were blotted and freed of any visible fat and connective tissue, frozen in liquid nitrogen (within ~20 s of being taken from the muscle) and stored at −80°C until further analysis.

Analyses

Blood samples were analysed for serum cortisol, testosterone, GH and IGF-1 at the Core Laboratory of McMaster University Medical Centre. All intra-assay coefficients of variation for these hormones were below 5% and all assays included standards and daily quality assurance procedures. Blood amino acids were analysed by HPLC as previously described (Moore *et al.* 2005). Plasma insulin was measured using a commercially available immunoassay kit (ALPCO Diagnostics, Salem, NH, USA) and blood glucose was measured using a standard spectrophotometric kit (Stanbio Laboratory, Boerne, TX, USA). Lactate was measured on neutralized, deproteinized whole blood using an enzymatic–colorimetric assay kit (Pointe Scientific, Inc., Canton, MI, USA).

As described previously (Moore *et al.* 2009*b*), approximately 20 mg of wet muscle was used to isolate free intracellular amino acids and the mixed-muscle protein bound fraction. A separate piece of muscle (~30 mg) was used to isolate, hydrolyse, purify, derivatize and analyse the myofibrillar protein fraction.

Western blots

Western blots for JAK2 and STAT3 proteins were conducted as follows. Muscle was homogenized with a glass pestle on ice in buffer (20 mM Tris-HCl, 1 mM Na_3VO_4, 50 mM NaF, 40 mM β-glycerolphosphate, 20 mM sodium pyrphosphate, 0.5% Triton X-100, 2 complete mini Roche protease inhibitor tabs, pH 7.2). Protein content was determined by the Bradford assay (Thermo Fisher Scientific, Rockford, IL, USA). Equal amounts of protein (75 μg) were separated on a 7.5% gel and transferred to a polyvinyl difluoride (PVDF) membrane (Millipore, Etobicoke, Canada). Membranes were blocked in 5% BSA and incubated with primary antibody (p-JAK2 Tyr1007/1008 1 : 500, p-STAT3 Tyr705 1 : 1000; Cell Signaling, Danvers, MA, USA) overnight at 4°C. After washing, membranes were incubated with secondary antibody (goat anti-rabbit HRP, 1 : 50,000; Abcam Inc., Cambridge, MA, USA). Phosphorylated protein levels were detected with ECL (SuperSignal West Dura; Thermo Fisher Scientific) and Alpha Innotech FluorChem SP (Alpha Innotech Corp., San Leandro, CA, USA). Membranes were stripped with Restore Western Blot Stripping Buffer (Thermo Fisher Scientific), re-probed with primary antibody (JAK2 D2E12 1 : 500, STAT3 1 : 1000) overnight and incubated with secondary antibody. Total protein detection was conducted as phosphorylated protein detection and quantified using AlphaEase FC Software, v. 5.0.2 (Alpha Innotech Corp.). The ratio of phosphorylated to total protein levels is presented. Western blotting of the remaining proteins was conducted as follows. Muscle samples were homogenized in ice-cold extraction buffer (10 μl mg^{-1}) containing 50 mM Tris-HCl (pH 7.4), 0.1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 0.1% 2-mercaptoethanol, 10 mM β-glycerophosphate, 50 mM NaF, 0.5 mM activated sodium orthovanadate (all chemicals from Sigma-Aldrich, Poole, UK) and a complete protease inhibitor cocktail tablet (Roche, West Sussex, UK). Homogenates were rotated on a vibrating platform for 10 min at 4°C, centrifuged at 10 000 *g* for 10 min at 4°C, before recovery of supernatants representing sarcoplasmic fractions. Bradford assays were used to determine sarcoplasmic protein concentrations after which samples were standardized to 1 mg ml^{-1} by dilution with Laemmli loading buffer in order to measure relative phosphorylated protein concentrations of elongation factor eEF2 Thr56, p70S6K Thr389, eukaryotic initiation factor 4E binding protein 1 (4EBP1) Thr37/46, acetyl-CoA carboxylase β (ACC-β) Ser729, Akt Ser473, proline-rich Akt substrate of 40 kilodaltons (PRAS40) Ser246 (New England Biolabs, Ipswich, MA, USA), and α-actin (Sigma-Aldrich). Samples were mixed and heated at 95°C for 7 min before 15 μg of protein per lane was loaded onto Criterion XT Bis-Tris 12% SDS-PAGE gels (Bio-Rad, Hemel Hempstead, UK) for electrophoresis at 200 V for ~60 min. Gels were equilibrated in transfer buffer (25 mM Tris, 192 mM glycine, 10% methanol) for 30 min before proteins were electroblotted onto 0.2 μm PVDF membranes (Bio-Rad) at 100 V for 30 min. After blocking with 5% low-fat milk

Table 1. Plasma amino acid, glucose and insulin concentrations

Time (min)	Pre	0	15	30	60	90	120	180	240
Glucose (mM)	4.8 ± 0.1	4.9 ± 0.2	4.8 ± 0.2	4.9 ± 0.3	4.8 ± 0.2	4.4 ± 0.3	4.5 ± 0.1	4.7 ± 0.1	4.6 ± 0.2
Insulin (μIU ml^{-1})	6.1 ± 0.6^{a}	8.5 ± 0.8^{a}	8.0 ± 0.5^{a}	7.4 ± 0.6^{a}	6.4 ± 0.4^{a}	20.0 ± 2.7^{b}	15.9 ± 1.4^{b}	6.4 ± 0.7^{a}	4.8 ± 0.6^{a}
$\sum$EAA (μm)	568 ± 102^{a}	578 ± 92^{a}	536 ± 78^{a}	579 ± 106^{a}	525 ± 59^{a}	838 ± 115^{bc}	963 ± 142^{bd}	799 ± 84^{bc}	658 ± 63^{ac}
$\sum$BCAA (μm)	316 ± 55^{ae}	304 ± 50^{a}	285 ± 44^{a}	317 ± 54^{ae}	262 ± 30^{a}	476 ± 60^{bd}	584 ± 80^{bc}	444 ± 46^{de}	368 ± 35^{ae}

Glucose and insulin: there was a main effect for time and condition for insulin, but no significant interaction; therefore values are means $\pm$ S.E.M. across LH and HH conditions. Means with different letters are significantly different from each other ($P < 0.001$). Amino Acids: there was no significant difference between conditions; therefore values are means $\pm$ S.E.M (in μM) for average essential amino acid (EAA) and branched-chain amino acid (BCAA) concentrations across LH and HH conditions. There was a main effect for time for EAA and BCAA ($P < 0.001$) where means with different letters are significantly different from each other ($P < 0.05$). Note: 25 g of protein were consumed at 60 min.

in TBS-T (Tris-buffered saline and 0.1% Tween-20, both Sigma-Aldrich) for 1 h, membranes were rotated overnight with primary antibody against the aforementioned targets at a concentration of 1 : 2000 at 4°C. Membranes were washed (3×5 min) with TBS-T and incubated for 60 min at room temperature with horseradish peroxidase (HRP)-conjugated anti-rabbit secondary antibody (New England Biolabs, Ipswich, MA, USA) before further washing (3×5 min) with TBS-T and incubation for 5 min with enhanced chemiluminescence (ECL) reagents (Immunstar kit; Bio-Rad). Blots were imaged and quantified by assessing peak density after ensuring bands were within the linear range of detection using the Chemidoc XRS system (Bio-Rad). Phosphorylation of signalling proteins was corrected for loading to α-actin.

Calculations

The rate of myofibrillar protein synthesis was calculated using the standard precursor–product method:

$$\text{FSR}\,(\%\,\text{h}^{-1}) = [E_{2\text{b}} - E_{1\text{b}}/E_{\text{ic}}t^{-1}] \times 100$$

where $E_{2\text{b}}$ and $E_{1\text{b}}$ are the bound protein enrichments from muscle at time 2 ($E_{2\text{b}}$) and plasma proteins or the previous muscle biopsy at time 1 (i.e. baseline; $E_{1\text{b}}$), and thus their difference is the change in bound protein enrichment between two time points; E_{ic} is the intracellular phenylalanine enrichment; and t is the tracer incorporation time. Since the number of biopsies we could ethically take from the relatively small biceps brachii muscle was limited and the basal enrichment of the heavy [*ring*-$^{13}C_6$]phenylalanine isotope in body proteins of 'tracer naive' subjects would have been undetectable, we used a mixed plasma protein fraction as the baseline enrichment from a pre-infusion blood sample in the first trial. In doing so we made an assumption that these subjects would have an m+6 phenylalanine enrichment of virtually zero and that would be equivalent in muscle, enrichment equal to that of the tracer and blood; this is an assumption that we have confirmed in our laboratory. The enrichment obtained from the pool of all plasma proteins therefore represents a basal measure of isotopic enrichment for m+6 from which the enriched measurement can be taken. The muscle protein enrichment from the resting arm in the first trial (which would then become the exercised arm in the subsequent trial) was subsequently used as the baseline enrichment in the second trial. We have used this approach previously and shown it to be robust against taking a subsequent biopsy in the second trial for baseline enrichment (Tang *et al.* 2007).

Statistics

This study was a within-subject repeated measures design. Blood analytes were analysed using two factor (time $\times$ condition) repeated measures analysis of variance (ANOVA) statistics. MPS and signalling protein data were analysed using one factor (condition) repeated measures ANOVA statistics. Tukey's honestly significant different *post hoc* test was used to determine differences between individual values if a significant main effect or interaction was detected in the ANOVA ($P < 0.05$). Values are expressed as means $\pm$ S.E.M.

Results

Blood amino acid concentrations

There was a main effect for time for branched-chain amino acids (BCAA) and essential amino acids (EAA) ($P < 0.001$). Protein ingestion 60 min post-exercise stimulated a rise in EAA and BCAA concentrations that were elevated at 90 min, peaked at 120 min and returned to basal levels by 240 min post-exercise. There were no differences between conditions. Table 1 shows the average BCAA and EAA concentrations from the two trials.

Blood glucose and plasma insulin

Fasted blood glucose was 4.5 ± 0.1 mM at rest and did not differ between conditions (Table 1). Fasted

plasma insulin concentration was 6.1 ± 0.4 μIU ml^{-1} and increased by ~230% 90 min post-exercise and returned to resting values by 180 min. There were significant main effects for condition (HH > LH, $P < 0.05$) and time ($P < 0.001$), but the interaction did not reach significance ($P > 0.05$).

Serum testosterone, cortisol, growth hormone, insulin-like growth factor-1 and blood lactate

The HH condition produced a large rise in blood lactate concentration (~9-fold increase) that was significantly greater than LH ($P < 0.001$) for 60 min post-exercise (Fig. 1*A*). The LH condition elicited low GH and IGF-1

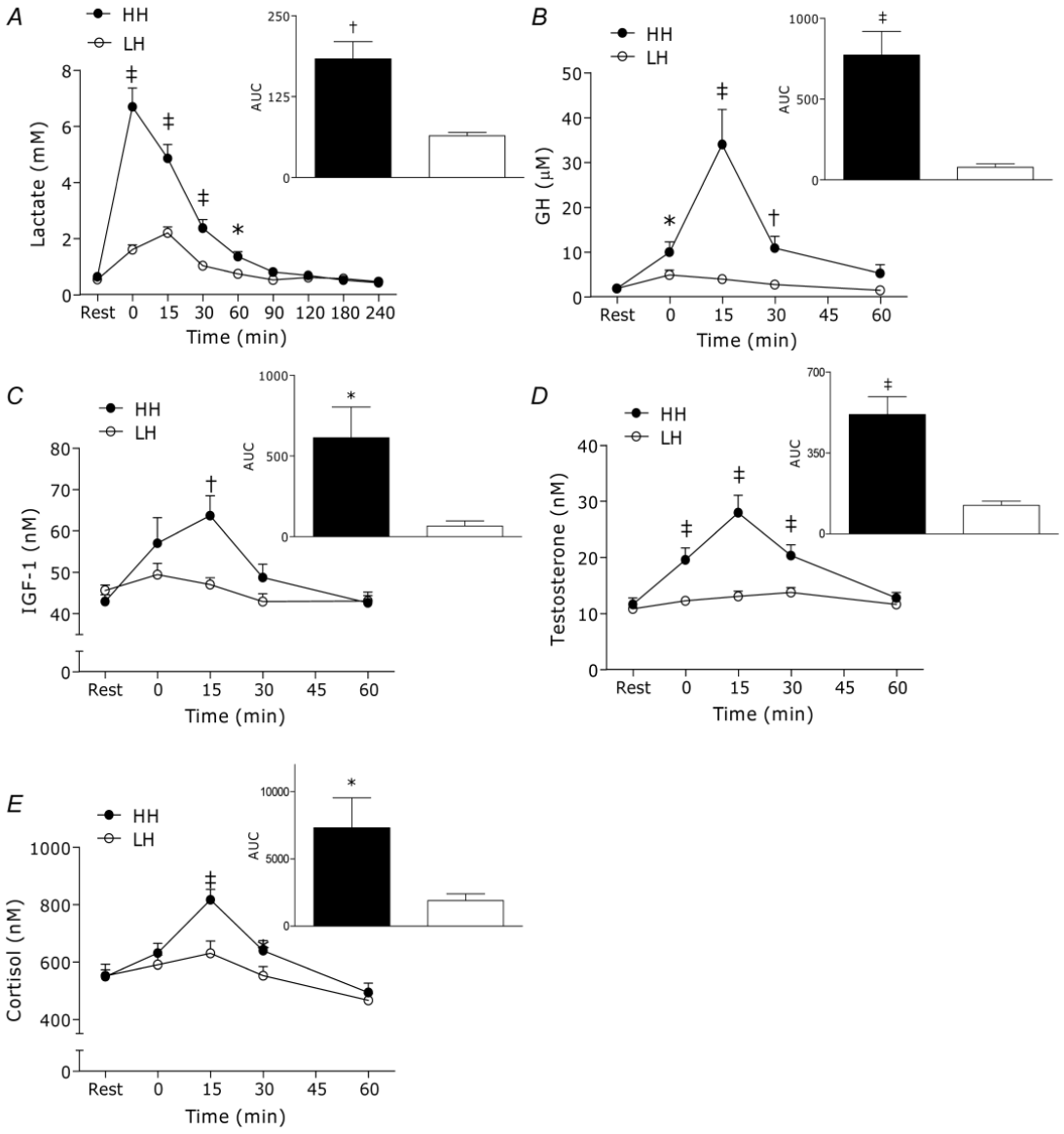

Figure 1. Whole blood lactate (*A*) and serum GH (*B*), IGF-1 (*C*) testosterone (*D*) and cortisol (*E*) concentrations at rest and after LH and HH exercise protocols
Inset: net area under the curve (rest = 0); filled bars – HH, open bars – LH. HH significantly greater than LH for corresponding time points and for AUC, *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$. Values are means ± S.E.M.

responses that were not different from baseline whereas the HH condition produced significantly greater GH and IGF-1 (GH: ∼8-fold greater at peak, $P < 0.001$, Fig. 1*B*; IGF-1: ∼1.3-fold greater at peak, $P < 0.05$, Fig. 1*C*). Testosterone and cortisol concentrations did not change from baseline following LH, but were significantly elevated after HH (main effects for condition: testosterone, $P < 0.001$, Fig. 1*D*; cortisol, $P < 0.05$, Fig. 1*E*).

Plasma and muscle intracellular free phenylalanine enrichment

Resting, LH and HH precursor phenylalanine enrichments were as follows: 3.8 ± 0.4, 3.7 ± 0.4, 4.1 ± 0.3 (tracer/tracee), respectively; $P > 0.05$. Plasma enrichments at 60, 120 and 180 min were 6.1 ± 0.2, 6.1 ± 0.2 and 6.7 ± 0.2, respectively. Linear regression analysis indicated that the slopes of the plasma enrichments were not significantly different from zero ($P > 0.05$), suggesting that isotopic plateau was achieved and that the use of the steady-state precursor product equation is appropriate.

Myofibrillar protein synthesis

Elbow flexor exercise elevated MPS by 78% (LH) and 61% (HH) ($P < 0.05$) with no difference between conditions ($P = 0.72$) (Fig. 2). Similar results were obtained for mixed muscle protein synthesis (see online Supplemental Material). There was no effect of trial order on MPS ($P > 0.05$). There was also no difference in resting MPS between conditions ($P > 0.05$).

Signalling proteins

JAK2 phosphorylation was not affected by exercise or by elevated hormone concentrations induced by the HH condition (Fig. 3*A*). There was a trend toward increased STAT3 phosphorylation in response to exercise ($P = 0.09$), but with no additive effect of elevated hormones (Fig. 3*B*). Phosphorylation of p70S6K increased ($P < 0.05$, Fig. 3*C*), and there was a trend toward decreased phosphorylation of eEF2 ($P = 0.09$, Fig. 3*D*), in response to exercise but there were no differences between conditions. The phosphorylation status of ACC-β, PRAS40, Akt and 4EBP1 was not different from rest after either exercise protocol or from each other (see Supplemental Material).

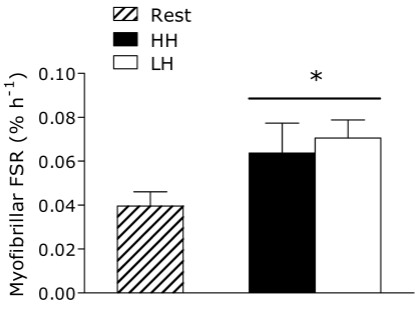

Figure 2. Rate of myofibrillar protein synthesis in the fed state at rest and following LH and HH exercise protocols
*Significantly different from rest, $P < 0.05$. Values are means ± S.E.M.

Discussion

We report here that, despite being exposed to substantial differences in purportedly anabolic hormones such as testosterone, GH, and IGF-1, the rate of MPS in identically exercised muscles was not different. These data demonstrate that local factors are paramount in determining not only the signalling pathway activation but also the response of MPS. Furthermore, our results indicate that increases in MPS are able to occur without increases in systemic anabolic hormone concentrations and are not enhanced by the acute elevation that can follow resistance exercise; this finding is in agreement with previous work from our lab showing that increases in circulating hormones are not necessary for hypertrophy (Wilkinson *et al.* 2006). If our results are broadly applicable and generally accepted models of protein accretion in humans are correct (Phillips, 2004; Rennie *et al.* 2004), then our results also bring into question the posited role of anabolic hormones, which are important during growth and development, in the process of muscular hypertrophy in adults (Kraemer & Ratamess, 2005). However, we do not know if or how the hormonal elevations may have affected muscle protein balance as a whole since we did not measure muscle protein breakdown.

The present study was conducted in the fed state. Participants consumed a whey protein supplement 1 h after exercise in an attempt to provide a substrate for any potential divergent signalling or synthetic responses initiated by the two different hormone environments. It is possible that the synthetic response from the protein dominated any potential subtle differences in MPS. In our minds, especially from an applied standpoint, this only emphasizes the importance of consuming a high-quality protein after resistance exercise rather than designing exercise regimes around the exercise-induced hormone response.

Arm exercise was selected to precede leg exercise so that there would be no possible confounding influence of central fatigue (i.e. participants would be able to exert maximal effort during arm exercise in both conditions without any residual fatigue from the leg exercises). We can only speculate, but we do not anticipate that our results would have changed significantly had leg

exercise preceded the arm exercise unless the muscle was somehow responsive to hormones at some critical threshold during contraction or immediately thereafter (presumably, the hormones would have peaked near the end of the arm exercise or closer to the zero time point). Recently, we measured the hormone responses to the same exercise protocols used in the present study before and after 15 weeks of training (unpublished data). While the responses between conditions were very different (like the present study), the responses within each condition across time were very similar (i.e. a divergent hormone profile was maintained). We are not aware of any data that show that training alters the synthetic sensitivity of the skeletal muscle to exercise-induced hormonal increases.

If hormones were driving increments in protein synthesis, we should expect to see an association between the magnitude of the exercise-induced hormone response and the acute MPS or signalling responses. In contrast, we found no enhancement of MPS or phosphorylation of key signalling proteins. Our data suggest that exercise-induced local mechanisms, rather than increases in serum hormone availability, are the activators of STAT3, p70S6K and eEF2 signalling and the subsequent acute elevation of MPS. Two studies, one in rodents (Baar & Esser, 1999) and the other in humans (Terzis *et al.* 2008), report that hypertrophy is strongly related to increases in phosphorylation of p70S6K; this is in agreement with the notion that signal activation initiates an increase in muscle protein synthesis which may lead to hypertrophy with training. The extent of p70S6K phosphorylation is also related to the extent of MPS 1–2 h after resistance exercise in young individuals (Kumar *et al.* 2009).

The underlying mechanisms affecting increments in hormone concentration were not measured in the present study, but the differential hormone response may be due to differences in hormone production/release (Kraemer, 2000), clearance (Kraemer, 2000) as well as shifts in plasma volume (Ploutz-Snyder *et al.* 1995). The ~9-fold

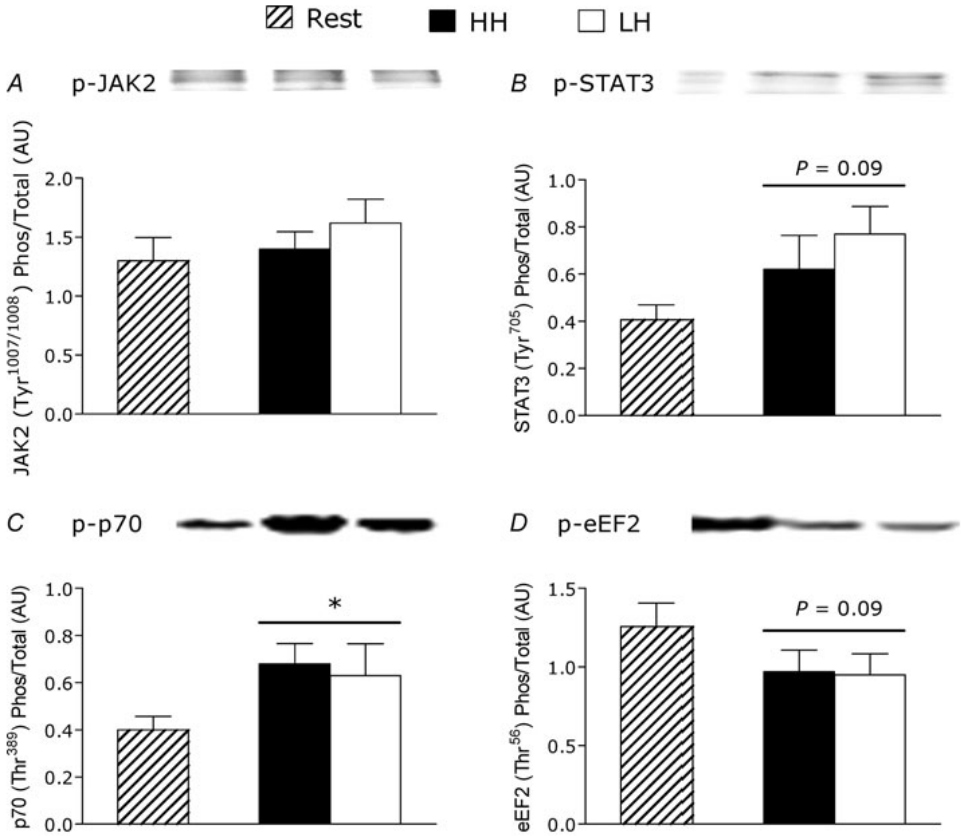

Figure 3. Phosphorylated to total protein ratio of JAK2 (*A*), STAT3 (*B*), p70S6K (*C*) and eEF2 (*D*) at rest and after LH and HH exercise protocols
*Significantly different from rest, $P < 0.05$. Values are means ± S.E.M.

increase in lactate in HH provides indirect support for the stimulatory action of lactate on GH (Godfrey *et al.* 2003) and testosterone (Lu *et al.* 1997).

In summary, transient resistance exercise-induced increases in endogenous purportedly anabolic hormones did not enhance anabolic signalling or the acute (4 h) post-exercise MPS response in the fed state. Post-exercise increases in these hormones cannot be used as proxy markers for hypertrophic potential in human skeletal muscle. In contrast, local mechanisms appear to be predominant in the acute post-exercise MPS response.

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Author contributions

D.W.D.W., G.W.K., D.R.M., J.E.T. and S.M.P. planned the study; D.W.D.W., G.W.K., N.A.B., J.P.P., S.K.B. and S.M.P. collected data; D.W.D.W., G.W.K., D.R.M., P.A., N.A.B., J.P.P., M.D. and S.M.P. analysed data; D.W.D.W., G.W.K., D.R.M., N.A.B., G.P., M.J.R., S.K.B. and S.M.P. wrote and edited the manuscript. M.J.R. and S.M.P. raised funds. None of the authors have a financial or personal conflict of interest to declare.

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CHAPTER 3

TITLE: Elevations in ostensibly anabolic hormones with resistance exercise enhance neither training-induced muscle hypertrophy nor strength of the elbow flexors

AUTHORS: West DW, Burd NA, Tang JE, Moore DR, Staples AW, Holwerda AM, Baker SK, & Phillips SM

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Elevations in ostensibly anabolic hormones with resistance exercise enhance neither training-induced muscle hypertrophy nor strength of the elbow flexors

Daniel W. D. West,[1] Nicholas A. Burd,[1] Jason E. Tang,[1] Daniel R. Moore,[1] Aaron W. Staples,[1] Andrew M. Holwerda,[1] Steven K. Baker,[2] and Stuart M. Phillips[1]

[1]*Exercise Metabolism Research Group, Department of Kinesiology; and* [2]*Department of Medicine, McMaster University, Hamilton, Ontario, Canada*

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West DW, Burd NA, Tang JE, Moore DR, Staples AW, Holwerda AM, Baker SK, Phillips SM. Elevations in ostensibly anabolic hormones with resistance exercise enhance neither training-induced muscle hypertrophy nor strength of the elbow flexors. *J Appl Physiol* 108: 60–67, 2010. First published November 12, 2009; doi:10.1152/japplphysiol.01147.2009.—The aim of our study was to determine whether resistance exercise-induced elevations in endogenous hormones enhance muscle strength and hypertrophy with training. Twelve healthy young men (21.8 ± 1.2 yr, body mass index = 23.1 ± 0.6 kg/m^2) trained their elbow flexors independently for 15 wk on separate days and under different hormonal milieu. In one training condition, participants performed isolated arm curl exercise designed to maintain basal hormone concentrations (low hormone, LH); in the other training condition, participants performed identical arm exercise to the LH condition followed immediately by a high volume of leg resistance exercise to elicit a large increase in endogenous hormones (high hormone, HH). There was no elevation in serum growth hormone (GH), insulin-like growth factor (IGF-1), or testosterone after the LH protocol but significant ($P < 0.001$) elevations in these hormones immediately and 15 and 30 min after the HH protocol. The hormone responses elicited by each respective exercise protocol late in the training period were similar to the response elicited early in the training period, indicating that a divergent postexercise hormone response was maintained over the training period. Muscle cross-sectional area (CSA) increased by 12% in LH and 10% in HH ($P < 0.001$) with no difference between conditions (condition × training interaction, $P = 0.25$). Similarly, type I ($P < 0.01$) and type II ($P < 0.001$) muscle fiber CSA increased with training with no effect of hormone elevation in the HH condition. Strength increased in both arms, but the increase was not different between the LH and HH conditions. We conclude that exposure of loaded muscle to acute exercise-induced elevations in endogenous anabolic hormones enhances neither muscle hypertrophy nor strength with resistance training in young men.

testosterone; growth hormone; insulin-like growth factor-1; anabolism

HORMONES such as testosterone, growth hormone (GH), and insulin-like growth factor (IGF-1) are important for skeletal muscle anabolism during growth and development (e.g., 23). It has been suggested that acute elevation of this triumvirate of hormones that occurs after performance of intense resistance exercise is a significant contributor to the gains in strength and hypertrophy that are observed with resistance training (14, 19, 24, 30). The magnitude of the increase in concentration of these hormones is largely dependent on parameters of the resistance exercise. Specifically, large elevations of systemic hormones are observed acutely postexercise after resistance exercise bouts consisting of a high amount of work (8, 9, 28). Additionally, higher intensity exercise (16, 19) with short rest intervals (4, 17) that is performed with large muscle groups (10, 19) are also associated with large rises in these hormones. In fact, training principles have been constructed to maximize the postexercise rise in these hormones based on the interpretation that exercise-induced increases in systemic hormones like testosterone and GH will enhance muscle hypertrophy (15, 27, 31). Specifically, it has been suggested that small exercising muscle groups (e.g., biceps brachii), which are incapable of inducing large increases in systemic anabolic hormones when used in isolation, should be trained concurrently with large exercising muscle masses that can elevate testosterone and GH (10, 19). However, the hypothesis that exercise-induced rises in testosterone and GH enhance increases in strength and muscle hypertrophy with training remains untested.

We have previously demonstrated that acute changes in muscle protein synthesis following resistance exercise and feeding (38) can qualitatively predict gains in muscle fiber cross-sectional area (CSA) and lean body mass (11). Recently, we have shown that exercise-induced hormones that are hypothesized to be anabolic and affect hypertrophy do not acutely enhance fed-state myofibrillar protein synthesis after acute resistance exercise (36). Despite the ability of the postexercise protein synthetic response (38) to predict phenotypic adaptations to training (11), it is unknown whether the synthetic responses that we observed after a single acute bout of exercise (36) would ultimately translate into similar increases in muscle size and strength when hormone availability is manipulated following repeated bouts of resistance exercise (i.e., training). Thus the aim of our present study was to determine if increases in muscle strength and hypertrophy are enhanced by exercise-induced increases in hormone availability with resistance training. Based on our previous acute finding that myofibrillar protein synthesis was not enhanced by acute elevations in circulating testosterone, GH, and IGF-1, we hypothesized that repeatedly elevating the acute postexercise availability of these hormones would not enhance the muscle hypertrophy and strength gains achieved from a progressive resistance training program. Previous work demonstrating an effect of elevated endogenous hormones on gains in isometric strength has used a similar design (10) . Thus, as a reasonable proof of principle study, if acute rises in GH, IGF-1, and testosterone were to result in differential phenotypic changes, then an inherently superior design for testing this thesis is a unilateral within-person design, where interindividual differences in potential for hypertrophy and strength gains, which are substantial (12), are minimized.

Address for reprint requests and other correspondence: S. M. Phillips, Exercise Metabolism Research Group, Dept. of Kinesiology McMaster Univ. 1280 Main St. West, Hamilton, ON, L8S 4K1 Canada (e-mail: phillis@mcmaster.ca).

METHODS

Subjects. Twelve healthy young men (21.8 ± 0.4 yr, 1.78 ± 0.02 m, 74.1 ± 3.3 kg; means ± SE) volunteered to participate in the study after being informed of the procedures and potential risks involved in the investigation. Subjects were recreationally active with no formal weightlifting experience. Participants provided consent to an agreement that was approved by the Research Ethics Board of Hamilton Health Sciences and that was written in accordance with standards set by the Declaration of Helsinki.

Experimental protocol. Using a within-person design, participants trained each arm on separate days under two different hormonal environments for 15 wk. In the low hormone condition (LH), one arm performed arm curl exercise only, while in the high hormone condition (HH) the contralateral arm performed the same arm curl exercise followed immediately by a bout of leg resistance exercises designed to elicit large increases in circulating hormones. In *weeks 1–6*, participants trained each arm three times over 2 wk; they trained in a manner that allowed 72 h between LH and HH training days (e.g., *week 1*: Monday*, Tuesday, Friday*; *week 2*: Monday, Thursday*, Friday; HH session days shown with asterisk). This approach was taken to ensure that the enhanced muscle protein synthetic response, which can be elevated in the untrained state for ~48 h (22, 26), occurred exclusively on the background of basal hormone concentrations during LH before the hormonal spike that was associated with exercise on the HH day 72 h later. In *weeks 7–15*, an extra training session was added to enhance the training stimulus so that each arm trained twice per week with at least 48 h following each arm-only trial (e.g., *week 7*: Monday*, Tuesday, Thursday*, Friday; HH session days shown with asterisk). We have previously shown that resistance training shortens the duration (i.e., <28 h) for which muscle protein synthesis is elevated after exercise (33). Therefore, despite the inclusion of an additional training session per week, the attenuated time course of muscle protein synthesis after exercise in a more trained state would still have provided enough recovery to ensure that the LH arm was not exposed to the hormonal milieu of HH during the skeletal muscle remodeling process. Participants consumed 18 g of whey protein immediately before exercise and 18 g at 90 min after arm exercise in each condition to support maximal rates of muscle protein synthesis both in the absence and presence of elevated hormone concentrations as well as to reduce variability in nutrition surrounding the exercise bout.

Training. Subjects were familiarized with each exercise before strength testing and beginning the training program. Exercise in LH consisted of three to four sets of 8–12 repetitions at a load that was ~95% of their 10-repetition maximum (RM) such that voluntary failure occurred during the final set. Exercise in HH was performed in the contralateral arm and consisted of identical arm exercise to LH but was followed by five sets of 10 repetitions of leg press and three sets of 12 repetitions of leg extension/leg curl "supersets" (1 set of each exercise, back-to-back, with no rest between sets) at ~90% of 10 RM. Between-set rest intervals for arm and leg exercises were 120 and 60 s, respectively. Participants followed a progressive training protocol with respect to the number of sets and repetitions and to the load that was lifted. Each training session was supervised individually, and compliance across 56 sessions (28 per arm) was 96%.

Strength testing. Isotonic 1 and 10 RMs were tested pre- and posttraining for the arm curl and leg exercises using standard procedures. Isometric maximal voluntary contraction (MVC) strength was tested using a custom dynamometer. To isolate the elbow flexors as the sole producers of force, participants were seated with their upper arm abducted in the horizontal plane and with a pad clamped firmly down on the top of their shoulder. The forearm was supinated and tightly fastened using straps on an aluminum plate that was attached to a steel shaft. The participant's arm was positioned at 120° (180° = full extension) with the elbow visually aligned with the axis of rotation. A strain gauge was used to detect torque produced by isometric elbow flexion. The signal from the strain gauge was amplified and filtered (DC to 200-Hz bandwidth) before being digitized (sample rate of 3 kHz; model DI420, Dataq Instruments, Akron, OH) and analyzed with custom LabView 7 Express software (National Instruments, Austin, TX).

Analyses. Blood samples were taken following the third as well as the final training session to characterize the hormonal response elicited by each condition (LH and HH) both early and late in the training period. Blood samples were analyzed for serum cortisol, testosterone, GH, dehydroepiandrosterone sulfate (DHEA-S) and IGF-1 using solid-phase, two-site chemiluminescence immunometric assays (Immulite; Intermedico, Holliston, MA). All intra-assay coefficients of variation for these hormones were below 5%, and all assays included standards and daily quality assurance procedures. Free testosterone was calculated from total testosterone and sex hormone binding globulin (21). Lactate was measured on neutralized deproteinized whole blood using an enzymatic-colorimetric assay kit (Pointe Scientific, Canton, MI). Plasma insulin concentration was determined using Coat-a-Count insulin kits (Diagnostic Products, Los Angeles, CA). Blood amino acids were analyzed by HPLC as previously described (25). Hematocrit was measured in triplicate using standard microcapillary methods. Change in plasma volume was calculated from hemoglobin concentration (cyanmethemoglobin technique, Pointe Scientific) and hematocrit using equations by Dill and Costill (7).

MRI. Participants underwent MRI scans using a 1-T extremity scanner (OrthOne, ONI Medical Systems, Wilmington, MA) at the Centre for Appendicular MRI Studies (Hamilton, ON, Canada). Fluorescent markers were placed over the elbow joint line and 12 cm proximally; coronal and sagittal scout scans were used to locate the markers and center the region of interest in the magnetic field. Participants lay with their arm abducted and their wrist in a neutral position so that their elbow flexors were not compressed. Beginning at the joint line, 16 axial scans were taken to determine elbow flexor CSA as a function of distance from the elbow joint line. Images were acquired using the following parameters: repetition time/echo time, 700 ms/21.8 ms; field of view, 14 cm; phase/frequency, 288; slice thickness, 2 mm; gap, 6 mm. Participants' arms were scanned before the beginning of the training program and ~72 h after the final training bout. Each image was circled three times by the same investigator using National Institutes of Health ImageJ software (version 1.38, Bethesda, MD); average coefficient of variation for measured CSA on repeated traces was 0.6%.

Muscle fiber CSA. Muscle biopsies were performed pre- and posttraining with a 5-mm Bergström needle that was custom modified for manual suction under local anesthesia (2% xylocaine). Muscle fibers were oriented vertically under a dissecting microscope and embedded in optimal cutting temperature (OCT) medium. The mounted muscle was frozen in isopentane cooled by liquid nitrogen and stored at −80°C until processing for CSA analysis. Cross sections (10 μm thick) were cut, mounted on glass slides, and stained using a myofibrillar ATPase histochemisty procedure at an acid preincubation pH of 4.6 to distinguish type I and type II fibers as described previously (13, 32). Pictures of the stained slides were taken using a light microscope and NIS Elements 3.0 Imaging Software (Nikon, NY). An average of 47 type I and 53 type II fibers were circled using ImagePro Plus software (version 4.5.1.22, Media Cybernetics, Bethesda, MD) to quantify CSA. During the course of analysis our freezer malfunctioned, which compromised the quality of some of our samples. Since the study was a within-subject design with pre- and posttraining biopsies, if one or more of four samples (pre-LH and pre-HH; post-LH and post-HH) was of poor quality it prevented us from being able to make within-subject comparisons. Thus we have only included data for subjects ($n = 7$) for whom we could obtain a complete data set from both arms pre- and posttraining.

Statistics. This study was a within-subject repeated-measures design. Blood lactate and serum hormone concentrations were analyzed

using three-factor repeated-measures ANOVA statistics with training (pre and post), condition (LH and HH), and time (preexercise and 0, 15, 30, and 60 min postexercise) as within-subject factors. Two-factor (training × condition) repeated-measures ANOVA was used to analyze area under the curve data; two-factor (time × condition) repeated-measures ANOVA was used to analyze hematocrit and plasma volume change early in the training period. One-factor (time) repeated-measures ANOVA was used to analyze mean insulin and amino acid concentrations across training in each condition. Hypertrophy and strength data were analyzed using two-factor (training × condition)

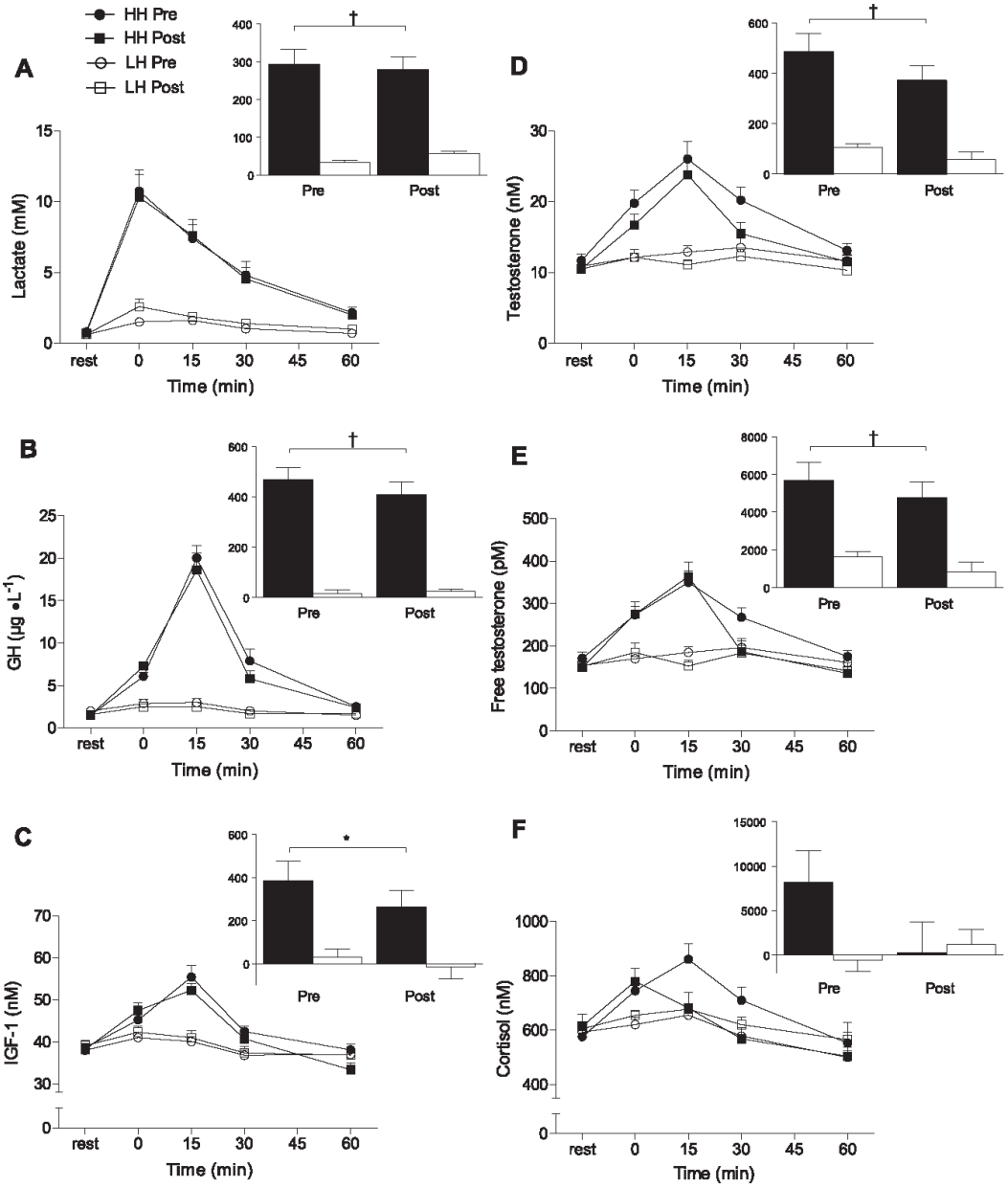

Fig. 1. Whole blood lactate (*A*) and serum growth hormone (GH; *B*), IGF-1 (*C*), total testosterone (*D*), free testosterone (*E*) and cortisol (*F*) concentrations at rest and after low hormone (LH) and high hormone (HH) exercise protocols. *Insets*: net area under the curve (AUC; rest = 0); closed bars, HH; open bars, LH. Significantly greater than LH for corresponding time points and for AUC, $*P < 0.01$, $\dagger P < 0.001$. Values are means ± SE.

Table 1. *Hematocrit and change in plasma volume*

	Pretraining	Time, min			
		0	15	30	60
Hematocrit, %					
HH	43.7 ± 1.2	49.5 ± 1.0‡	46.2 ± 1.6†	44.9 ± 1.3*	45.0 ± 1.2
LH	43.1 ± 0.8	46.0 ± 0.9	43.4 ± 1.2	42.8 ± 1.1	43.4 ± 1.3
Plasma volume, %Δ					
HH		−19.8 ± 1.0†	−9.1 ± 2.2	−5.6 ± 1.4	−5.8 ± 1.9
LH		−10.0 ± 2.8	−4.1 ± 1.2	−3.2 ± 2.2	−4.8 ± 2.1

Values are means ± SE. Hematocrit: there was a greater increase in hematocrit after the high hormone (HH) protocol (condition × time interaction, $P < 0.001$). Different from low hormone (LH) at same time: *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$. Plasma volume: there was a greater decrease in plasma volume after the HH protocol (condition × time interaction, $P < 0.001$). †Different from LH at same time, $P < 0.01$.

repeated-measures ANOVA. Where ANOVA revealed significance ($P < 0.05$), Tukey's post hoc test was used to identify pairwise differences. Values are expressed as means ± SE.

RESULTS

Blood analyses. LH elicited a minimal rise in lactate concentration (~2 mmol/l at peak), whereas HH elicited a marked increase (~10.5 mmol/l at peak); nearly identical responses were elicited before and after training within each condition (Fig. 1*A*). Figure 1, *B–E,* shows the hormone responses of GH, IGF-1, and total and free testosterone, respectively, after each condition during the first and last weeks of training. There was no change in the concentration of any of these hormones after exercise in LH. In contrast, there was a marked increase in GH, IGF-1, and total and free testosterone that peaked ~15 min after HH ($P < 0.001$); this divergent pattern in hormone availability was present both at the beginning and end of 15 wk of training. Cortisol concentrations were similar to basal levels after LH and were markedly elevated after HH; there was an attenuation of the cortisol response after HH at the end of the training period. DHEA-S exhibited a pattern broadly similar to other hormones with a significant rise at 0 and 15 min in HH compared with LH and with no training-induced changes in basal levels or in the pattern of response (data not shown). Hematocrit increased, and plasma volume decreased, to a greater extent after HH compared with after LH (Table 1).

Insulin concentration peaked 30 and 45 min after the preexercise protein drink in LH and HH, respectively (Table 2). Amino acids appeared in the blood at a similar rate. In LH, amino acids reached a plateau and concentrations remained elevated at 75 min postdrink ($P < 0.001$). In HH, amino acids peaked 30 min postdrink, and then declined, before appearing to reach a steady-state above baseline 90 min postdrink ($P < 0.05$; Table 2). Note that the values in Table 2 do not reflect the second 18-g protein drink that was given 90 min after arm exercise in each condition.

Strength. Isometric strength increased 20 ± 4% (range: 3–49%) in LH and 19 ± 3% (range: 2–34%) in HH with training ($P < 0.001$, Fig. 2*A*), but there were no differences between conditions (condition × training interaction, $P = 0.65$). Similarly, 1 RM increased 23 ± 6% (2–56%) in LH and 25 ± 5% (2–53%) in HH ($P < 0.001$, Fig. 2*B*) with no effect of condition ($P = 0.43$). The 10 RM increased 46 ± 3% (33–62%) in LH and 47 ± 6% (20–100%) in HH ($P < 0.001$, Fig. 2*C*), but there were no differences between conditions ($P = 0.63$).

Muscle fiber and elbow flexor CSA. Type I muscle fiber CSA increased 9 ± 3% (0–16%) in LH and 11 ± 4% (0–26%) in HH with training ($P < 0.01$, Fig. 3A) while type II muscle fiber CSA increased 21 ± 4% (8–42%) in LH and 24 ± 6% (9–53%) in HH ($P < 0.001$, Fig. 3B); there were no differences between conditions for either fiber type (condition ×

Table 2. *Plasma insulin and blood amino acid concentrations*

	Pretraining	Time, min					
		15	30	45	60	75	90
Insulin							
HH	7.9 ± 0.7^{a}		9.4 ± 0.9^{ab}	12.5 ± 1.8^{b}	10.3 ± 1.4^{ab}		8.2 ± 0.5^{a}
LH	8.0 ± 0.6^{a}	11.4 ± 1.1^{ab}	15.9 ± 1.8^{c}	12.6 ± 1.1^{bc}		8.7 ± 0.7^{a}	
BCAA							
HH	395 ± 13^{a}		566 ± 26^{c}	510 ± 21^{bc}	452 ± 23^{ab}		487.0 ± 34^{bc}
LH	394 ± 17^{a}	486 ± 24^{ab}	563 ± 36^{bc}	618 ± 39^{c}		608 ± 45^{c}	
EAA							
HH	711 ± 25^{a}		983 ± 47^{c}	884 ± 37^{bc}	798 ± 40^{ab}		885 ± 61^{bc}
LH	728 ± 35^{a}	851 ± 38^{ab}	1,009 ± 62^{bc}	1,114 ± 67^{c}		1,096 ± 755^{c}	
Total AA							
HH	1,958 ± 66^{a}		2,611 ± 122^{c}	2,452 ± 104^{bc}	2,132 ± 150^{ab}		2,356 ± 129^{bc}
LH	1,966 ± 99^{a}	2,206 ± 95^{ab}	2,367 ± 135^{bc}	2,548 ± 161^{bc}		2,494 ± 170^{bc}	

Values are means ± SE in μM for amino acids (AA) and in μIU/ml for insulin. Blood sample time points in this table are referenced to the protein drink that participants consumed immediately before exercise and therefore do not match the time points in the hormone graphs that are referenced to the end of exercise (e.g., Fig. 1). Because the length of the HH exercise bout was ~15 min. longer than the LH bout, blood samples are only available for select time points and were analyzed within condition. For amino acids, there was a main effect for time in each condition for branched chain AA (BCAA), essential AA (EAA), and total AA (all $P < 0.001$) where different superscript letters indicate significantly different means ($P < 0.05$). For insulin, there was a main effect for time in HH and LH ($P < 0.05$ and 0.001, respectively) where different superscript letters indicate significantly different means ($P < 0.05$).

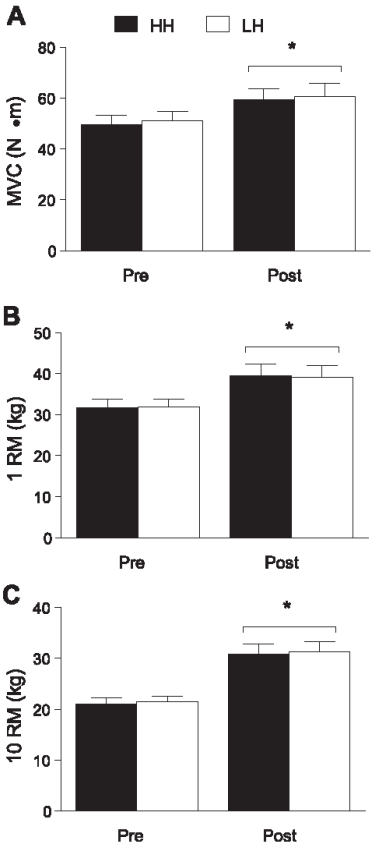

Fig. 2. Maximal voluntary contraction (MVC; *A*), one-repetition maximum (1 RM; *B*), and 10-repetition maximum (10 RM; *C*) before and after training in LH and HH. *Main effect of training, $P < 0.001$; there were no interactions (training $\times$ condition) for any strength measure ($P = 0.65$, 0.43, 0.63 for MVC, 1 RM and 10 RM, respectively). Values are means ± SE.

training interaction, $P \geq 0.55$). Elbow flexor CSA increased 12 ± 2% (3–18%) in LH and 10 ± 2% (4–20%) in HH with training ($P < 0.001$), with no differences between conditions (condition $\times$ training interaction, $P = 0.27$; Fig. 3*C*). Figure 3*D* shows elbow flexor CSA as a function of the distance from the elbow joint line for 12 serial slices for each condition; there were no differences when accounting for the position of the measurement.

DISCUSSION

In the present study, we were able to effectively manipulate endogenous hormone concentrations so that, during the acute postexercise time period when amino acids were readily available for protein synthesis, one arm was repeatedly exposed to marked increases in GH, IGF-1, as well as total and free testosterone concentration. The contralateral arm was exposed to only basal levels of these hormones. Despite vast differences in hormone availability in the immediate postexercise period, we found no differences in the increases in strength or hypertrophy in muscle exercised under low or high hormone conditions after 15 wk of resistance training. These findings are in agreement with our hypothesis and previous work showing that exercise-induced hormone elevations do not stimulate myofibrillar protein synthesis (36) and are not necessary for hypertrophy (37). Thus our data (36 and present observations), when viewed collectively, lead us to conclude that local mechanisms are of far greater relevance in regulating muscle protein accretion occurring with resistance training and that acute changes in hormones, such as GH, IGF-1, and testosterone, do not predict or in any way reflect a capacity for hypertrophy.

We found no additional strength improvements in the arm trained under the HH condition, which is in contrast to a study (10) that is commonly cited (e.g., 14, 18–20, 30, 31) to support the thesis that exercise-induced increases in hormone availability enhance training adaptations. However, the finding of greater isometric strength gains due to physiological hormone elevations in the previous study (10) may have been related to differences in baseline strength between different groups training with either low or high hormone concentrations. In the present study there was no difference between arms in any of our measures of strength before or after training. Moreover, in agreement with our data, Hansen and co-workers (10) reported no effect of exercise-induced rises in hormone levels on dynamic strength gains with training. Since our study was a within-subject design, we can expect that a portion of the increase in strength was due to cross-education effects, i.e., increased strength in the limb that is contralateral to the training limb, which is ~7% of initial strength in the elbow flexors (5). Additionally, any contribution to increased strength due to cross-education would have likely benefited both arms similarly and is a reflection of increased motorneuron output rather than muscular adaptations (6).

In addition to measuring changes in strength, we quantified whole muscle and fiber CSA to determine if exercise-induced elevations in circulating hormones enhances muscle hypertrophy with resistance training. We found no effect of elevated hormones on the degree of muscle hypertrophy measured by either MRI or histochemical staining with 15 wk of training. Instead, our data showed virtually identical increases in both muscle fiber and whole muscle CSA regardless of hormonal condition. These findings agree with the notion that local and not systemic factors are responsible for initiating signaling responses in type I and II fibers in response to resistance exercise (34).

By strictly supervising each session of a progressive training program, we successfully maintained divergent hormone profiles in the acute recovery period after exercise. That is, LH and HH elicited low and high hormone (and lactate) responses, respectively, at both the beginning and through to the end of training. The mechanisms that drive increases in exercise-induced hormones, as well as the biological relevance of exercise-induced hormones, are unclear but are more likely related to metabolic stress and/or fuel mobilization rather than muscle anabolism. It is important to note that the transient physiological increase in hormone availability that can occur after resistance exercise is in contrast to the continued marked elevation that is observed with pharmacological administration, which can have an effect on overall muscle mass depending on the hormone. For example, supraphysiological doses of testosterone are clearly potently anabolic to skeletal muscle (3)

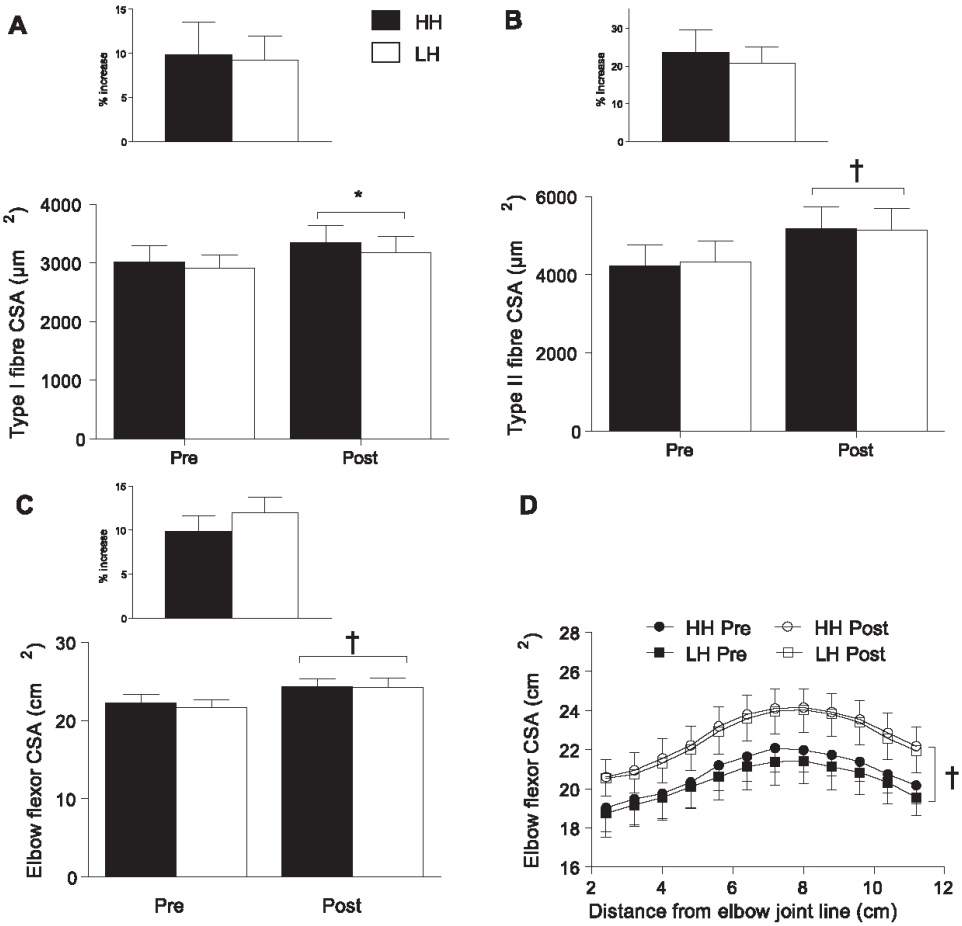

Fig. 3. Type I (*A*) and II fiber (*B*) cross-sectional area (CSA) of the biceps brachii before (Pre) and after (Post) training in LH and HH; main effect of training, $*P < 0.01$, $\dagger P < 0.001$. Elbow flexor CSA before and after training (*C*) in LH and HH; main effect of training, $\dagger P < 0.001$. Elbow flexor CSA as a function of distance from the elbow joint line before and after training (*D*) in LH and HH; main effect of training, $\dagger P < 0.001$. There were no interactions (training × condition) for either fiber or whole muscle CSA (type I, $P = 66$; type II, $P = 0.55$; CSA, $P = 0.27$). Values are means ± SE.

whereas GH supplementation has little effect on the exercise-induced increase in muscle mass (39). Furthermore, there is evidence that a minimal basal level of testosterone is required to support strength and hypertrophy gains, which are otherwise attenuated (20). Therefore, the hormone-sensitive processes that underpin muscle anabolism at hypo- and supraphysiological hormone levels are not being activated appreciably by exercise-induced increases in hormone availability or at least do not result in any measurable enhancement of strength or hypertrophy.

In our view, resistance exercise provides an intrinsic stimulus to the working muscle, which drives hypertrophy, and whereas physiological systemic hormone concentrations may be permissive for the hypertrophic process, exercise-induced elevations do not enhance or in any way predict hypertrophy. Resistance exercise results in the phosphorylation of critically important signaling pathway proteins that are correlated with the extent of muscle hypertrophy in rodents (2) as well as humans (35). These data (2, 35) suggest that local mechanisms within the muscle are of paramount importance in determining muscle hypertrophy. Clearly further research is required to clarify how these mechanisms, possibly in concert with local growth factors (1), combine with other mechanical signals to stimulate muscle protein synthesis and facilitate muscle protein accretion. Ultimately, muscle hypertrophy is specific to the trained muscle group, and strength adaptations are specific to the characteristics of the training regime (29); our data suggest that these adaptations are dissociated from acute exercise-induced hormonal rises.

In summary, transient resistance exercise-induced increases in endogenous purportedly anabolic hormones do not enhance muscle strength or hypertrophy following 15 wk of resistance training. Instead, our data are consistent with the notion that local mechanisms are of primary relevance in producing gains

in muscle strength and hypertrophy with resistance training. These findings, combined with our previous work (36, 37), provide multiple lines of evidence that exercise-induced elevations of purportedly anabolic hormones are not necessary for, and do not enhance, muscle anabolism in young men. Our data indicate that exercise-induced changes in concentrations of systemic hormones do not reflect the underlying processes of muscle protein accretion and cannot be used as a proxy marker of muscle hypertrophy.

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DISCLOSURES

No conflicts of interest are declared by the authors.

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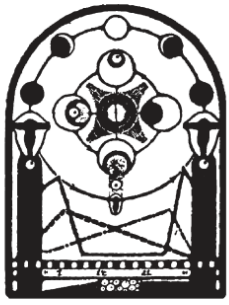

CHAPTER 4

TITLE: Sex-based comparisons of myofibrillar protein synthesis after resistance exercise in the fed state

AUTHORS: West DW, Burd NA, Churchward-Venne TA, Camera DM, Mitchell CJ, Baker SK, Hawley JA, Coffey VG, & Phillips SM

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Sex-based comparisons of myofibrillar protein synthesis after resistance exercise in the fed state

Daniel W. D. West,[1] Nicholas A. Burd,[1] Tyler A. Churchward-Venne,[1] Donny M. Camera,[2] Cameron J. Mitchell,[1] Steven K. Baker,[3] John A. Hawley,[2] Vernon G. Coffey,[2] and Stuart M. Phillips[1]

[1]*Department of Kinesiology, McMaster University, Hamilton, Ontario, Canada;* [2]*Health Innovations Research Institute, School of Medical Sciences, RMIT University, Victoria, Australia; and* [3]*Department of Medicine, McMaster University, Hamilton, Ontario, Canada*

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West DW, Burd NA, Churchward-Venne TA, Camera DM, Mitchell CJ, Baker SK, Hawley JA, Coffey VG, Phillips SM. Sex-based comparisons of myofibrillar protein synthesis after resistance exercise in the fed state. *J Appl Physiol* 112: 1805–1813, 2012. First published March 1, 2012; doi:10.1152/japplphysiol.00170.2012.—We made sex-based comparisons of rates of myofibrillar protein synthesis (MPS) and anabolic signaling after a single bout of high-intensity resistance exercise. Eight men (20 ± 10 yr, BMI = 24.3 ± 2.4) and eight women (22 ± 1.8 yr, BMI = 23.0 ± 1.9) underwent primed constant infusions of L-[*ring*-$^{13}C_6$]phenylalanine on consecutive days with serial muscle biopsies. Biopsies were taken from the vastus lateralis at rest and 1, 3, 5, 24, 26, and 28 h after exercise. Twenty-five grams of whey protein was ingested immediately and 26 h after exercise. We also measured exercise-induced serum testosterone because it is purported to contribute to increases in myofibrillar protein synthesis (MPS) postexercise and its absence has been hypothesized to attenuate adaptative responses to resistance exercise in women. The exercise-induced area under the testosterone curve was 45-fold greater in men than women in the early (1 h) recovery period following exercise ($P < 0.001$). MPS was elevated similarly in men and women (2.3- and 2.7-fold, respectively) 1–5 h postexercise and after protein ingestion following 24 h recovery. Phosphorylation of mTOR^{Ser2448} was elevated to a greater extent in men than women acutely after exercise ($P = 0.003$), whereas increased phosphorylation of p70S6K1^{Thr389} was not different between sexes. Androgen receptor content was greater in men (main effect for sex, $P = 0.049$). Atrogin-1 mRNA abundance was decreased after 5 h recovery in both men and women ($P < 0.001$), and MuRF-1 expression was elevated in men after protein ingestion following 24 h recovery ($P = 0.003$). These results demonstrate minor sex-based differences in signaling responses and no difference in the MPS response to resistance exercise in the fed state. Interestingly, our data demonstrate that exercise-induced increases in MPS are dissociated from postexercise testosteronemia and that stimulation of MPS occurs effectively with low systemic testosterone concentrations in women.

muscle protein synthesis; testosterone

THERE IS A PAUCITY of sex-based comparisons research on skeletal muscle protein turnover after feeding and exercise combined. To date, studies have reported basal postabsorptive rates of muscle protein synthesis that are similar (15, 21) or higher (18) in women compared with men. Dreyer and colleagues (13) reported similar increases between men and women in muscle protein synthesis and mTOR signaling in a 2-h period after exercise in the fasted state. Smith and colleagues (46) reported no major sex differences in rates of muscle protein synthesis in response to hyperinsulinemic-hyperaminoacidemic clamps. However, there is a lack of data that has compared the combined effects of resistance exercise and dietary protein consumption on anabolic mechanisms in skeletal muscle of men and women. Here we superimpose the anabolic effect of whey protein (50, 52) on the anabolic effect of resistance exercise (7, 41) and thus make sex-based comparisons under conditions that would be expected to promote rates of muscle protein synthesis that are near a physiological maximum. Examining rates that are near maximal may provide the best chance of revealing sex-based based differences in the ability to elevate muscle protein synthesis compared with the submaximal stimulus of feeding or exercise alone. Furthermore, to our knowledge, no study has made sex-based comparisons of muscle protein synthesis in the immediate and later (e.g., 24 h) stages of recovery after resistance exercise. Therefore, the primary aim of our study was to determine the impact of resistance exercise and protein feeding on rates of myofibrillar protein synthesis (MPS) and the molecular anabolic response during early (1–5 h) and late (24–28 h) recovery periods in men and women.

A secondary aim of this study was related to the potential impact of physiological testosterone to anabolic responses in men and women. Although evidence is conflicting (43, 53, 54) and there is a lack of direct evidence, it is frequently stated that short-term elevations in testosterone following a bout of heavy resistance exercise promote protein synthesis protein synthesis, leading to protein accretion and hypertrophy (for review, see Ref. 26). Women have testosterone concentrations that are ~10- to 15-fold lower than men and do not experience significant elevations in postexercise testosterone compared with men (24, 25). This divergent postexercise testosteronemia has led to speculation that women may have an attenuated potential for resistance exercise-induced hypertrophy (44). Therefore, a secondary aim of our study was to test the hypothesis that low serum testosterone in women may attenuate anabolic responses after resistance examine compared with men.

Herein, we quantify changes in phosphorylation of the mTOR signaling pathway to gain insight into cellular processes involved in regulating the anabolic response to resistance exercise (28, 36). Finally, as an [albeit imperfect (5)] complement to the anabolic markers that we measured, we quantified changes in the mRNA abundance of MuRF-1 and atrogin-1 to determine if there were sex-based differences in these ubiquitin ligase genes that have been shown to regulate myofibrillar proteolysis.

Address for reprint requests and other correspondence: S. M. Phillips, Exercise Metabolism Research Group, Dept. of Kinesiology, McMaster Univ., 1280 Main St. West Hamilton, Ontario, Canada (e-mail: phillis@mcmaster.ca).

Table 1. *Participant characteristics*

	Men (n = 8)	Women (n = 8)
Age, yr	20.0 ± 1.1	22.0 ± 1.8*
Height, m	1.80 ± 0.06	1.71 ± 0.07*
Weight, kg	77.0 ± 10.5	67.1 ± 5.6*
Body mass index, kg·m^{-2}	24.3 ± 2.4	23.0 ± 1.9
Body fat %	15.6 ± 3.5	23.1 ± 4.1*
Thigh fat %	17.7 ± 3.8	28.6 ± 4.7*
Thigh lean mass, kg	9.2 ± 1.2	7.2 ± 0.8*
MVC (knee ext), Nm	233 ± 116	161 ± 36
Normalized strength, Nm · g lean mass^{-1} · cm^{-1}	1.14 ± 0.4	0.86 ± 0.38

Values are means ± SD. *Significant difference from men, $P < 0.05$. Data were analyzed by unpaired t-tests. MVC, maximal voluntary contraction.

METHODS

Participants. Eight men and eight women (Table 1), who were habitually engaging in two to five sessions of physical activity per week including no more than two lower body resistance training sessions, were recruited. Dual-energy x-ray absorptiometry (DEXA; QDR-4500A; Hologic, Waltham, MA) scans were taken of participants. The thigh was delineated as a region of interest and a ratio of isometric knee extension strength (Biodex System 3; Shirley, NY) to lean mass per unit length of the region of interest was calculated. This measure served as a marker of specific strength (strength per unit lean mass) and, together with a survey of physical activity habits, was used to match male and female participants. Equal numbers of women who were and who were not taking an oral contraceptive (n = 4 on low dose combination of ethinyl estradiol and norgestimate) were recruited. It has been shown that menstrual cycle hormones do not affect rates of MPS at rest or after an acute bout of exercise (34). All participants gave written informed consent to a protocol approved by the Hamilton Health Sciences/Faculty of Health Sciences Research board and which was prepared in accordance with the Declaration of Helsinki and with current Canadian tri-council government funding agency guidelines for use of human participants in research [Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (10a); http://www.pre.ethics.gc.ca/eng/policy-politique/initiatives/tcps2-eptc2/Default/].

Experimental protocol. Participants were familiarized with the exercises and strength tested at least 1 wk before the trial to determine the exercise load to be used during the trial. Participants consumed a controlled diet the day prior to the trial that was prepared according to an energy intake calculated from the Harris-Benedict equation (activity factor range = 1.4 to 1.55) that provided 15, 30, and 55% of energy from protein, fat, and carbohydrate, respectively. These macronutrient ratios were similar to those reported by the participants in 3-day diet logs. The infusion protocol is shown in Fig. 1. Participants arrived at the lab at 0600 after an overnight fast and had a catheter inserted into an antecubital vein. A baseline blood sample was drawn before a 0.9% saline drip was started to keep the catheter patent for repeated blood sampling. A second catheter was placed in the opposite arm for a primed continuous infusion (0.05 μmol·kg^{-1}·min^{-1}; 2.0 μmol/kg prime) of L-[*ring*-$^{13}C_6$]phenylalanine (Cambridge Isotope Laboratories, Woburn, MA) that was passed through a 0.2-μm filter. After ~3 h of tracer incorporation, a resting biopsy was taken from the vastus lateralis for the subsequent estimation of resting rates of MPS. After the biopsy, participants performed a bout of intense, high-volume lower body exercise consisting of 5 sets of 10 repetitions of leg press at ~90% of 10 repetition maximum and 3 sets of 12 repetitions of leg extension/leg curl supersets (1 set of each exercise, back-to-back, with no rest between sets) at ~90% of 12 RM (53, 54). Between-set rest intervals were 60 s. After exercise completion, participants consumed a drink containing 25 g of whey protein (Alacen 352, Fonterra, Palmerston North, New Zealand) and rested in a supine position for the remainder of the trial. Rather than normalizing the dose of the protein supplement to a given parameter (e.g., lean mass, body mass, body surface area, blood volume), we decided to simply administer an equal 25-g dose to both men and women. On the basis of a previous finding (37), we would expect that this dose would be sufficient to support maximal rates of protein synthesis in both sexes. Drinks were enriched to 4% with tracer according to a phenylalanine content of 3.5% in whey protein (10) to minimize disturbances in isotopic equilibrium. Further biopsies were obtained after 1, 3, and 5 h postexercise (*day 1*; see Fig. 1). After the last biopsy, participants were provided with a meal from their controlled diet and given the remainder of the food to consume over the course of the day. The following morning, participants arrived at the lab after an overnight fast and underwent a second primed, constant infusion (*day 2*) with muscle biopsies obtained 24, 26, and 28 h after the exercise bout performed on *day 1*. Muscle biopsies obtained on *day 2* were taken at 1 and 3 h after the start of the infusion to measure 24-h postexercise fasting MPS. Whey protein (25 g) was consumed after the second biopsy on *day 2* and a final biopsy was taken 2 h later to measure MPS in the fed state.

Blood analyses. Serum samples were analyzed for the concentrations of growth hormone, IGF-1, total testosterone, cortisol, and estradiol from time points acutely (pre to 60 min) and 24 h after exercise using solid-phase, two-site chemiluminescence immunometric assays (Immulite; Intermedico, Holliston, MA). The exercise-induced area under the hormone curve (AUC) was calculated by subtracting pre-exercise values (set as the baseline) from postexercise values and determining the AUC. Lactate and glucose concentrations were measured on whole blood deproteinized with perchloric acid and

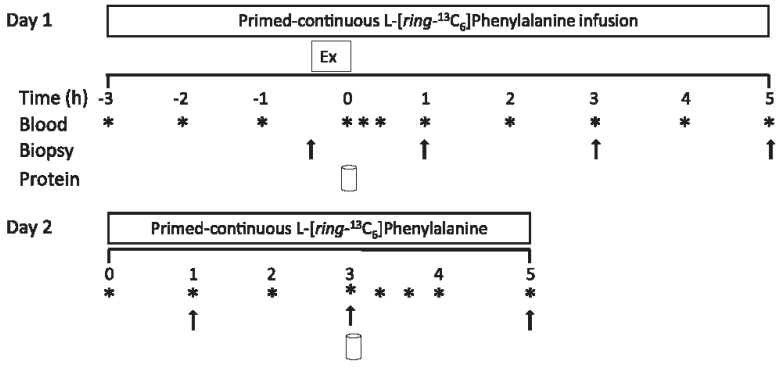

Fig. 1. Schematic diagram of the infusion protocol. A resting fasted biopsy (vastus lateralis) was taken to determine resting fasted myofibrillar protein synthesis (MPS), after which a bout of bilateral lower body resistance exercise was performed. A drink containing 25 g of whey protein was ingested immediately after exercise (0 h). Blood draws were obtained periodically throughout the trial and muscle biopsies were taken at 1, 3, and 5 h postexercise. The next day, participants underwent biopsies at 24 and 26 h postexercise in the fasted state, were fed 25 g of protein at 26 h, and a final biopsy was obtained at 28 h.

neutralized with potassium bicarbonate, using an enzymatic-colorimetric assays (lactate: Pointe Scientific, Canton, MI; glucose: Stanbio Laboratory, Boerne, TX). Blood amino acid concentrations were analyzed by high-performance liquid chromatography as previously described (38). Plasma L-[*ring*-$^{13}C_6$]phenylalanine enrichments were determined as previously described (16).

Calculations. Because all participants were tracer-naive, we calculated a resting fractional synthetic rate (FSR) from naturally abundant ^{13}C enrichments, determined from a baseline preinfusion plasma sample and a single biopsy taken following a period of tracer incorporation (10). This method assumes that the ^{13}C enrichment of a mixed plasma protein fraction reflects the ^{13}C enrichment of muscle protein (19). This method is appropriate because it has been shown (47) that obtaining the first (t_1) biopsy immediately before the start of a primed constant infusion results in rates of muscle protein synthesis that are the same as rates that are calculated from a t_1 biopsy obtained 60 min after the start of the tracer infusion.

Rates of myofibrillar protein synthesis were calculated using the standard precursor-product method: FSR (%/h) = $(E_{2b} - E_{1b}/E_p \times t^{-1}) \times 100$, where E_{2b} and E_{1b} are the bound protein enrichments from muscle at *time 2* (E_{2b}) and plasma proteins or the previous muscle biopsy at *time 1* (i.e., baseline; E_{1b}), E_p is the precursor L-[*ring*-$^{13}C_6$]phenylalanine enrichment and t is the tracer incorporation time.

Gene expression. A small piece (~10–15 mg) of frozen wet muscle tissue was soaked overnight in RNAlater-ICE (Applied Biosystems, Foster City, CA) at −20°C. Tissue was homogenized in 500 μl TRIzol Reagent (Invitrogen, Carlsbad, CA), and isolated RNA was eluted through a spin cartridge according to manufacturer instructions (PureLink RNA Minikit, Invitrogen, Foster City, CA). Extracted RNA was quantified using a QUANT-iT analyzer kit (Invitrogen, cat. no. Q32852) according to the manufacturer's directions and then diluted appropriately with nuclease-free water to yield a total volume of 20 μl. First-strand complementary DNA (cDNA) synthesis was performed using commercially available TaqMan Reverse Transcription Reagents (Invitrogen, Melbourne, Victoria, Australia) on a BioRad thermal cycler (BioRad, Gladesville, Australia). Serial dilutions (100, 10, 1, 0.1, 0.01 ng) of template RNA (AMBION; cat. no. AM7982) were included for calculation of a standard curve for real-time quantitative RT-PCR. Quantification of mRNA (in duplicate) was performed on a 72-well Roto-Gene 3000 Centrifugal Real-Time Cycler (Corbett Research, Mortlake, NSW, Australia). Taqman FAM-labeled primer/probes for MuRF-1, Atrogin-1, and androgen receptor were used in a final reaction volume of 20 μl. The amplification calculated by Rotor-Gene software was specific and efficient (1.05 ± 0.05) for all genes. Threshold cycle data were analyzed using the $2^{-\Delta\Delta}$ C_T method (31), with genes of interest expressed as fold-changes from baseline and normalized to housekeeping gene GAPDH expression.

Western blot analysis. A piece of frozen wet muscle (~50 mg) was homogenized by hand with a Dounce glass homogenizer on ice in a buffer (10 μl/mg, pH 7.4) containing (in mM): 10 Tris (pH 7.2), 250 sucrose, 10 NaCl, 3 $MgCl_2$, 1 dithiothreitol, 1 EGTA, 1 EDTA, 0.3% vol/vol Triton-X 100, and protease/phosphatase inhibitor cocktail tablets (Complete Protease Inhibitor Mini-Tabs, Roche, Indianapolis, IN; PhosSTOP, Roche Applied Science, Mannhein, Germany). The homogenate was transferred to an Eppendorf tube and spun at 15,000 *g* for 10 min at 4°C. The supernatant was removed for protein concentration determination using a standard protein assay kit (Pierce Biotechnology, Rockford, IL) and subsequent Western blotting analysis. Cell lysates were prepared in Laemmli sample buffer (30) and were loaded (40 μg protein) on 10% sodium dodecyl sulfate polyacrylamide gels for separation by electrophoresis. Proteins were transferred to polyvinylidene fluoride membranes, blocked for 1 h in 5% (wt/vol) non-fat milk prepared in Tris-buffered saline with Tween (TBST; 10 mM Tris·HCl, 100 mM NaCl, and 0.02% Tween 20). Membranes were incubated in primary antibody (1:1,000) overnight at 4°C: phospho-Akt^{Ser473} (#9271), phosphor-mTOR^{Ser2448} (#2971), phospho-p70S6K^{Thr389} (#9205), and total androgen receptor (#3202S) were from Cell Signaling Technology (Danvers, MA). Androgen receptor molecular weight (110 kDa) was confirmed using an LNCaP cell lysate as a positive control. Membranes were then washed in TBST and incubated in secondary antibody (1:2,000) for 1 h at room temperature before washing and detection by chemiluminescence imaging (SuperSignal Chemiluminescent Substrate, Pierce Biotechnology, Rockford, IL; FluorChem, Alpha Innotech, Santa Clara, CA). Blots were quantified by assessing peak density using Image J software (National Institutes of Health). All time points for each participant were run on the same gel, and data are expressed relative to α-tubulin (1:2,000, T6074, Sigma-Alderich, St. Louis, MO).

Protein-bound enrichments. Myofibrillar proteins were extracted from frozen wet muscle (~50 mg) as previously described (38). The resulting protein pellet was hydrolyzed overnight 6 M HCl at 110°C, and the free amino acids were purified using cation-exchange chromatography (Dowex 50WX8–200 resin Sigma-Aldrich) and converted to their *N*-acetyl-n-propyl ester derivatives for analysis by gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS: Hewlett Packard 6890; IRMS model Delta Plus XP, Thermo Finnagan, Waltham, MA).

Statistics. Anthropometry and AUC measures were analyzed by unpaired *t*-tests. Blood analytes, MPS, protein and gene expression were analyzed (separate analyses performed for *days 1* and *2*) by two-factor (sex and time) ANOVA with Tukey post hoc tests where

Table 2. *Blood glucose, insulin, and amino acid concentrations in the rested fasted state and after resistance exercise plus ingestion of 25 g of whey protein (day 1)*

	Rested Fasted	Postexercise					
Time (min):	−60	0	15	30	60	90	120
Glucose, mmol/l							
Women	4.5 ± 0.3	5.2 ± 0.6*	5.2 ± 0.8*	4.6 ± 0.4	4.3 ± 0.2	4.0 ± 0.3	4.3 ± 0.2
Men	4.5 ± 0.4	5.3 ± 0.6*	5.2 ± 0.7*	5.1 ± 0.8	4.7 ± 0.1	4.5 ± 0.5	4.5 ± 0.5
Insulin, μIU/ml							
Women	3.5 ± 2.1	6.3 ± 3.6	9.9 ± 6.0	17.8 ± 11.5*	19.9 ± 13.2*	8.4 ± 5.4	7.5 ± 5.6
Men	5.1 ± 3.7	5.0 ± 2.4	9.4 ± 7.9	13.8 ± 11.0*	19.2 ± 7.5*	13.0 ± 4.2	9.9 ± 3.0
Total AA, μmol/l							
Women	2169 ± 468	2092 ± 456	—	2425 ± 854	3202 ± 746*	2702 ± 597*	2577 ± 560
Men	1967 ± 444	2340 ± 200	—	2488 ± 306	2994 ± 417*	2712 ± 535*	2691 ± 337

Values are means ± SD (n = 8 in each group). Sex × time interactions for glucose, insulin, and total amino acids, respectively: P = 0.69, 0.96, 0.84. Main effects for time (all $P < 0.001$); *values are significantly different from pre-exercise, men and women combined, $P < 0.05$. Total AA, summed total amino acid concentrations (Asp, Ser, Asn, Glu, Gly, Gln, His, Tau, Thr, Arg, Ala, Pro, Tyr, Cys, Val, Met, Ile, Leu, Lys, Lys, Phe). Drink comprised 25 g of whey protein isolate.

significant ($P \leq 0.05$) main effects or interactions were indicated. In the figures, significant main effects are described in the legends and symbols indicate between-sex differences that are the result of a significant post hoc test. Statistical analyses were performed using SigmaStat version 3.1 software (Systat Software, Point Richmond, CA). All data are expressed as means ± SD.

RESULTS

Participant characteristics. Participant characteristics are shown in Table 1. Women had a greater percentage of total body and thigh fat than men and had a lower lean body mass and thigh lean mass (all $P < 0.01$). Body mass index was not different between men and women (24.3 ± 2.4 vs. 23.0 ± 1.9, respectively; $P = 0.25$). The ratio of isometric strength to thigh lean mass (which was normalized to femur length) was not different between men and women (1.14 ± 0.4 vs. 0.86 ± 0.38, $P = 0.37$).

Blood metabolites and serum hormones. Blood glucose concentration was elevated above resting concentrations immediately and 15 min after exercise (Table 2, main effect for time, $P < 0.001$); glucose concentrations were unchanged on *day 2* (data not shown). Plasma insulin concentrations were elevated 30 and 60 min after protein ingestion on both *day 1* (Table 2, main effect for time, $P < 0.001$) and *day 2* (data not shown). Blood amino acids were elevated similarly in men and women at 60 and 90 min after the 25-g protein drink ($P < 0.001$; sex × time interaction, $P = 0.84$). Blood lactate concentrations increased from 0.6 mM in both men and women to 10.7 mM in men and to 7.1 mM in women immediately postexercise (sex × time interaction, $P < 0.001$), resulting in

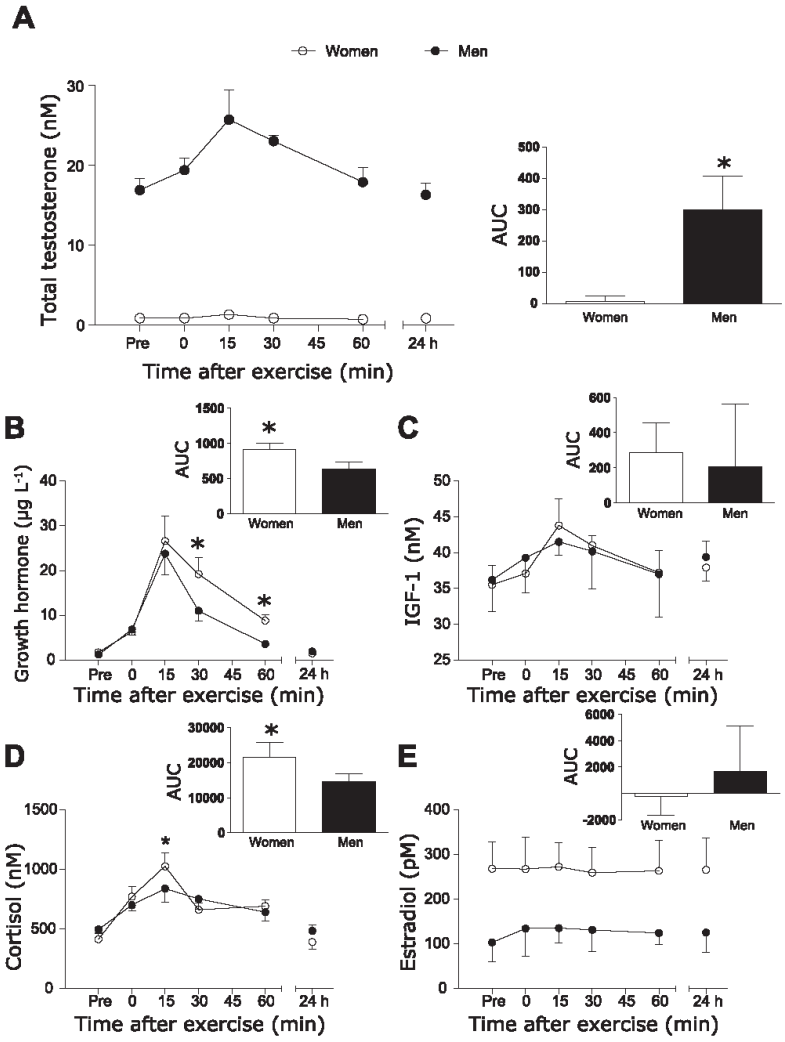

Fig. 2. Serum postexercise testosterone (*A*), growth hormone (GH) (*B*), IGF-1 (*C*), cortisol (*D*), and estradiol (*E*) concentrations in men and women. *Insets* are net areas under the curve where pre-exercise (Pre) = 0; *significant difference between sexes, $P < 0.001$. *Significant difference between sexes at the same time point, $P < 0.01$. Values are means ± SD, $n = 8$ in each group.

a greater area under the curve in men ($P = 0.02$). Serum concentrations of total testosterone, growth hormone, IGF-1, cortisol, and estradiol are shown in Fig. 2 *A, B, C, D,* and *E*, respectively. Fifteen minute postexercise, testosterone increased from 17 to 26 nM in men and from 0.8 to 1.3 nM in women (sex × time interaction, $P < 0.001$); this resulted in an exercise-induced area under the testosterone curve that was ~45-fold greater in men ($P < 0.001$). Postexercise growth hormone concentration peaked similarly in men and women at 15 min, with a more prolonged elevation in women such that concentrations were greater in women at 30 and 60 min (both $P < 0.01$; sex × time interaction, $P < 0.001$), resulting in a greater AUC ($P < 0.001$). IGF-1 was similarly elevated in men and women (time effect, $P < 0.001$; sex × time interaction, $P = 0.55$). Cortisol concentration peaked at a higher concentration 15 min post-exercise in women compared with men (sex × time interaction, $P < 0.001$), resulting in a greater AUC in women ($P < 0.001$). Estradiol concentrations were higher at all time points in women and remained unchanged in the acute post-exercise period (sex effect, $P < 0.001$; sex × time interaction, $P = 0.60$).

Myofibrillar protein synthesis. Plasma phenylalanine tracer-to-tracee ratios during fasted, 1- to 3-h, 3- to 5-h, 24- to 26-h, and 26- to 28-h periods, respectively, for men and women (respectively) were 0.052 and 0.051, 0.057 and 0.054, 0.61 and 0.061, 0.071 and 0.068, 0.053 and 0.062, 0.059 and 0.060. Basal rates of MPS were similar between men (0.021 ± 0.007%/h) and women (0.020 ± 0.008). MPS was elevated acutely in men and women at 1–3 h ($P = 0.018$) and 3–5 h ($P = 0.021$) after exercise and protein consumption (Fig. 3*A*) and at 26–28 h in the fed state ($P < 0.001$). There was a trend for a sex × time interaction for MPS acutely after exercise ($P = 0.087$) that appeared to be attributable not to the magnitude of the response but more to subtle temporal differences (Fig. 3*A*); MPS rates calculated across the aggregate 1- to 5-h time period were not different between sexes (*inset* of Fig. 3*A*).

Cell signaling. Akt^{Ser473} phosphorylation increased at 1 h ($P < 0.001$, main effect for time; Fig. 4*A*) and to a greater extent in men (sex × time interaction, $P = 0.018$). Phosphorylation of $mTOR^{Ser2448}$ was increased at 1, 3, and 5 h ($P < 0.001$; Fig. 4*B*); there was a main effect for sex (men > women, $P = 0.003$). Phosphorylation of $mTOR^{Ser2448}$ was elevated similarly between sexes after next-day protein feeding, ~26 h after the exercise bout (sex × time interaction, $P = 0.49$; main effect for time, 28 > 26 h, $P = 0.006$, data not shown). Phosphorylation of $p70S6K1^{Thr389}$ increased at 1, 3, and 5 h (all $P < 0.001$; sex × time interaction, $P = 0.13$; Fig. 4*C*) and there was a significant interaction with next-day feeding (28 > 26 h in women only, sex × time interaction, $P = 0.016$; data not shown). Androgen receptor content was greater overall in men ($P = 0.049$) but there was no significant interaction ($P = 0.47$; Fig. 4*D*).

Gene expression. MuRF-1 mRNA was not different from rest in men and women 1 and 5 h after exercise (sex × time interaction, $P = 0.58$; Fig. 5*A*); MuRF-1 increased at 28 h in men only (sex × time interaction, $P = 0.003$). Atrogin-1 mRNA decreased irrespective of sex at 5 h compared with rest ($P < 0.001$) 1 and 5 h ($P = 0.016$) (sex × time interaction, $P = 0.98$; Fig. 5*B*); Atrogin-1 increased at 28 h (time effect, $P = 0.002$; Fig. 5*B*) with a trend ($P = 0.09$) toward a greater increase in men. Androgen receptor mRNA was not different from rest in men and women at 1 and 5 h after exercise (sex × time interaction, $P = 0.38$; Fig. 5*C*) and was increased at 28 h compared with 26 h (time effect, $P < 0.022$; sex × time interaction, $P = 0.16$; Fig. 5*C*).

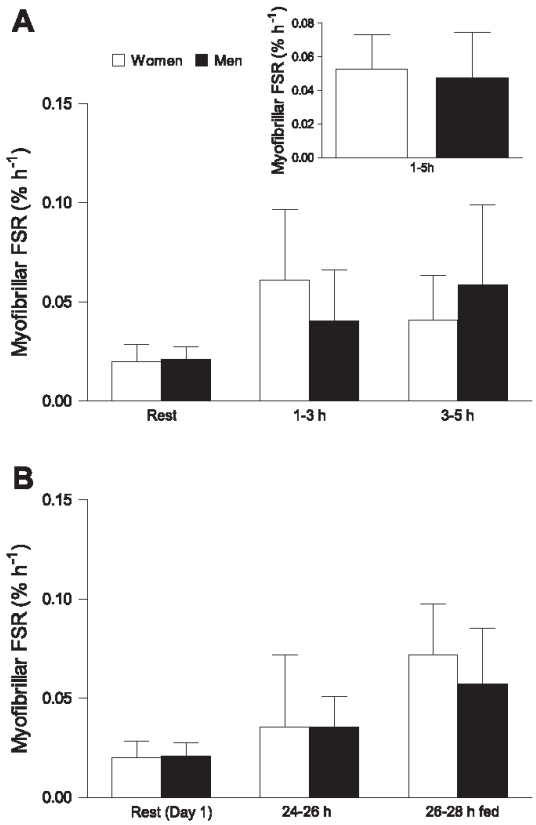

Fig. 3. Myofibrillar fractional synthetic rates (FSR) in the rested fasted state, 1–3 and 3–5 h after resistance exercise and protein ingestion (*A*). Myofibrillar FSR 24–26 h postexercise and 26–28 h postexercise after 25 g of protein ingested at 26 h (*B*). *Inset* in *A* is the aggregate FSR from 1–5 h calculated from enrichments obtained from biopsies taken at 1 and 5 h. 1–3 h and 3–5 h > rest, $P < 0.001$; sex × time interaction, $P = 0.087$. The rested fasted rate from *day 1 A* is simply redrawn in *B* for comparisons sake. 26–28 h > 24–26 h, $P < 0.001$; sex × time interaction, $P = 0.56$. Values are means ± SD, $n = 8$ in each group.

DISCUSSION

The present study reports similar rates of MPS and anabolic signaling responses between men and women after resistance exercise and bolus protein ingestion early (1–5 h) and late (24–28 h) in postexercise recovery. The increase in MPS at 1–5 h was similar between men and women and is in support of previous findings showing comparable changes in men and women with feeding and exercise individually (13, 46). The sustained increase in MPS in the 3- to 5-h recovery period observed, a time when amino acids had returned to basal concentrations, is in agreement with our previous observations (38, 51). This observation indicates that the resistance exercise

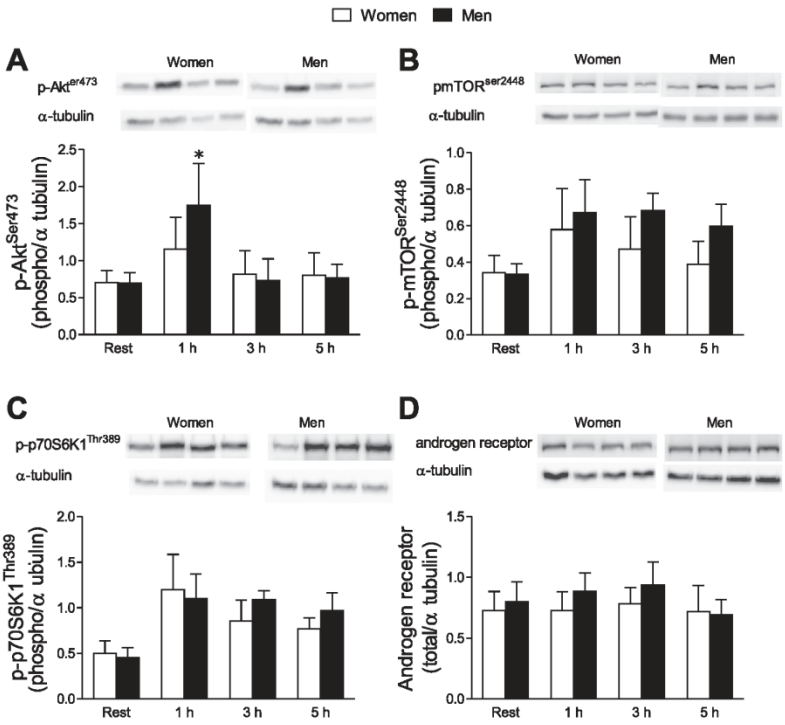

Fig. 4. Akt^{Ser473} (*A*), mTOR^{Ser2448} (*B*), and p70S6K1^{Thr389} (*C*) protein phosphorylation and total androgen receptor protein content (*D*). For each target, the P value for the sex × time interaction is given, followed by a description of the significant effects. p-Akt^{Ser473}: $P = 0.018$; men > women at 1 h ($P < 0.001$); 1 h > rest, 3, 5 h ($P < 0.01$). p-mTOR^{Ser2448}: $P = 0.16$; 1, 3, 5 h > rest ($P < 0.001$); men > women ($P = 0.003$). p-p70S6K1^{Thr389}: $P = 0.13$; 1, 3, 5 h > rest ($P < 0.001$). Androgen receptor: $P = 0.47$; men > women ($P = 0.049$). *Significant difference between sexes at the same time point, $P < 0.001$. Data are expressed relative to α-tubulin. Values are means ± SD, $n = 8$ in each group.

stimulus, not feeding, is responsible for sustained increases in fractional synthetic rate (4, 9).

The phosphorylation profiles of the signaling proteins we measured were also broadly similar between men and women (Fig. 4). However, we found that mTOR^{Ser2448} was phosphorylated to a greater extent in men during the acute recovery period after resistance exercise, which is contrast to the equal increase between sexes previously reported (13). Despite these differences in mTOR^{Ser2448} phosphorylation, both studies report similar (in magnitude and between sexes) increases in p70S6K1 phosphorylation acutely after resistance exercise. This may indicate that the level of mTOR activation in women was sufficient to result in downstream effects on p70S6K1, changes in the phosphorylation status of which may better indicate mTOR activation than phosphorylation changes at the particular Serine^{2448} site (45). The reason(s) for differences in mTOR phosphorylation are unclear; both groups were fed identical amounts of protein, had similar aminoacidemia and insulin responses (Table 2), and performed the exercise bout at the same relative intensity. Men have a greater percentage type IIA fiber area on average (48) and mTOR has been reported to be localized in type IIA fibers (40), which could potentially account for some of the differences observed. Nevertheless, on the basis of the small differences in muscle protein synthesis between fiber types (12, 35) this would not be expected to have a major influence.

The optimal approach to capture mechanistic insight from changes in the signaling of individual proteins remains elusive and it has been demonstrated that there is often a disconnect between signaling and muscle protein synthesis in both magnitude (17) and time course (2). In light of this disconnect, it is worth recognizing that the phosphorylation of signaling proteins is a temporal snapshot of the propagated signal for translation initiation. It is also unclear if there is a minimum threshold signal required to initiate and completely activate or turn on translation. If there is such a threshold then it seems plausible that greater phosphorylation above such a threshold would be unlikely to further amplify the signal/lead to increased rates of translation. Nonetheless, it has been demonstrated that the elevations in protein synthesis that are mediated by amino acids (11) and contraction (14) are dependent on mTOR activation, which regulates translation initiation (22).

In addition to sex-based comparisons of anabolic indices we compared the mRNA response of genes that regulate muscle protein breakdown. The ubiquitin ligases MuRF-1 and atrogin-1 are associated with skeletal muscle atrophy (8) but their specific role in muscle remodeling and exercise-induced adaptation is yet to be defined. Our data are consistent with other studies (23, 32) in reporting an attenuation in atrogin-1 expression after acute resistance exercise, a decrease that we found was similar in men and women. Collectively, the present and previous (32) data are in agreement with the notion that, despite being thought to perform similar roles in ubiquitin-proteosome-mediated protein breakdown, MuRF1 and atrogin-1 can be differentially regulated after acute resistance exercise and may function independently (39). Surprisingly, there was an increase in atrogin-1 and MuRF-1 expression with protein feeding ~24 h after exercise. No study has made direct measures of muscle

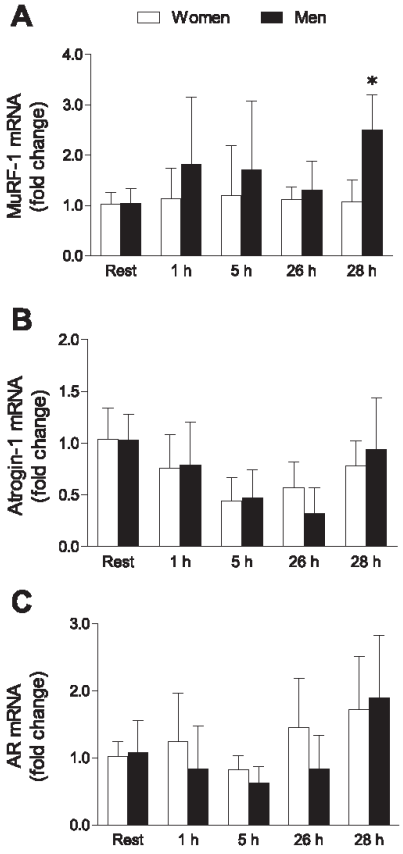

Fig. 5. MuRF-1 (*A*), atrogin-1 (*B*), and androgen receptor (*C*) gene expression in the rested fasted state; 1, 5, and 26 h after resistance exercise plus 25 g of protein; and at 28 h (second 25 g protein bolus given at 26 h). Sex × time interactions and significant post hoc effects (respectively) are as follows. MuRF-1: *day 1*, $P = 0.58$; *day 2*, $P = 0.003$, men > women at 28 h ($P < 0.001$). Atrogin-1: *day 1*, $P = 0.98$, time effect $P = 0.001$, rest and 1 h > 5 h ($P = 0.001$ and 0.016, respectively); *day 2*, $P = 0.092$, time effect 28 h > 26 h, $P = 0.002$. Androgen receptor: *day 1*, $P = 0.38$; *day 2*, $P = 0.16$, time effect 28 h > 26 h, $P = 0.022$. Threshold cycle data were analyzed using $2^{-\Delta\Delta C_T}$ method (31) with glyceraldehyde-3-phosphate dehydrogenase as a housekeeping gene. Values are means ± SD, $n = 8$ in each group.

protein breakdown under circumstances similar to the present study, making it somewhat difficult to place the finding in context. Whether the provision of exogenous amino acids permits the muscle to return to a state of increased remodeling (we know that MPS is elevated), including increased breakdown, after an overnight fast requires further investigation. We recognize that there is a lack of data that links gene-based markers of proteolysis to an actual phenotypic change. Reasons for elevated MuRF-1 mRNA in men compared with women with next-day protein feeding are not clear, although it is interesting to note that estradiol supplementation in men attenuates exercise-induced muscle inflammation (33) and that MuRF-1 is responsive to local inflammation (39). However, inflammation was not measured in the present study and how female sex steroids affect sex-based differences in postexercise muscle inflammation (42, 49) and muscle protein breakdown is presently unclear.

Our finding of increased androgen receptor mRNA abundance at 28 h into recovery after resistance exercise adds to studies showing increases 48 h postexercise (6, 20, 56). Androgen receptor expression, which is reported to be affected by feeding (27) as well as mechanical load (6), did not appear to be affected by differences in acute postexercise testosterone concentration between men and women.

A secondary aim of this study was to examine the potential impact of sex-based differences in post-exercise testosteronemia. We report that despite dramatic (45-fold) differences in exercise-induced serum testosterone, men and women had similar increases in MPS after resistance exercise and protein ingestion. Our data are in contrast to the theory that the absence of a transient acute hypertestosteronemia in response to resistance exercise in women compared with men limits their potential for hypertrophy (44). Although not causative, the clear dissociation of the postexercise rise in MPS and systemic testosterone suggests that the anabolic response to resistance exercise does not rely on postexercise hypertestosteronemia and can occur effectively despite low systemic testosteronemia in women. Our present and previous (53–55) work suggests that intramuscular mechanisms are predominantly driving the synthesis of myofibrillar proteins after resistance exercise and that exercise-induced testosteronemia is not required for this process. Whereas our sex-based model suggests postexercise testosteronemia is not a prime mediator of postexercise MPS, the potential role of intramuscular androgen-related events [testosterone and dihydrotestosterone can be synthesized in peripheral, non-gonadal tissue (1, 29, 57)] is unclear. Thus testosterone concentrations in systemic circulation may not be a good indication of the potential impact of androgens on muscle tissue (29).

In conclusion, we report similar increases in rates of MPS between men and women after resistance exercise with protein ingestion during the early (1–5 h) and late (24 h) recovery period. Interestingly, the postexercise testosterone responses in women, which were 45-fold lower than men, did not appear to attenuate increases in MPS or p70S6K1 phosphorylation from occurring. Indeed, the clear dissociation between the testosterone response and the MPS response that we observed, suggests that postexercise testosteronemia is not likely a predominant driver of MPS. Further studies that measure intramuscular androgen intracrinology and receptor activity are necessary to elucidate the potential role for physiological androgen-related mechanisms in regulating skeletal muscle mass in men and women.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: D.W.W., N.A.B., and S.M.P. conception and design of research; D.W.W., N.A.B., T.A.C.-V., C.J.M., S.K.B., and S.M.P. performed experiments; D.W.W., N.A.B., D.M.C., V.G.C., and S.M.P. analyzed data; D.W.W., N.A.B., D.M.C., J.A.H., V.G.C., and S.M.P. interpreted results of experiments; D.W.W. prepared figures; D.W.W. drafted manuscript; D.W.W., N.A.B., T.A.C.-V., D.M.C., C.J.M., S.K.B., J.A.H., V.G.C., and S.M.P. edited and revised manuscript; D.W.W., N.A.B., T.A.C.-V., D.M.C., C.J.M., S.K.B., J.A.H., V.G.C., and S.M.P. approved final version of manuscript.

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CHAPTER 5: DISCUSSION AND CONCLUSIONS

5.1 INTRODUCTION

This chapter begins by briefly outlining the rationale for studying testosterone as a primary hormone of interest in this thesis. Next is a discussion of our experimental models. First, the unilateral model that was used in the studies of Chapters 2 and 3 to create divergent hormone availability will be discussed. While these studies have been discussed to some extent elsewhere (7, 61, 64), special attention is now given to the recent supposition that the order of exercise is important for the efficacy of hormone hypothesis (small muscle groups trained before large muscle groups will be deprived of hormone-rich blood). Second, the sex-based comparisons model used in the study in Chapter 4 is discussed, along with studies by others that have made sex-based comparisons in muscle protein synthesis. The next part of the present chapter draws attention to the distinction between physiological versus non-physiological testosterone concentrations and how they relate to skeletal muscle protein metabolism. Finally, the biological functions of the exercise-induced hormone response are explored along with areas for future research.

The physiological/in vivo nature of the present thesis meant that the endogenous hormones generally thought to be most related to muscle anabolism (testosterone, growth hormone, IGF-1 and cortisol) were examined concurrently. During the time studies for this thesis were conducted, evidence has accumulated (13, 18, 39), that adds

to previous evidence (36, 40, 55, 65), to suggest that growth hormone and systemic IGF-1 do not play a significant role in stimulating the accretion of contractile muscle protein. Recognition of this evidence has been discussed elsewhere (64) and is becoming more accepted, although not by all (25). In contrast, due to the obvious potency of exogenous testosterone for muscular hypertrophy (3, 4), exercise-induced changes in testosterone remain highly scrutinized (9, 57). In our sex-based comparisons study (Chapter 4), outcomes (MPS, signalling, gene expression) can only truly be attributed to the sum of the variables measured (e.g., hormones) (or other variables not measured). However, we contend that the 45-fold divergence in exercise-induced testosterone, which was targeted *a priori* based on literature values, distinguishes it from other exercise-induced hormones (discussed further below). For these reasons, more attention will be paid to testosterone in the present chapter.

5.2 'MAXIMIZING' THE EXERCISE-INDUCED TESTOSTERONE RESPONSE: THE INFLUENCE OF EXERCISE ORDER

Studies (48, 49) show that when an exercise is performed last in a training session, its performance is negatively affected (e.g., decreased number of repetitions at the training load); this is true for large and small muscle groups. Therefore, since the primary outcome measures in Chapter 3 were the strength and hypertrophy of the elbow flexors, we exercised these muscles first to eliminate any possibility of central fatigue associated with prior exercise that may hinder training volume and, theoretically,

gains in strength and hypertrophy. The purpose of Chapter 3 was to compare the outcomes mentioned above in the context of either a low (~basal) or a high hormone systemic hormone environment. We observed no enhancement of strength or hypertrophy in the high hormone group and thus concluded that the post-exercise increase in testosterone and growth hormone were not important factors in determining the responses to training. Since this publication, another study (43) has been published using a similar design, but reached an opposing conclusion to ours that post-exercise hormonal increases underpinned greater adaptations in certain indices of strength and hypertrophy. The authors suggest that this disparity in findings was due to between-study differences in exercise order (i.e., they trained 'legs first, then arm' whereas we trained 'arm first, then legs').

As mentioned, we elected the 'arm first, legs second' design so that the arms, which were being compared for gains in strength and hypertrophy, were able to do identical amounts of work since in both conditions, the arm was being trained first in an unfatigued state. Indeed, the authors in the study by Ronnestad et al. (43) report that their subjects reported perceptions of greater fatigue after legs-then-arm exercise and attribute central fatigue to equal training loads between conditions throughout the training program, before greater adaptations in the hormone-enhanced arm were apparently revealed post-training. Other methodological concerns about this study have been raised also (38); nevertheless, the assertion (30, 43, 57) remains that an arm-then-legs exercise order could have deprived the arm of testosterone-rich blood. Therefore

we tested this theory by determining the testosterone potential/availability to elbow flexor muscles performing exercise before or after an intense leg workout. We hypothesized that a calculated testosterone delivery (blood flow to the muscle × testosterone concentration) would serve as a best estimate of the delivery of testosterone-rich blood.

The unpublished data set from this experiment is presented as Appendix 2. The experiment was conducted by DWD West, LM Cotie, CJ Mitchell, TA Churchward-Venne, MJ MacDonald and SM Phillips. Only basic details are outlined herein in order to provide a general orientation to the experiment and its findings. Seven men with previous weight-training experience performed two acute trials in a randomized cross-over fashion whereby unilateral arm resistance exercise was performed before or after bilateral leg exercise (Appendix 2, Figure 1). Blood samples for testosterone analysis as well as brachial artery blood flow measures (Doppler ultrasound) were obtained immediately after arm exercise and leg exercise, and at intervals in post-exercise recovery.

Figure 2 in Appendix 2 shows the total and free testosterone concentrations in each condition. Figure 3 in Appendix 2 shows the effect of the exercise conditions on brachial artery diameter and blood flow. Elbow flexor resistance exercise increased brachial artery blood flow 4-fold and brachial blood flow remained at the elevated level in the face of subsequent intense leg exercise. Intense leg exercise increased brachial blood flow 3-fold, which was further elevated after subsequent arm exercise. Figure 4 in

Appendix 2 shows the estimated delivery of total and free testosterone to muscles supplied by the brachial artery. The data suggests that arm muscles supplied by the brachial artery were delivered similar quantities of testosterone-rich blood when exercised before or after intense leg exercise. Therefore, we conclude that claims (30, 43, 57) that exercise order and subsequent effects on testosterone delivery account for the lack of hormonal enhancement of hypertrophy (Chapter 4) appear unfounded. We also propose that measuring blood flow in conjunction with hormone concentration may provide a more physiologically relevant measure of hormonal exposure to a given tissue than traditional measures of hormone concentration alone.

5.3 SEXUAL DIMORPHISMS IN EXERCISE-INDUCED TESTOSTERONE: EFFECTS ON MUSCLE PROTEIN SYNTHESIS

There were two main aims of our sex-based comparisons study: i) to compare men and women after resistance exercise and protein ingestion in both early and late post-exercise recovery, and ii) to use sex-based dimorphism as a model of divergent exercise-induced testosteronemia. As mentioned, we contend that the sheer differences in exercise-induced testosterone, which was targeted *a priori* based on previous testosterone literature, distinguishes it from other exercise-induced hormones, and sheds light as to whether or not exercise-induced testosterone is crucial to the anabolic response to resistance exercise. Notably, testosterone is distinguished from other exercise-induced hormones for two reasons discussed below.

First, the aforementioned clear divergence in exercise-induced responses between men and women is far above differences of other exercise-induced hormones, which appeared to be reasonably similar between men and women. Specifically, of the exercise-induced hormone responses measured, GH was 1.4-fold greater in women, IGF-1 was 1.4-fold greater in women, cortisol was 1.5-fold greater in women, estradiol was 7-fold greater in men, and testosterone was 45-fold greater in men. Thus, while we cannot make cause and effect statements because we have not isolated or pharmacologically manipulated testosterone independently, we contend that there should be obvious between-sex differences in our measures of MPS if the testosterone response is truly integral to the anabolic response to resistance exercise.

Second, there is clear evidence that testosterone is anabolic toward skeletal muscle in pharmacological doses (17), a conclusion that is supported through a mechanistic characterization of its action, thus giving it a plausible anabolic role in an exercise scenario. In contrast to testosterone, the same cannot be said about growth hormone or IGF-1 which, even when administered to supraphysiological doses, do not stimulate myofibrillar protein synthesis (13).

Previous studies that have made sex-based comparisons in muscle protein synthesis generally do not report any major differences after resistance exercise (14), feeding (50) or, as we have shown (60), feeding plus resistance exercise, a scenario in which rates of muscle protein synthesis are presumably near a physiological maximum. Despite one report of greater rates of muscle protein synthesis in women than men (23),

the majority of studies examining sex-differences in fasting basal rates of muscle protein synthesis have reported no differences (14, 19, 24, 37, 50, 60), at least in young and middle-aged adults. Nevertheless, mandates for sex-based research by National funding agencies continue to prompt men versus women comparisons in muscle physiology; notably, a recent editorial (52) on our work (60) urges researchers to examine how sex-differences influence skeletal muscle in aging, obesity and other conditions of muscle atrophy. Indeed, research to date on sexual dimorphism in muscle protein synthesis suggests that elderly women have, compared with elderly men, greater rates in the basal postabsorptive state (51, 53), and a blunted response to feeding (51) as well as exercise (53). The reasons for age-dependent sex-based differences in muscle protein synthesis are unclear, but differences only seem to appear after middle age once muscle mass is in decline (e.g., the elderly), and may be associated with obesity (53). Theories have been proposed to partially account for age-related sex-based differences. Greater basal postabsorptive rates of muscle protein synthesis in elderly women could be related to a reduction in the apparently suppressive effect of estrogens on muscle protein synthesis (51); however, caution is warranted here since the suppressive effects of estrogens on muscle protein synthesis are characterized from animal models and appears to be in conflict with human data (see paragraph below). The blunted response to feeding in elderly women appears to be related to a failure to activate certain signalling proteins that regulate translation initiation (51), but the upstream regulatory factors remain unclear. Similarly, reasons why elderly women are also resistant to an

exercise stimulus, insofar as their ability to elevate muscle protein synthesis (53), are unclear. Clearly more research is needed in to understand sexual dimorphisms in protein metabolism in skeletal muscle.

In Chapter 4, concentrations of estradiol, which were higher in women as expected, were not responsive to exercise in either sex (Figure 2E in Chapter 4). While beyond the scope of this thesis, the chronic effect of estrogens to muscle anabolism warrants further research since hormone replacement therapy generally (albeit not definitively (56)) appears to enhance muscle strength in postmenopausal women (21, 42). Apparently, however, estrogen replacement therapy depresses resting MPS and yet is necessary for an exercise-induced elevation in protein synthesis in postmenopausal women (22). In general, as with sex-based comparisons of muscle protein synthesis, more research is required to understand how estrogen regulates human skeletal muscle and how age may affect this regulation.

In summary, we examined rates of myofibrillar protein synthesis in men and women that were presumably near a physiological ceiling after resistance exercise and whey protein ingestion. Our finding of similar rates between sexes is consistent with other comparisons of submaximal rates of muscle protein synthesis in young men and women. Notably, the 45-fold difference in the exercise-induced testosterone response did not prevent an elevation of MPS from occurring in women to similar extent as men. However, despite similar rates of MPS between sexes in younger persons, future research is required to understand why differences seem to appear with aging (51, 53).

Furthermore, further examination of how estrogens and testosterone precursors can regulate skeletal muscle in young and old is warranted.

5.4 TESTOSTERONE AND SKELETAL MUSCLE: CONCENTRATION AND TIME COURSE MATTER

As discussed in the introduction, studies of testosterone administration have revealed mechanisms of action and often pronounced effects of exogenous testosterone on skeletal muscle. The collective mechanisms and effects of exogenous testosterone administration on skeletal muscle are frequently cited and serve as a foundation to design studies and interpret findings, as well as to prescribe exercise programs with the rationale that rises in post-exercise testosterone are likely to have a similar effects to exogenously administered testosterone or its analogues. However, a fact that often goes unmentioned or unrealized is that there are stark differences between exogenous and endogenous testosterone in concentration and pattern of availability. We have previously discussed these differences in general terms (64); these points are now illustrated using a detailed example. Figure 1 in this chapter illustrates the pharmokinetics of a single 200 mg intramuscular injection of testosterone enanthate (12), a relatively modest dose. [Note: 200 mg of testosterone enanthate injected bi-weekly is a standard conservative dose prescribed for testosterone replacement therapy (41); 600 mg doses produce testosterone concentrations of at least 100 nM (2) and represents a dose that is equivalent to about 7-10 times normal weekly production of 42

mg wk^{-1} (1)]. As illustrated, after the injection, testosterone increases ~200% to ~45 nM and remains chronically elevated 7 d post injection. By contrast, endogenous testosterone concentrations follow a circadian rhythm, oscillating from ~22 nM (0600 h) to 13 nM (1800 h) (11). The exercise-induced increase on day 4 represents an 18 – 100 % increase (depending on initial concentrations) that lasts for ~20-30 min. Therefore, by comparison, the testosterone availability, on an area under the curve basis and using the nadir of 13 nM as the baseline, and assuming that the exercise bout does not alter 24 h secretion (27), the exercise-induced increase in testosterone on day 4 represents a 3.6% increase above normal resting conditions. By contrast the testosterone that is available in circulation to the muscle on day 4 from the moderately-dosed injection of testosterone enanthate is 458% greater than normal resting conditions.

The purpose of this discussion is not to say that transient periods of testosteronemia are altogether irrelevant ; however, because the purported effects of exercise-induced testosterone are frequently based on effects from exogenous administration studies, then comparisons of the shared variables, testosterone concentration and time course, are warranted. Essentially, if testosterone is being treated as the common independent variable between studies, then we question why it is reasonable to contend that there are similar effects on a dependent variable (e.g., hypertrophy) when such a disparity in the independent variable (testosterone concentration and time course) exists.

Rather than assuming that the effects of testosterone are consistent across a range of concentrations, we propose that the testosterone-protein accretion curve is better described by distinguishing whether the testosterone condition is in a hypophysiological, physiological or supraphysiological. Figure 2 in this chapter is a crude theoretical schematic that illustrates this possibility and summarizes the implications of the differences between changes in testosterone that are in the normal physiological range and those that are considerably higher or lower. Specifically, the physiological changes such as those that occur after exercise probably do not represent a substantial stimulus to protein synthesis for two main reasons. Firstly, the testosterone responses are of similar magnitude to the changes of the normal diurnal range (as shown in Figure 1 of this chapter). And secondly, that the changes are very transient which is in contrast to pharmacological interventions which induce long-lasting changes in testosterone and which represent a persistent perturbation to homeostasis. The supraphysiological arm in Figure 2 of this chapter showing increased protein accretion after testosterone administration is well-supported by work by Bhasin and colleagues (3) who have shown a dose response between testosterone and change in muscle size and lean body mass. The hypophysiological arm requires further research but stems from data showing that suppression of endogenous testosterone reduces muscle and lean body mass in patients undergoing androgen deprivation therapy (54) and attenuates training adaptations to strength training in healthy young men (29). The work in the present thesis proposes the

flat physiological range, illustrating a null effect of physiological changes in testosterone concentration on muscle protein accretion.

While our data in Chapter 3 showed no enhancement of exercise-induced testosterone on strength and hypertrophy, research on the therapeutic effects of pharmacological testosterone will continue to be an area of intense research. For example, more research is needed to explain the recent finding (46) that rates of muscle protein synthesis can remain persistently elevated even when exogenous testosterone is cycled off for a month and returns to basal concentrations. Furthermore, ongoing clinical trials for new modes of testosterone delivery (e.g., nasal sprays) and selective androgen receptor modulators promise to keep the anabolic effects of testosterone at the forefront of skeletal muscle therapies.

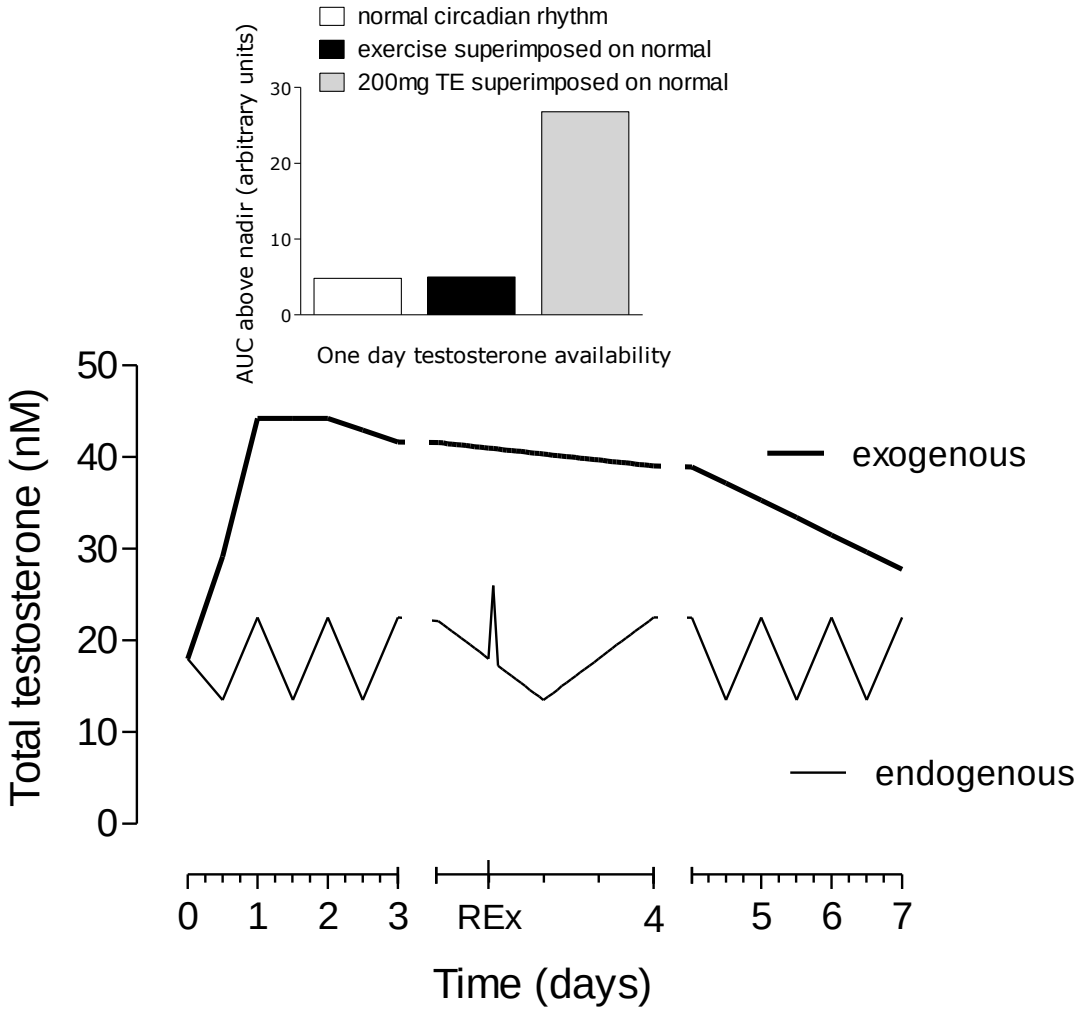

Figure 1. Exogenous and endogenous exercise-induced testosterone concentrations over the course of a week. Dark line at top of figure: exogenous testosterone after a single 200 mg intramuscular injection of testosterone enanthate (from Dobs et al., 1999, J.Clin.Endocrinol.Metab.). Note: 200 mg is a relatively mild dose; a dose of 600 mg induces peak testosterone of at least 100 nM (Bhasin et al., 1996, N.Engl.J.Med). Light line at bottom of figure: endogenous testosterone showing the circadian rhythm (peak =

0600 h, trough = 1800 h) (Diver et al., 2003, Clin.Endocrinol(Oxf). and testosterone after a bout of heavy resistance exercise at noon on Day 4 (minor ticks on X-axis indicate 6 h intervals).

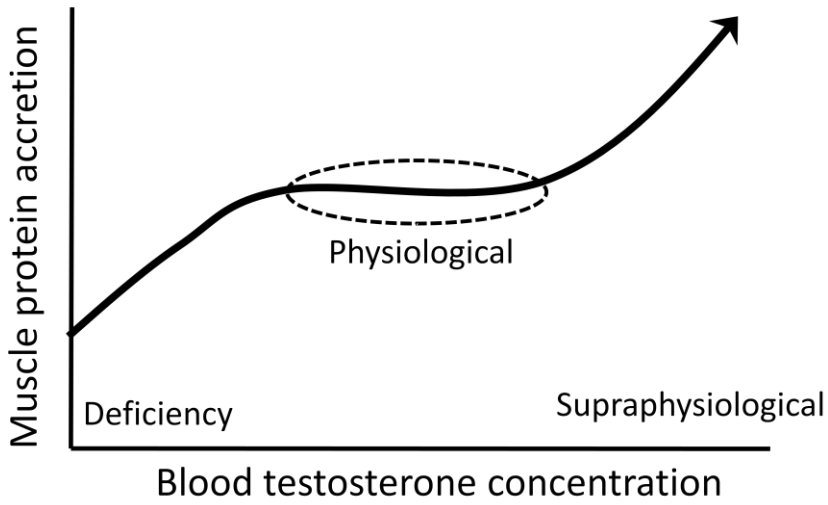

Figure 2. A simplified schematic drawn to illustrate, in very general terms, the hypothesized relationship between testosterone concentration and muscle protein accretion. The flat physiological section of the curve implies that physiological fluctuations in testosterone, including exercise-induced increases observed in studies within the present thesis, do not have a significant impact on muscle protein accretion.

5.5 BIOLOGICAL ROLES OF EXERCISE-INDUCED HORMONES AND FUTURE RESEARCH

The primary purpose of our studies was to investigate a potential contribution of exercise-induced hormones to skeletal muscle protein synthesis and hypertrophy. The fact that our data does not support a significant role for hormones in enhancing these

variables naturally raises the following question: What is the biological 'reason' or 'purpose' for the exercise-induced hormone response? Our studies were not designed to investigate other possible biological roles of the exercise-induced hormone response, which remain poorly understood. Nevertheless, the question is pertinent and often raised, motivating the following discussion.

While the biological reasons/purposes for exercise-induced hormonal elevations are largely unknown, possibilities include one or more of the following: i) substrate mobilization (59), ii) supporting remodelling or growth of connective tissue (6, 13), and/or iii) a biologically/evolutionarily-conserved 'fight or flight' stress hormone response to a stressor or challenge. There is at least some support for each of these possibilities; however, only the first possibility will be briefly discussed here. Readers can refer to other sources on psychoendocrinology as it may relate to the testosterone response (8). The effects, if any, of acute changes in GH/IGF-1 on connective tissue remodelling should also be examined. Undertaking cell culture experiments in which multiple cell types (e.g., fibroblasts, myoblasts and adipocytes) are treated with exercise-induced hormone-rich serum might serve as a starting point in determining how various tissues may be affected by an exercise-induced hormone response.

As mentioned, a potential reason/purpose/function of the collective acute exercise hormone response is that it is the product of a sympathetic nervous system response to acute exercise and helps mobilize energy stores. Early support for this notion comes from the observation (32) that adrenalectomized rats and rats with

sympathetic blockade have a poor exercise tolerance, and this poor tolerance is associated with impaired mobilization of glucose from glycogen and free fatty acids from triglyceride stores. There is evidence of hormonal interplay to produce available energy substrates. For example, physiologic epinephrine transiently increases hepatic glucose output and decreases disposal, and cortisol extends the duration of epinephrine's hyperglycaemic effect (47).

Much of what is known about hormones is derived from studies that use supraphysiological hormone doses as an intervention. The exercise-induced hormone response might be better replicated by giving individuals a low dose pharmacological 'pulse' of a single, or a combination of, hormones to match the exercise-induced response (magnitude and time course). This approach has been used to some extent to study growth hormone, and demonstrated a dose-response relationship between physiological growth hormone pulses and circulating free fatty acids and glycerol, as well as reductions in glucose uptake (non-dose dependent) (34). The decrease in glucose uptake was rapid and persistently depressed shortly after peak GH (34), whereas peak lipolysis was delayed, occurring approximately 2 h after peak GH (33). Thus, acute GH administration results in increased rates of lipid oxidation that may compensate for decreased rates of glucose oxidation from reduced uptake (34). The observation that GH is increased to a greater extent after acute cycling exercise at 70% VO_2 max (presumably a stimulus that does not induce hypertrophy) than after acute resistance exercise (presumably a hypertrophy-inducing stimulus) provides indirect but credible evidence

that the biological role of GH may be related to substrate use/mobilization rather than hypertrophy (20).

Alternatively to a pharmacological 'pulse', if off-target effects could be avoided, the exercise-induced hormone response could be suppressed and the effects examined. For example, what would be the effect of suppressing the exercise-induced growth hormone response using a selective growth hormone antagonist (45)? In either the hormone suppression or supplementation scenario, characteristic markers of potential effects of the hormone (e.g., cortisol: protein balance, glucose production, lipolysis, hyper-alaninemia/glutaminemia; GH: myofibrillar versus collagen synthesis, IGF-1 gene expression, lipolysis) could be investigated to help determine biological effects of the exercise-induced hormone response.

Another question concerning the biological effects of exercise-induced hormones is whether there is a role of the exercise-induced rise in cortisol other than a classical proteolytic effect. Whereas we have discussed the effects of GH and testosterone on skeletal muscle elsewhere (6, 64), cortisol is briefly highlighted here. It was outlined in the introduction of this thesis that cortisolemia is often examined after exercise, and that increased concentrations are often interpreted to be driving muscle catabolism. Instead, Viru and colleagues (58) contend that cortisol is essential for adaptation and perhaps "guilty by association with maladaptation". But even this latter point seems to be at odds with empirical data since cortisol concentrations are often elevated the most after resistance exercise schemes shown to elicit robust hypertrophy (26). A study from

our lab (63) correlated changes in exercise-induced hormones with changes in strength and hypertrophy in a large cohort (*n* = 56) after resistance training. While the data is associative, the cortisol response was significantly correlated (albeit weakly) with gains in lean body mass. Conversely, exercise-induced changes in testosterone, GH and IGF-1 responses were not. Similarly, the strongest correlation between these hormones and change in type I fibre area was with cortisol. These observations, combined with the report that cortisol infusion has no effect on fasting muscle net protein balance versus overnight fast (16), make it unlikely that exercise-induced cortisol is catabolic and detrimental to skeletal muscle adaptation.

While an impact of exercise-induced cortisol on net protein balance and/or hypertrophy has not been demonstrated, perhaps cortisol plays other biological roles in the context of exercise. One possibility is to assist in substrate mobilization for energy. Glucocorticoid treatment increases arterial amino acid concentrations (including large increases in the gluconeogenic amino acid alanine) and blood glucose (~25% increase) (31). Cortisol also extends the duration of physiologic epinephrine's hyperglycaemic action (47). In this way, cortisol appears to play a supportive role for the gluconeogenic action of epinephrine and glucagon by permitting activation of hepatic gluconeogenesis (15). In the physiological context, carbohydrate ingestion blunts the cortisol response to resistance exercise (5), suggesting that its secretion is sensitive to energy (and perhaps glucose per se) status of the body and not a marked driver of muscle protein catabolism. Collectively, these observations suggest that exercise-induced cortisol could contribute

to the maintenance of glucose homeostasis during or in the acute post-exercise period, and perhaps to a greater extent in unaccustomed exercise as we observed (62).

In general, one aspect that seems to have hindered our understanding of how exercise-induced hormones interact with skeletal muscle is that a descriptive approach has often been favoured. Oftentimes researchers have only measured systemic hormone concentrations and simply projected the physiological impact, or rationalized an outcome measure in a post-hoc manner using hormonal mechanisms that were demonstrated in extreme/pharmacological models. Indeed, assuming that the effects of pharmacological testosterone are the same in an exercise-induced context is raised as a criticism in this thesis and elsewhere (64). Part of the uncertainty of the role of exercise-induced hormones is derived from the inherent difficulty in isolating and determining the effects of physiological changes in hormone concentration. Measuring hormone concentrations concomitant with physiological measures of variables that can be affected by hormones will help, especially when an *a priori* hypothesis is established.

Finally, perhaps the mechanistic advantages of extreme models can be combined with a physiological approach to advance our understanding of the effects of exercise-induced hormones. While extreme models can complicate interpretations of normal physiology, they might be able to provide insight as to whether a given hormone has the same action in normal physiological and exercise circumstances based on whether 'telltale' characteristics are observed. For example, a sign of glucocorticoid activity in skeletal muscle is a reduction in glutamine content due to a hallmark efflux of glutamine

from the muscle cell (35) such that the large intracellular store is reduced, dramatically so in extreme cases (44). Glucocorticoids are also characteristically anti-inflammatory, suppressing expression of genes involved in initiating an inflammatory response (10). In the physiological scenario, however, the main question is whether exercise-induced increases in cortisol generate traces of these signs. Perhaps it is possible through 'omic' profiling methodologies to identify effects of exercise-induced cortisol, or other exercise-induced hormones, through an omic signature.

5.6 CONCLUSIONS AND IMPLICATIONS

This thesis contains data from three independent experiments that have demonstrated that the hormonal milieu generated by an acute bout of resistance exercise is not an important regulator of myofibrillar protein synthesis or hypertrophy. Furthermore, studying rates of MPS, at a physiologic exercise- and feeding- induced maximum, in men and women demonstrated no significant sex-based differences despite a 45-fold greater post-exercise testosterone response in men. The lack of an observable deficit in the ability of young women to elevate MPS after resistance exercise with feeding, despite virtually no testosterone response, brings into question the relevance of the exercise-induced testosterone response as mediator or indicator of post-exercise skeletal muscle anabolism.

The collective findings of this thesis have implications for both applied and basic sciences standpoints. From an applied standpoint, it means that exercise programs

should not be designed based on nuances in the post-exercise hormonal milieu. Further, it means that small muscle groups do not need to be paired with large muscle groups to benefit from hormonal elevations stimulated by large muscle group exercise. Thus, in general, a broad 'message' from this thesis is that, for the average person, resistance exercise does not need to be complicated, at least not from a program design standpoint in attempting to optimize hormonal patterns. This message is based on the fact that our data are contrary to principles (assumptions?) that are currently widely used to create exercise programs that are based on post-exercise hormone responses (28).

Our data also provide mechanistic information that the anabolic capacity of women is not impaired by their lack of an acute testosterone response to an exercise bout. Instead, intramuscular processes that are common to men and women are likely responsible for eliciting adaptation after resistance exercise. As a result of the collective findings of this thesis, we suggest that local processes, intrinsic to the muscle fibres themselves and directly linked to their mechanical loading, are primarily responsible for contraction-induced muscle protein synthesis and accretion. While a full analysis of mechanotranduction in skeletal muscle is beyond the scope of the present discussion, several potential targets include the following: stretch-activated calcium signalling, growth factors, and structural complexes that transmit tension. Thus, a natural progression from this thesis work is to attempt to define how mechanotransduction regulates skeletal muscle mass.

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APPENDIX 1: EVIDENCE OF THE PREVALENT VIEWPOINT (THESIS) THAT EXERCISE-INDUCED HORMONE RESPONSES MEDIATE ADAPTATIONS TO RESISTANCE TRAINING

Below is a sampling of quotations/articles, presented arbitrarily in a reverse chronological order, that heed a hormone hypothesis. That is, they assume (i.e., supporting data or references are not provided) that the exercise-induced response of a given hormone mediates adaptations to resistance training. In general, these articles often make statements about an effect of a given hormone in *some* circumstance, one that is often non-physiological, thereby (apparently) justifying the purpose and subsequent findings of the given study.

For example:

1. Testosterone/growth hormone is a potent stimulator of protein synthesis (references optional).

2. Physiological hormone responses may modulate anabolism in post-exercise recovery (this point is optional; one or no original research articles cited).

3. Therefore, we examined the acute pattern of testosterone/growth hormone after resistance exercise.

Note: Below, numbers in parentheses that precede the abbreviated citation refer the reader to the full citation below, whereas numbers in parentheses that are within the quotations are citations from the original articles.

(5) Giannoulis et al., 2012, Endocrine Reviews

“GH, IGF-I, and testosterone (T) are directly involved in muscle adaptation to exercise because they promote muscle protein synthesis, whereas T and locally expressed IGF-I have been reported to activate muscle stem cells.”

(4) DeFrietas et al., 2011, EJAP

“…studies have shown that the total amount of muscle mass recruited and/or the total work performed affects the systemic anabolic hormone responses that are necessary for muscle growth.”

(10) Nakamura, 2011, Med.Sci.Sports Exerc.

“The responses of anabolic hormones to acute resistance exercise were different depending on the menstrual cycle state, suggesting that menstrual cycle state may influence the exercise training–induced skeletal muscular adaptation. Thus, it would be possible that training programs for eumenorrheic women might be timed in accordance with the menstrual cycle to maximize anabolic effects.”

(13) Schoenfeld, 2010, J.Strength.Cond.Res.

“However, acute [testosterone] responses are limited in women and the elderly, mitigating the hypertrophic potential in these populations (61,90,130).”

“Resultant to metabolic buildup, moderate repetition range training has been shown to maximize the acute anabolic hormonal response of exercise. Both testosterone and GH are acutely elevated to a greater degree from routines employing moderate rep sets as compared to those using lower repetitions (57,90,92,94,114), thereby increasing the potential for downstream cellular interactions that facilitate remodelling of muscle tissue.”

“Moreover, split routines can serve to increase muscular metabolic stress by prolonging the training stimulus within a given muscle group, potentially heightening acute anabolic hormonal secretions, cell swelling, and muscle ischemia.”

“Multijoint exercises recruit large amounts of muscle mass to carry out work. This has an impact on the anabolic hormonal response to training.”

“Multiple sets should be employed in the context of a split training routine to heighten the anabolic milieu.”

(14) Vingren et al 2010, Sports Med.

“Testosterone is important for the desired adaptations to resistance exercise and training; in fact, testosterone is considered the major promoter of muscle growth and subsequent increase in muscle strength in response to resistance training in men…In general, the variables within the acute programme variable domains must be selected such that the resistance exercise session contains high volume and metabolic demand in order to induce an acute testosterone response.”

(9) Migiano et al., 2009, J.Strength.Cond.Res.

“It is important to pay attention to the amount of muscle mass utilized in a resistance exercise protocol to optimize endocrine signaling.”

(12) Roberts et al., 2009, J.Strength.Cond.Res.

“Additionally, resistance training involving large muscle groups transiently increases the postexercise concentrations of free testosterone, total testosterone, and human growth hormone (hGH), but this response is blunted in older men (19). These hormonal decrements are hypothesized to lower resistance training–induced increases in strength and muscle mass (19).”

(2) Copeland et al., 2008, Int. J. Sports Med.

“Exercise-induced responses in the IGF-I system likely play a role in stimulating training adaptations, which means knowledge of how different exercise protocols influence the IGF system is potentially valuable. If we can manipulate training programs to provide optimal stimulation of the IGF-I system this may facilitate greater improvements in muscle strength, body composition and fitness.”

(1) Baker et al., 2006, J.Strength.Cond.Res.

“On a practical application level, older men can complete a high-intensity resistance exercise program resulting in spikes in T that may attenuate age-related muscle and BMD loss.”

(3) Crewther et al., 2006, Sports Med.

“The design of the resistance exercise programme, or scheme design, underpins the adaptive response to resistance training by modifying the acute hormonal responses. Consequently, examining the hormonal response to different strength and power schemes would provide a better understanding of the hormonal contribution to adaptation associated with the repeated application of these lifting methods.”

(8) Kraemer and Ratamess, 2005, Sports Med.

“In general, the acute response is dependent upon the stimulus (e.g., intensity, volume, muscle mass involvement, rest intervals, frequency) and may be the most critical element to tissue remodelling.”

(7) Kraemer et al., 2004, Med.Sci.Sports Exerc.

“Deadlifts (25), squat jumps (105), and Olympic lifts (67) have produced greater acute 22-kDa growth hormone and testosterone responses compared with exercises such as the bench press and seated shoulder press. Thus, the amount of muscle mass involved in a movement significantly impacts the acute metabolic demands and anabolic hormonal response, which have direct implications for resistance training programs targeting improvements in local muscle endurance, lean body mass, and reductions in body fat.”

(15) Willoughby et al., 2004, Med.Sci.Sports Exerc.

“Increased serum testosterone (TST) occurs in response to resistance exercise and is associated with increased muscle mass.”

(11) Nindl, 2000, Growth Horm.IGF.Res.

“It is important to know the impact of exercise on immunofunctional growth hormone as exercise mediated increases in growth hormone are thought to modulate the anabolic and metabolic outcomes of physical activity.”

(6) Kraemer, 2000, Essentials of Strength and Conditioning

"It is important for strength and conditioning professionals to have a basic understanding of the hormonal responses to resistance exercise. Such knowledge increases insight into how exercise prescription can enable hormones to mediate optimal adaptations to RE (76, 91)."

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APPENDIX 2: SUPPLEMENTARY DATA RELATING TO THE EXPERIMENTAL DESIGN DISCUSSION IN CHAPTER 5

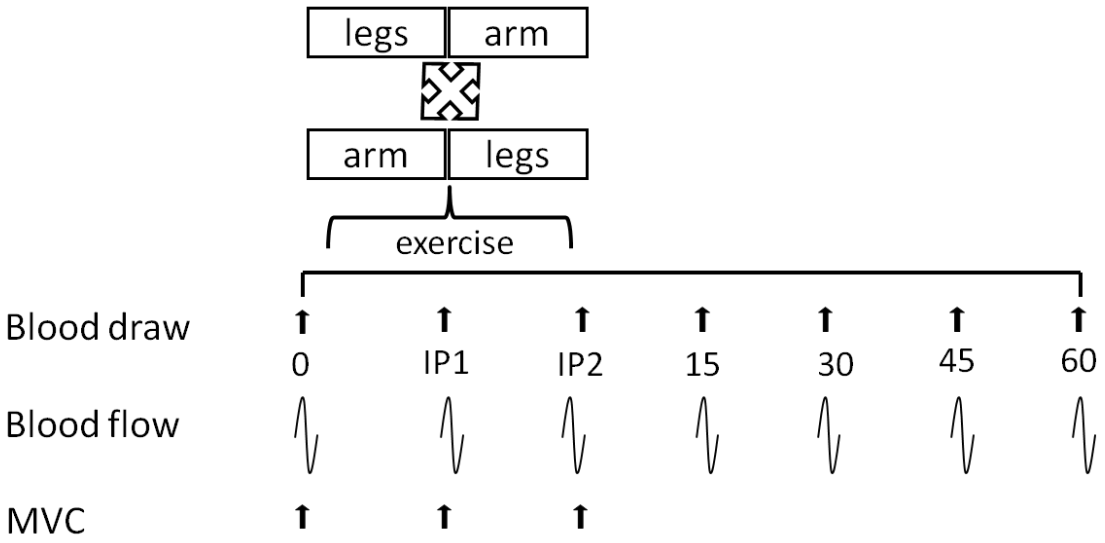

Figure 3. Schematic of the experimental protocol. The study was a within-subject cross-over design. Leg exercise was bilateral, consisting of 5 sets × 10 reps leg press, 3 × 12 hamstring curl – knee extension superset. Arm exercise was unilateral, consisting of 4 sets × 8-12 reps elbow flexion.

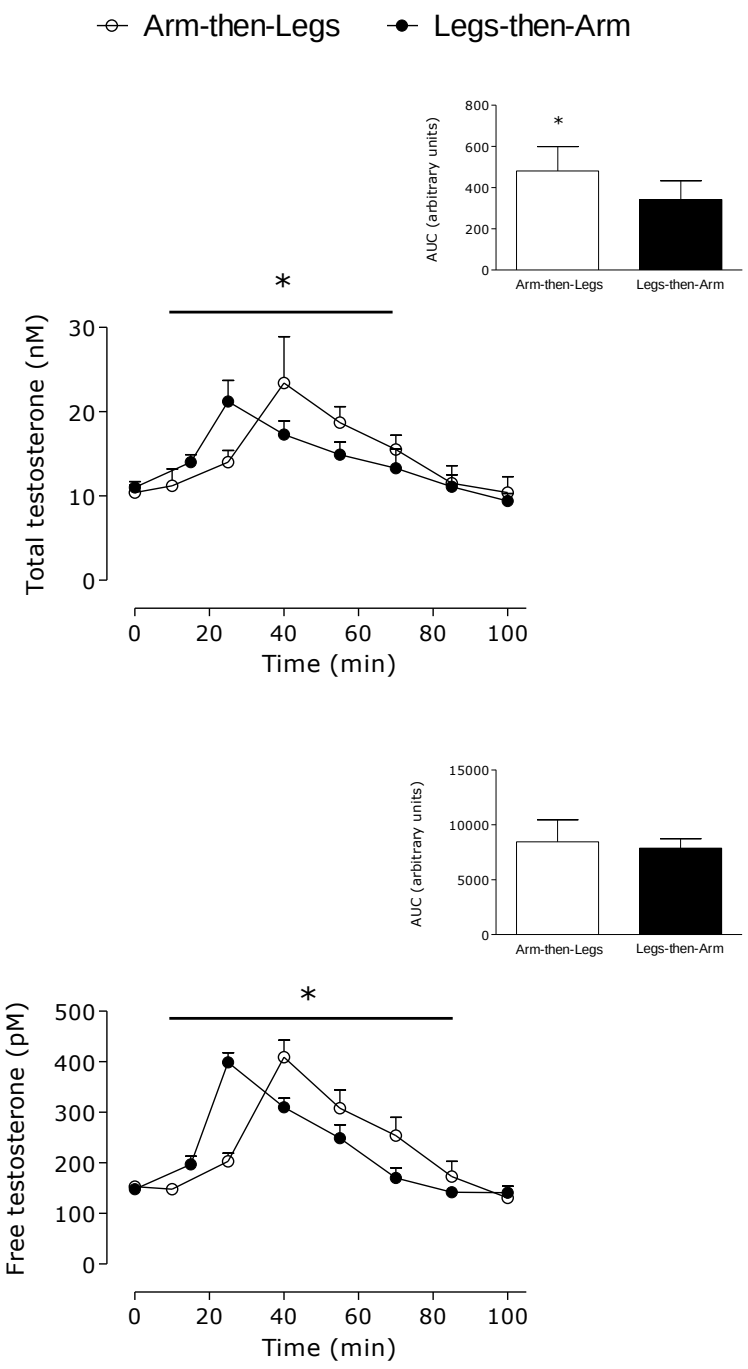

Figure 4. Total (top) and free (bottom) testosterone concentrations. *Significant difference ($P < 0.05$) between conditions at same time point.

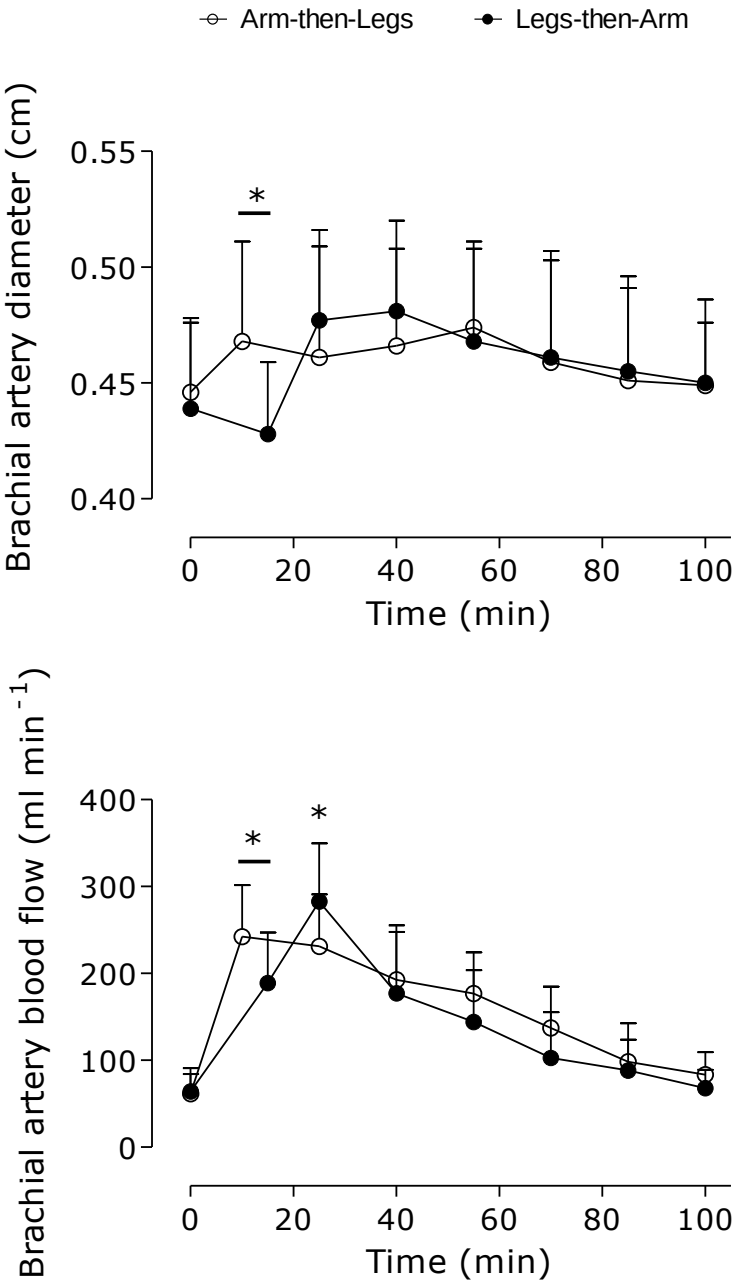

Figure 5. Brachial artery diameter (top) and blood flow (bottom). *Significant difference ($P < 0.05$) between conditions at same time point.

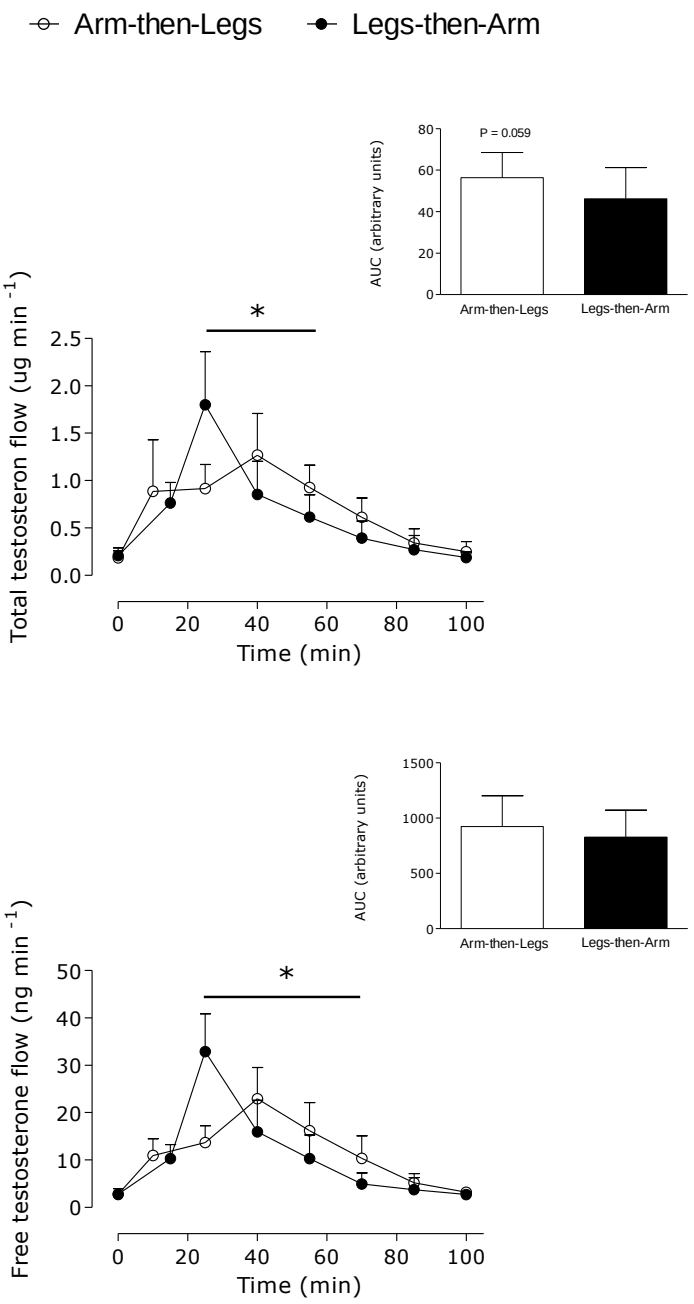

Figure 6. Estimated delivery of total (top) and free (bottom) testosterone to muscles supplied by the brachial artery. *Significant difference ($P < 0.05$) between conditions at same time point.