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Concurrent aerobic exercise interferes with the satellite cell response to acute resistance exercise in MHC I muscle fibers

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Concurrent Aerobic Exercise Interferes With the Satellite Cell Response to Acute Resistance Exercise in MHC I Muscle Fibers

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ABSTRACT

Purpose: Concurrent training attenuates hypertrophy compared to resistance training alone, and does so in a fiber-type specific manner. The mechanism responsible for this 'interference' is unclear, and satellite cell physiology, an important hypertrophic factor, has not been examined within this context. Therefore, the purpose of this investigation was to assess the fiber-type specific satellite cell response to acute resistance, aerobic and concurrent exercise. **Methods:** Eight recreationally active college-aged males (23±1 yrs, 83.4±3.6 kg, 181±2 cm, and 48.5±1.6 ml/kg/min) performed 3 sets of 10 repetitions with a fourth set ≥ 10 repetitions at 75% of 1RM for both unilateral leg extensions and unilateral leg press for acute resistance exercise. Ten days later subjects performed the same resistance exercise with the opposite leg followed by 90 minutes of cycling at 60% VO_{2max} to represent acute concurrent and aerobic exercise. Muscle biopsies were obtained immediately before and 4 days after each exercise session. Muscle samples were cross sectioned and stained with for NCAM, Ki-67, DAPI and MHC I via immunohistochemistry to assess satellite cells, activated satellite cells and fiber-type, respectively. **Results:** Total satellite cell number per fiber increased only in response to acute resistance exercise (+38±10%, $p < 0.05$), with no change following acute aerobic or concurrent exercise. Changes in total satellite cell number per fiber between resistance, aerobic and concurrent exercise differed only in MHC I fibers ($p < 0.05$), with no satellite cell number per fiber by mode interaction observed in non-MHC I muscle fibers. No changes in activated satellite cells were observed under any condition. **Conclusion:** Acute concurrent exercise blunts the satellite cell response of resistance exercise alone, and does so in a fiber-type specific manner by negating the satellite cell response in MHC

I, but not non-MHC I fibers. These results suggest that the interference effect of concurrent resistance training on MHC I hypertrophy may be regulated at the satellite cell level.

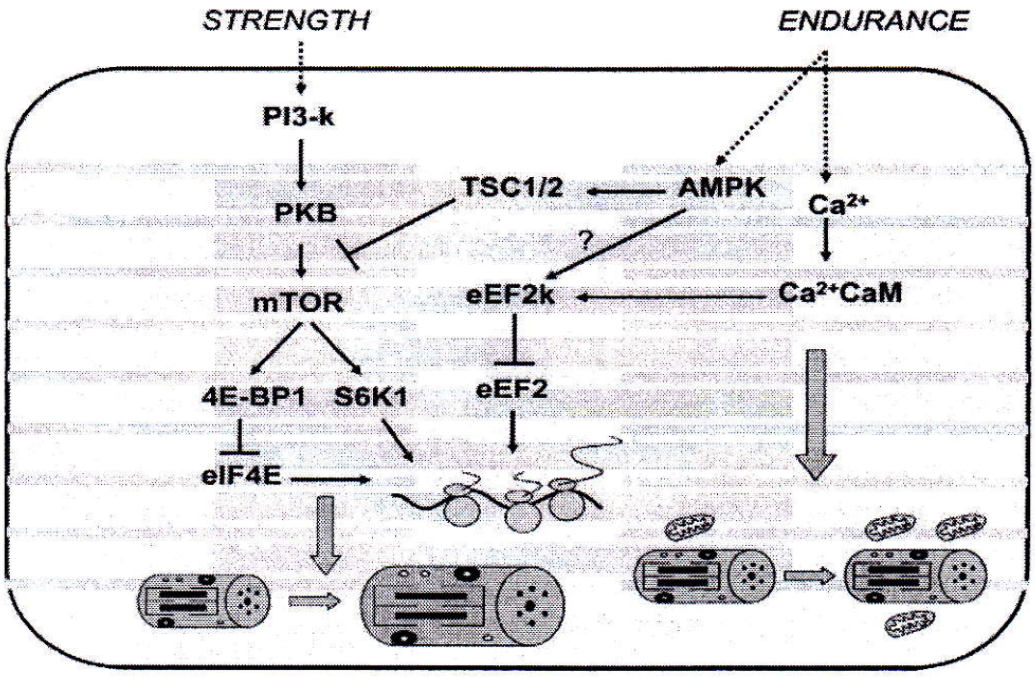
CHAPTER ONE - INTRODUCTION

Resistance exercise training (RE) increases myocellular and whole muscle size and strength. Concurrent training, RE combined with aerobic exercise (AE), has been shown to disrupt maximum muscle size and strength gains elicited by RE alone (22, 26, 27). Skeletal muscle size and strength greatly affect sports performance, occupational performance, and quality of life. Skeletal muscle health places a marked burden on the healthcare system. For instance, sarcopenia, the age-related loss of muscle mass and function, costs the US Healthcare system an estimated $18.5 billion annually (30). Concurrent training is common among athletes, individuals in weight loss programs, astronauts in space and older individuals. It would therefore be beneficial to design concurrent training programs that elicit size and strength gains more similar on magnitude to RE alone. However, to accomplish this, it is necessary to gain a more complete understanding of the mechanisms that underlie concurrent training adaptations.

The molecular regulation of muscle growth has gained considerable attention over the past 15 years. RE-induced hypertrophy primarily results from an accumulation of myofibrillar protein, which is facilitated through alterations in skeletal muscle contractile protein balance. This level of specificity is accomplished through a highly sophisticated network of intracellular molecular signals. Concurrent training has been hypothesized to 'confuse' the signaling events necessary for maximum hypertrophy. For example, AMP-activated-protein-kinase (AMPK), an inhibitor of muscle protein synthesis (e.g. mTOR pathway), is activated in response endurance exercise (50, 64), which would theoretically make it more difficult to accumulate intracellular proteins (Figure 1.1). Although an

attractive hypothesis, Tipton et al. demonstrated that post-exercise *mixed* muscle protein synthesis was heighted with concurrent training compared to RE alone (65). Carrithers et al. was more specific and found that the rate of post-exercise myofibrillar protein synthesis does not differ between a concurrent exercise stimulus and RE alone (10). These data suggest that mechanisms other than post-exercise protein synthesis rates are responsible for the interference effect of concurrent training.

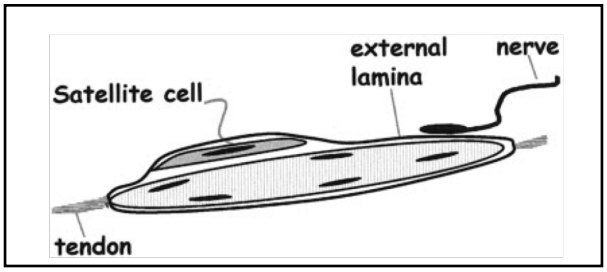
Figure 1.1



Proposed intracellular signaling for strength and endurance exercise, highlighting the incompatible cellular responses (50)

Recent data has implicated the role of satellite cells in skeletal muscle hypertrophy (37, 52). For example, the ablation of satellite cells has been shown to inhibit muscle growth in a resistance-training model (37). The term 'satellite' cell was coined to describe their spatial position around muscle cells, just like satellites in orbit around the earth (1). During muscle fiber hypertrophy (the only multinucleated cell in humans) the area surrounding each nucleus, or myonuclear domain, expands while satellite cells differentiate into the fiber in a presumable attempt to maintain a healthy domain. To accomplish this, satellite cells are first stimulated from a quiescent (inactive) state to an active state. They then proliferate (divide) and differentiate into the myofiber as a new nucleus. In response to acute RE satellite cells proliferate up to 8 days after a single bout (18), with no such evidence following acute AE. To our knowledge, no studies have examined satellite cell proliferation in response to concurrent training. Although their *magnitude* of importance is contentious, satellite cells are active during skeletal muscle hypertrophy in a presumable attempt to maintain a healthy myonuclear domain. It is therefore possible that concurrent exercise disrupts the satellite cell response to RE, consequently diminishing the magnitude of hypertrophy elicited by RE alone.

Figure 1.2



Anatomical orientation of satellite cells (69)

Some of the molecules that regulate and promote muscle growth also regulate satellite cell activity (25), namely the two splice variants of IGF-1, and myostatin. In response to mechanical loading (e.g. exercise), mechano-growth factor (MGF) is synthesized in skeletal muscle (56), and IGF-1Ea is produced in the liver and circulated (15, 25). MGF stimulates satellite cell activation and proliferation (16, 25) while myostatin is a negative regulator of muscle fiber hypertrophy (9, 34, 71) and satellite cell proliferation (34, 46). The genes that encode these proteins are differentially expressed in response to RE and AE training. Both respond to acute resistance exercise in a manner that initiates satellite cell activity and promotes hypertrophy (19, 25, 34, 38, 47, 56). Specifically, in response to acute RE, MGF mRNA is upregulated in skeletal muscle (19, 34, 47, 56), and has been shown to remain elevated up to 120 hours after a single bout, peaking around 24 hours (47). Myostatin decreases in response to both acute RE (34, 38, 59) and AE (38, 43), however the response is exaggerated after RE (38). Interestingly, acute concurrent exercise has been shown to attenuate the gene expression of both IGF-1Ea and MGF (13, 14). The post-exercise behavior of genes that regulate skeletal muscle hypertrophy and satellite cell activity suggest that concurrent exercise could attenuate the satellite cell response to RE alone.

Skeletal muscle is comprised of two primary types of muscle fibers: myosin heavy chain I (MHC I, slow twitch) and MHC IIa (fast twitch) fibers, with each making different but important functional contributions to whole muscle. Concurrent training has been shown to completely negate the MHC I hypertrophy that is traditionally observed with RE alone and to attenuate MHC IIa fiber hypertrophy (35, 45, 57, 58) Satellite cell numbers do not differ between MCH I and MHC IIa fibers in young untrained individuals

(31) and nothing is known about fiber-type specific satellite cell proliferation in response to acute exercise. It is plausible that satellite cell proliferation is mediated in a fiber-type specific manner similar to the aforementioned hypertrophic tendencies of each fiber type.

Purpose

The purpose of this study is to investigate potential mechanisms responsible for the attenuated growth response to concurrent AE and RE training when compared to RE training alone.

Aims and Hypothesis

Aim 1 - Quantify the exercise-provoked increase in satellite cell number following RE, AE and CE.

Hypothesis 1 - The exercise-provoked increase in satellite cell number will be mode-dependant. Specifically, the extent of the satellite cell response will rank as follows: resistance exercise > concurrent exercise > aerobic exercise.

Aim 2 – Quantify the exercise-provoked increase in *active* satellite cells following RE, AE and CE.

Hypothesis 2 – The exercise-provoked increase in *active* satellite cells will be mode-dependant. Specifically, the extent of the satellite cell response will rank as follows: resistance exercise > concurrent exercise > aerobic exercise.

Aim 3 - Quantify the exercise-provoked increase in satellite cell number in both MHC I and MHC II fibers.

Hypothesis 3 - The exercise-provoked increase in satellite cell number will vary according to fiber type. Specifically, the exercise-provoked increase in satellite cell number will be greater in MHC II fibers compared to MHC I fibers.

Aim 4 – Quantify the exercise-provoked increase in *active* satellite cells in both MHC I and MHC II fibers.

Hypothesis 4 – The exercise-provoked increase in *active* satellite cells will vary according to fiber type. Specifically, the exercise-provoked increase in *active* satellite cells will be greater in MHC II fibers compared to MHC I fibers.

Significance

Adaptations elicited by AE and RE training generally do not compliment each other at the hormonal, single fiber or whole muscle level, and the maximum training adaptations to both are attenuated when performed together (64). However, the molecular physiology that regulates this phenomenon is unknown. The mechanisms by which AE interferes with the responses elicited by RE have significant implications not only for sports performance, but for the maintenance of astronauts' health while in space and countermeasures against the age related loss of skeletal muscle size and strength. Understanding the mechanisms responsible for the attenuated training responses to concurrent exercise may aid in the design of more effective interventions that elicit size

and strength gains from concurrent training programs that are similar in magnitude to RE alone.

CHAPTER TWO – REVIEW OF LITERATURE

Objectives

The objectives of this chapter are to provide an overview of: 1) the effects of concurrent training on muscle size and strength, 2) the effect of acute concurrent exercise on protein synthesis, 3) the role of satellite cells during hypertrophy, 4) the hormonal regulation of satellite cells, 5) the hormonal response to acute resistance, aerobic and concurrent exercise, 6) the response of satellite cells to acute exercise, 7) the satellite cell response to chronic exercise, 8) and the fiber-type specific response of satellite cells to chronic exercise.

Skeletal Muscle Size and Strength Adaptations to Concurrent, Aerobic and Resistance Training

Chronic resistance exercise elicits significant gains in whole muscle size (6, 51) and strength (26, 27, 35) while aerobic training confers minimal, if any gains (7, 20, 27). Concurrent training, a combination of resistance and aerobic training, has been shown to attenuate hypertrophy and strength gains compared to resistance training alone (7, 20, 24, 27, 35). Interestingly, the impact of concurrent training at the cellular level appears to occur in a fiber-type specific manner. Specifically, skeletal muscle is comprised of two primary muscle fiber types: slow twitch (MHC I) and fast twitch (MHC II). Although not with out some contention (51), it largely appears that resistance training elicits significant increases in both MHC I and II fiber cross sectional area (CSA) (7, 24, 35, 45, 58),

whereas concurrent training increases CSA only in MHC IIa and blunts MHC I hypertrophy (7, 12, 24, 35, 45, 58).

Hickson and colleagues were the first to demonstrate that concurrent exercise can attenuate the strength gains elicited by resistance training alone (27). The authors investigated the impact of aerobic, resistance and concurrent training on peak squat strength and reported that resistance training elicited an increase of 44%, whereas concurrent training improved strength by only 25%. Others have documented similar findings, with squat strength increasing by 47% and 34% in response to resistance and concurrent training, respectively (68), bench press strength increasing 21% and 1% in response to resistance and concurrent training, respectively (26) and chest press strength increasing 24% and 19% in response to resistance and concurrent training, respectively (20). Aerobic training does not typically result in large strength gains (26, 27). However, a 23% increase in squat strength from 12 weeks of aerobic training has been reported (68). In contrast to reports of a concurrent training interference effect on strength, McCarthy et al. reported no interference in strength gains when resistance was performed concurrently with endurance (44). This finding is generally supported by Sale et al., but one leg served as resistance and the contralateral leg served as concurrent, so the possibility of an inhibitory systemic effect of concurrent exercise cannot be ignored (62).

Resistance training is the most effective method of increasing whole muscle size (6). In contrast, aerobic training elicits no increase in whole muscle size (45) with the most notable adaptation to aerobic training being an increase in aerobic power (20). Interestingly, concurrent training has been shown to interfere with the adaptations elicited by both resistance and aerobic training alone (6, 20, 27, 35). The interference effect that

concurrent training has on whole muscle growth has provided mixed results. Izquierdo et al. found no difference in the magnitude of hypertrophy of the vastus lateralis in response to 16 weeks of resistance, concurrent and aerobic training. However, the authors did report attenuated hypertrophy in the biceps brachii following concurrent training compared to resistance training (29). In this model, aerobic training performed only by the lower body interfered with the adaptations of a muscle in the upper body. This provides strong evidence of a systemic interference rather than a localized interference. In contrast, one study detected no significant differences in the whole muscle size gains of the quadriceps femoris between resistance and concurrent training groups following 10-weeks of concurrent training (45). However, 10 weeks may not be a sufficient duration to allow for differences in whole muscle size adaptations to be revealed. Differences in the time course of the adaptations have been noted, with a non significant plateau in size gains elicited by concurrent training, although there was no difference in the overall effect between concurrent and resistance training (6).

Resistance training results in significant hypertrophy of both MHC I and IIa fibers (7, 24, 35, 45, 58), with some data indicating greater hypertrophy in MHC I fibers compared to MHC IIa (24, 58). In contrast, aerobic exercise does not typically increase the size of either fiber type (7, 45, 58), although there are exceptions (35). Studies investigating fiber type specific size adaptations to resistance and concurrent training convincingly indicate that the interference effect of concurrent training is manifested in a fiber-type specific manner. Although Bell et al. found that both MHC I and MHC IIa muscle fiber hypertrophy is blunted with concurrent training (7), others show minimal or no MHC I hypertrophy (12, 24, 35, 45, 58). For example, similar MHC IIa muscle fiber

growth has been observed between resistance (26%) and concurrent (22%) training programs, with a concomitant attenuation of growth in MHC I fibers (concurrent vs. resistance = 13% vs. 46%) (24). McCarthy (45), Putnam (58) and Kraemer (35) all reported similar increases in the cross sectional area of MHC IIa fibers from concurrent and resistance training programs, MHC I fiber growth only resulting from resistance training and no change in MHC I fiber cross sectional area in response to concurrent training.

The interference between resistance and endurance training when performed together has profound effects on exercise-induced adaptations when compared to resistance training alone. The literature consistently reports attenuated whole muscle strength in response to concurrent compared to resistance training alone. Although reports of attenuated whole muscle size gains are contentious, the fiber type specific interferences are compelling and appear to prevent the hypertrophic effect of resistance training on MHC I fibers.

Table 2.1 Skeletal Muscle Strength Adaptations to Concurrent, Aerobic and Resistance Training

Author/Year	Subjects	Duration	Group/Design	Variables	Results
Bell 2000 (6)	45 males/females	12 Weeks	RE • 3 d/wk • 2-6 sets • 4-10 reps • 70-85% 1RM	LP 1RM QF 1RM	+56% +40%
			AE • cycle ergometer • 3 d/wk • 30-42 min/session at VT	LP 1RM QF 1RM	No Change No Change
			CE • the above protocols performed on alternate days	LP 1RM QF 1RM	+53% +28%
Bell 1991 (5)	31 subjects with previous RE or AE experience	12 Weeks	RE • Low velocity resistance • 3 d/wk	QF PF	+11%
			CE • RE protocol + 3 days/week endurance	QF PF	+8%
Dolezal 1998 (20)	30 physically active men	10 weeks	RE • 3 d/wk	MAP 1RM CP 1RM PS	-0.2% +24% +23%
			AE • 3 d/wk • Jogging	MAP 1RM CP 1RM PS	+13%* No Change No Change
			CE • Combination of both RE and AE	MAP 1RM CP 1RM PS	+7% +19% +12%
Hickson 1980 (27)	recreationally active men	10 Weeks	AE • 6 d/wk • Alt days of cycling and running	Parallel Squat Strength	No Change
			RE 5 d/wk lower body lifts		+44%
			CE RE+AE separated by 2 hours rest		+34%

Table 2.1 Skeletal Muscle Strength Adaptations to Concurrent, Aerobic and Resistance Training (Continued)

Author/Year	Subjects	Duration	Group/Design	Variables	Results
Hennessy 1994 (26)	56 Rugby Players	8 Weeks	RE • d/wk • 2-6 set • 4-10 reps • 70-85% 1RM	Bench Press Strength	+21%
				Squat Strength	+18%
			AE • cycle ergometer • 3 d/wk • 30-42 min at VT	Bench Press Strength	No Change
				Squat Strength	No Change
			CE RE and AE on alternating days	Bench Press Strength	+1%
				Squat Strength	+5%
Izquierdo 2005 (29)	31 men 40-46 years old	16 weeks	RE • 2 d/wk	HS Strength	+45%
				CP Strength	+37%
			CE • RE 1 d/wk • AE 1 d/wk	HS Strength	+37%
				CP Strength	+15%
			AE • 2 d/wk	HS Strength	+9%
				CP Strength	No Change
McCarthy 1995 (44)	30 Sedentary adult males	10 weeks	RE • 3 d/wk • 8 exercises 3 x 5-7 reps	1RM Squat	+23%
				1RM CP	+18%
				IKE	+12%
				VJ	+6%
			AE • 3 d/wk • 50 min cycling 70% HRR	1RM Squat	No Change
				1RM CP	No Change
				IKE	No Change
				VJ	No Change
			CE • RE+AE 10-20min rest between	1RM Squat	+22%
				1RM CP	+18%
				IKE	+7%
				VJ	+9%

Table 2.1 Skeletal Muscle Strength Adaptations to Concurrent, Aerobic and Resistance Training (Continued)

Author	Subjects	Duration	Group/Design	Variables	Results
Kraemer 1995 (35)	35 physically active Army soldiers	12 weeks	CE • RE protocol • AE Protocol • 4 d/wk • Separated by 5-6 h	CP Strength LP Strength	+23% +15%
			CE / UB RE Only RE protocol for UB only	CP Strength LP Strength	+27% No Change
			RE • 3x10 reps 5 UB / 4 LB	CP Strength LP Strength	+30% +29%
			AE • Long runs 2 d/wk Intervals 2 d/wk	CP Strength LP Strength	No Change No Change
Nelson 1990 (51)	14 active healthy men	20 weeks / 4 training sessions per week	Increased Torque Endurance Increased Torque + Endurance	QF strength QF strength QF strength	+29% No Change +34%
Sale 1990 (62)	22 weeks	21 Weeks	RE one leg, CE on the other AE one leg, CE on the other	Strength Endurance Strength Endurance	No interference with strength or endurance
Wood 2001 (68)	36 old but healthy individuals	12 Weeks	AE • 3 d/wk • 45 min • 60-75% HRR RE • 3 d/wk • 8 exercises • 1-2x8-12 reps CE • 3 d/wk • AE limited to 30 min • RE limited to 1 set	5 RM tests for leg ext, leg curl, seated row, chest press, lateral raise, seated dip and bicep curl	+23% except for chest press +47% +34%

RE = Resistance Exercise, AE = Aerobic Exercise, CE = Concurrent resistance and aerobic exercise, RM = Repetition Maximum, Ext = Extension, VT = Ventilatory Threshold, Reps = Repetitions, LP = Leg Press, Alt = alternating, QF = Quadriceps Femoris, HHR = Heart Rate Reserve, UB = Upperbody, LB = Lower Body, PF = Peak Force, CP = Chest Press, HS = Half Squat, d/wk = Days per Weeks, IKE = isometric knee extensions, VJ = vertical jump

Table 2.2 Skeletal Muscle Size Adaptations to Concurrent, Aerobic and Resistance Training

Author	Subjects	Duration	Group/Design	Variables	Results
Bell 1991 (5)	31 subjects with previous CE or RE experience	12 Weeks	RE • Low velocity resistance • 3 d/wk	QF CSA	+5.4%
			CE RE protocol + 3 d/wk endurance	QF CSA	+4.16
Bell 2000 (6)	45 males/females	12 Weeks	RE • 3 d/wk • 2-6 sets • 4-10 reps • 70-85% 1RM	MHC I CSA	+27%
				MHC IIa CSA	+28%
			AE • cycle ergometer • 3 d/wk • 30-42 min per session at VT	MHC I CSA	No Change
				MHC IIa CSA	No Change
			CE • RE and AE protocols performed on alternate days	MHC I CSA	+11%
				MHC IIa CSA	+14%
Chilibeck 2002 (11)	10 untrained; 5 males and 5 females	12 weeks	CE • Periodized training program	MHC I CSA	No Change
				MHC IIa CSA	+15%
Hakkinen 2003 (24)	27 healthy males	21 Weeks	RE • 2 d/wk • 3-5 sets • 8-12 reps • 50-85% 1RM	MHC I CSA	+46%
				MHC IIa CSA	+26%
			CE Above resistance program with 2 d/wk running and cycling	MHC I CSA	+13%
				MHC IIa CSA	+22%

Table 2.2 Skeletal Muscle Size Adaptations to Concurrent, Aerobic and Resistance Training (Continued)

Author	Subjects	Duration	Group/Design	Variables	Results
Izquierdo 2005 (29)	31 men 40-46 years old	16 weeks	RE • 2 d/wk	BB CSA	+9%
				QF CSA	+14%
			CE • RE 1 d/wk • AE 1 d/wk	BB CSA	No Change
				QF CSA	+12%
			AE • 2 d/wk	BB CSA	No Change
				QF CSA	+10%
Kraemer 1995 (35)	35 physically active Army soldiers	12 weeks	CE • RE protocol • AE Protocol • 4 d/wk • Separated by 5-6 h	MHC I CSA	No Change
				MHC IIa CSA	+18%
			CE / UB RE RE protocol for UB only	MHC I CSA	No Change
				MHC IIa CSA	No Change
			RE • 3x10 reps • 5 UB / 4 LB	MHC I CSA	+11%
				MHC IIa CSA	+21%
			AE • Long runs 2 d/wk • Intervals 2 d/wk	MHC I CSA	-11%
				MHC IIa CSA	No Change
McCarthy 2002 (45)	30 Sedentary adult males	10 Weeks	RE • 3 d/wk • 8 exercises • 3 x 5-7 reps	MHC I CSA	+19%
				MHC IIa CSA	+24%
				QF CSA	+6%
			AE • 3 d/wk • 50 min cycling 70% HRR	MHC I CSA	No Change
				MHC IIa CSA	No Change
				QF CSA	No Change
			CE • RE+AE • 10-20min rest between	MHC I CSA	No Change
				MHC IIa CSA	+28%
				QF CSA	+9%
Nelson 1990 (51)	14 active healthy men	20 weeks / 4 training sessions per week	Increased Torque	MHC I CSA	+10%
				MHC IIa CSA	-4.8%
			Endurance	MHC I CSA	+8.1%
				MHC IIa CSA	+5%
			Increased Torque + Endurance	MHC I CSA	+25%
				MHC IIa CSA	-20%

Table 2.2 Skeletal Muscle Size Adaptations to Concurrent, Aerobic and Resistance Training (Continued)

Author	Subjects	Duration	Group/Design	Variables	Results
Putman 2004 (58)	24 males/16 femals	12 weeks	RE • 3 d/wk • 4 exercises • 2-6 x 4-10 reps • 70-85% 1RM	MHC I CSA	+17%
				MHC IIa CSA	+13%
			AE • Cycle ergometer • 3 d/wk • 30-42min at VT	MHC I CSA	No Change
				MHC IIa CSA	No Change
			CE • RE+AE on Alt days	MHC I CSA	No Change
				MHC IIa CSA	+18%

RE = Resistance Exercise, AE = Aerobic Exercise, CE = Concurrent resistance and aerobic exercise, MHC I = Myosin Heavy Chain I, MHC II = Myosin Heavy Chain II, CSA = Cross Sectional Area, RM = Repetition Maximum, Ext = Extension, VT = Ventilatory Threshold, Reps = Repetitions, LP = Leg Press, Alt = alternating, QF = Quadriceps Femoris, HHR = Heart Rate Reserve, UB = Upperbody, LB = Lower Body, d/wk = Days per Week

The Effects of Concurrent Exercise on Protein Balance

The proposed mechanism underlying the effects of concurrent exercise centers around the molecular pathway involved in muscle protein synthesis (50), which is exemplified in Figure 1. Specifically, interference caused by aerobic exercise hypothetically inhibits the protein synthesis initiated by resistance exercise through the upregulation of AMPK, which inhibits the activity of protein kinase B (PKB), a critical step in the pathway leading to protein synthesis. This proposed mechanism is supported by Baar et al. who further explains that PKB is up-regulated by resistance exercise and may inhibit the downstream pathway initiated by endurance exercise that leads to mitochondrial biogenesis via the phosphorylation of FOXO1 (2). This potentially explains the attenuated endurance gains reported by Nelson et al. (51). Muscle fiber

hypertrophy typically results from an accumulation of myofibrillar proteins and interference in molecular pathways initiated by resistance and endurance exercise appears to be the most likely culprit for the attenuated hypertrophic response of concurrent exercise. However, two studies reported no difference in mixed muscle (65) or myofibrillar (10) protein synthesis in response to resistance and concurrent exercise. Specifically, Tipton and colleagues were the first to address the impact of concurrent exercise on protein synthesis, and reported that concurrent exercise increased mixed muscle protein synthesis, with no increase following resistance exercise (65). Most relevant to the current project, Carrithers found that myofibrillar protein synthesis increased to a similar extent following resistance and concurrent exercise suggesting that early post-exercise FSR does not explain the attenuated response (10).

Dolezal and colleagues assessed urinary nitrogen in response to resistance, endurance and concurrent training and found that endurance training significantly increased urinary nitrogen while resistance and concurrent training had no effect (20). Increased urinary nitrogen is indicative of a negative protein balance. With no differences in urinary nitrogen between resistance and concurrent training it can be inferred that protein degradation is not differentially affected and cannot explain the differential adaptations. Thus, the data above suggests that another factor is likely responsible for the attenuated growth response that is characteristic of concurrent training.

Table 2.3: The Effects of Concurrent Exercise on Protein Balance

Author	Subjects	Group/Design	Variables	Results
Tipton 1996 (65)	7 collegiate swimmers	RE • 3 x 6 reps upper-body lifts • 3 x 10 reps lower body lifts • 65% 1RM	Change in FSR of Deltoid	No Change
		AE • 1.5 hours of high intensity work		No Change
		CE • RE and AE		+81%
Carrithers 2007 (9)	6 men and 6 women	RE • 3 x 10 reps + set to failure • 85% 1RM for leg ext and leg press CE • RE + 90 mins cycling at 60% VO_{2max}	Myofibrillar synthetic rate of Vastus Lateralis	No difference between groups
Dolezal 1998 (20)	30 physically active men	10 weeks RE • 3 days per week	Urinary Urea Nitrogen	No Change
		10 weeks AE • 3 days jogging per week	Urinary Urea Nitrogen	Increased
		10 weeks CE • Combination of both RE and EE	Urinary Urea Nitrogen	No Change

RE = Resistance Exercise, AE = Aerobic Exercise, CE = Concurrent Resistance and Aerobic Exercise, Reps = Repetitions, RM = Repetition Maximum, Mins = Minutes, FSR = fractional synthetic rate, BMR = Basal Metabolic Rate

The Role of Satellite Cells in Skeletal Muscle Growth

As skeletal muscle hypertrophies, satellite cells differentiate and migrate into the myofiber as a new nucleus in a presumable attempt to maintain myonuclear domain, which is defined as the area within the muscle fiber that each myonuclei is responsible for. The process by which satellite cells become nuclei begins with proliferation, or division, followed by differentiation, or the migration of the satellite cell into myofiber. The precise role that satellite cells have in muscle growth processes is unclear. However, the ablation of satellite cells prevents muscle fiber growth suggesting at least that satellite cell differentiation is required for muscle hypertrophy.

Barton-Davis et al. provided evidence for the importance of satellite cells during hormonally mediated skeletal muscle growth. The authors demonstrated that the ablation of satellite cells significantly reduces muscle mass while also inhibiting the anabolic effects of IGF-1 (4). The irradiation of satellite cells appears to elicit the same results in mechanically loaded muscle. Following ablation of the tibialis anterior to overload the extensor digitorum longus (EDL) in rats, Rosenblatt et al. assessed hypertrophy of the EDL with and without the irradiation of satellite cells. In agreement with Barton-Davis, mechanical load-induced hypertrophy of the EDL was inhibited by satellite cell irradiation (60). Further evidence of satellite cell mediated hypertrophy was provided by Li et al. who demonstrated that the irradiation of satellite cells in sedentary control mice decreases muscle mass, while irradiation again inhibited mechanical load induced hypertrophy in exercising mice (37). These studies provide evidence that satellite cells are required for significant hypertrophy, at least in animal models.

Table 2.4: The Role of Satellite Cells in Skeletal Muscle Growth

Author/Year	Methods	Groups	Results	Conclusion
Barton-Davis 1999 (4)	Mice were subjects to one of 3 treatments and then compared to a control group to determine the role of satellite cells in IGF-1 induced muscle hypertrophy	SC proliferation inhibited by GR	Decrease in muscle mass	Hypertrophy is predominantly mediated by satellite cell activation
		Viral-mediated gene transfer of IGF-1	Increase in muscle mass	
		Both Treatments together	Hypertrophy was prevented	
Rosenblatt 1994 (60)	Ablation of TA was used to overlaod the EDL and induce hypertrophy in rats. Irradiation of satellite cells was used to determine their role in overload induced hypertrophy	Irradiation + Ablation	No change in muscle mass	Satellite cells are required for over-load induced muscle hypertrophy
		Irradiation only	No change in muscle mass	
		Ablation only	Increase in muscle mass	
		Control	No Change	
Li 2006 (37)	X-ray irradiation of satellite cells was used to determine their role in running induced hypertrophy in mice. Measurements of muscle weight of the soleus and planatris were taken at 2 and 4 weeks	Running + Irradiation	Attenuated hypertrophy compared to non-irradiated	Satellite cells are required for exercise induced muscle hypertrophy
		Running	Increase in muscle mass	
		Sedentary + Irradiation	less muscle mass than controls in the soleus, no difference in the plantaris	

TA = Tibialis Anterior, EDL = Extensor Digitorum Longus, SC = satellite cell, GR = gamma radiation

Molecular Regulation of Satellite Cells

Many of the hormones that regulate hypertrophy also regulate satellite cell activity. Specifically, insulin-like growth factor and myostatin have been reported to strongly influence muscle size alterations and satellite cell activity. The IGF-1 isoform mechano-growth factor (MGF) initiates both hypertrophy and satellite cell proliferation, while the liver produced isoform (IGF-1Ea) stimulates terminal differentiation (70). Myostatin disrupts both by inhibiting the IGF-1 signaling pathway (71).

Hill et al. assessed the timecourse of MGF and IGF-1Ea mRNA, and satellite cell activity following electrical stimulation (28). Hill found that the upregulation of MGF mRNA immediately preceded satellite cell proliferation. Although MGF protein was not assessed, the mRNA response suggests that MGF may play a role in stimulating proliferation. Additionally, the IGF-1Ea mRNA response was delayed, suggesting that the liver produced isoform my play a unique role in determining the fate of satellite cells. Perhaps a stronger case for the role of the IGF-1 isoforms in satellite cell activity was demonstrated by subjecting mouse skeletal muscle cells to MGF and IGF-1Ea in vitro (70). When subjected to MGF, satellite cells began to proliferate, while IGF-1Ea stimulated their differentiation into new myoblasts. Another study by the same group exposed the same line of mouse skeletal muscle cells to myostatin and found that satellite cells ceased to proliferate, and the PI3k/Akt pathway initiated by IGF-1 was completely inhibited (71). When taken together, these studies exemplify the effects that IGF-1 and myostatin have on satellite cell proliferation and differentiation.

Table 2.5: Molecular Regulation of Satellite Cells

Author/Year	Design	Results
Yang 2002 (70)	C2C12 line of mouse SM cells in vitro were subjected to MGF and IGF-1Ea	• MGF stimulated proliferation • IGF-1Ea stimulated differentiation
Yang 2007 (71)	C2C12 line of mouse SM in vitro were subjected to myostatin	• Cells ceased to proliferate • PI3k/Akt pathway inhibited
Hill 2003 (28)	Rats were subjected to electrical stimulation of the tibialis; time courses for MGF, IGF-1Ea and satellite cell proliferation were assessed	• Expression of MGF mRNA immediately preceded satellite cell proliferation • IGF-1Ea mRNA upregulation delayed

MGF = Mechano Growth Factor, IGF = Insulin-like Growth Factor, SM = skeletal muscle

IGF-1 Isoforms and Myostatin mRNA Responses to Acute Exercise

In the process of making a specific protein, DNA is transcribed into messenger RNA (mRNA), and then translated into protein. The upregulation of mRNA does not always translate to a proportional increase in the hormone itself, but does indicate cellular intent. Of particular interest to this study are myostatin, a negative regulator of muscle mass and satellite cell activity, and the isoforms of IGF-1, which are positive regulators of muscle mass and satellite cell activity.

The IGF-1 and myostatin mRNA responses to exercise behave in a manner that theoretically promotes hypertrophy and satellite cell activity in response to resistance training, with an attenuated response to concurrent. MGF mRNA is significantly upregulated following acute resistance exercise (25, 34, 47), while myostatin mRNA is significantly downregulated (34, 38, 59). The decrease in myostatin mRNA in response to acute aerobic exercise is approximately half the response observed following acute

resistance exercise (38). Interestingly, the expression of IGF-1 mRNA is attenuated in response to concurrent exercise (14). In contradiction, one study reported no change in IGF-1 mRNA (3), and another reported lower mRNA levels following acute exercise (8).

IGF-1 and myostatin mRNA responses have also been observed in animal models. Matsaka et al found that the myostatin mRNA response to aerobic exercise in mice is similar to the response to resistance exercise in humans, in that myostatin was significantly reduced after an acute bout of swimming (43). This response was also noted after 3 and 5 days of training.

In summary, if myostatin and IGF-1 protein behave in accordance with their respective mRNA responses, satellite cell activity may follow the hormonal and hypertrophic tendencies of resistance and concurrent training. When taken together, it seems plausible that the magnitude of the satellite cell response to acute exercise would display a similar pattern as the hypertrophic responses of resistance and concurrent training. Specifically, satellite cell activity should increase to a greater extent following resistance exercise compared to concurrent, and to a greater extent following concurrent exercise than aerobic.

Table 2.6: IGF-1 Isoforms and Myostatin mRNA Responses to Acute Exercise

Author/Year	Subjects	Methods	Groups	Variables	Results
McKay 2008 (47)	8 males	• 30 x 10 maximal IC of the VL at 180 deg/second. • Bx were taken pre-ex, and 4, 24, 72 and 120 hours post-ex.	MGF mRNA	Expression after acute resistance exercise	• Increase in MGF mRNA • Peaked at 24 hours post exercise
			IGF-1Ea mRNA		• Increase in IGF-1Ea mRNA • Peaked at 72 hours
Hameed 2003 (25)	8 young and 7 old males	• 10 x 6 reps of LE at 80% 1RM. • Bx were taken pre-ex and 2.5 hours post-ex.	Young	MGF mRNA	Increase
				IGF-1Ea mRNA	No change
			Old	MGF mRNA	No change
				IGF-1Ea mRNA	No change
Raue 2006 (59)	8 young and six old females	• 3 x 10 reps at 70% 1RM for LE • Bx taken pre-ex and 4 hours post-Ex	Young	Change in Myo mRNA expression	Myo mRNA down regulated 2.2 fold in both groups
			Old		
Coffey 2009 (12)	6 males	• Cross over design • RE (8x5 LE at 80% 1RM) followed by SE (10x6 sec sprints) and vice-versa • Bx were taken from the VL pre and 15 min and 3 hours post Ex to assess IGF-1 mRNA expression	RE followed by SE	IGF-1 mRNA Expression	Non-Significant Decrease ($p = 0.06$)
				MGF mRNA Expression	No Change
			SE followed by RE	IGF-1 mRNA Expression	Non-Significant Decrease ($p = 0.06$)
				MGF mRNA Expression	No Change

Table 2.6: IGF-1 Isoforms and Myostatin mRNA Responses to Acute Exercise (Continued)

Author/Year	Subjects	Methods	Groups	Variables	Results
Kim 2005 (34)	• 10 young males • 10 young females • 9 old males • 9 old females	• 3 x 12 reps for squat, LP and LE • Bx taken pre-ex and 24 hours post-ex to assess the change in Myo and MGF mRNA expression	Young Males	Myo	-56%
				MGF	+91%
			Young Females	Myo	-48%
				MGF	No Change
			Old Males	Myo	-40%
				MGF	No Change
			Old Females	Myo	No Change
				MGF	No Change
Louis 2007 (38)	12 healthy subjects	• 2 women and 4 men performed RE • 1 woman and 5 men performed running. • Bx were take pre and 1,2,4,8,12 and 24 hours post-ex to determine the time course of Myo mRNA expression	Resistance Exercise	Myo mRNA Expression post-ex	6.3 fold decrease from 1 to 24 hours post exercise
			Running		3.6 fold decrease 8-12 hours post exercise
Coffey 2009 (13)	8 males	• Cross over design • 4 subjects; RE then AE • 4 subjects; AE then RE • Switch treatments after 1 week • RE consisted of 8X5 LE at 80% 1RM • AE consisted of 30 min of cycling at 70% VO_{2max}. • Bx were taken from the VL pre and 15 min and 3 hours post Ex to assess IGF-1 mRNA expression	RE followed by AE	IGF-1 mRNA Expression	No Change
			AE followed by RE		• Non significant increase • attenuated response compared to RE followed by AE

Table 2.6: IGF-1 Isoforms and Myostatin mRNA Responses to Acute Exercise (Continued)

Author/Year	Subjects	Methods	Groups	Variables	Results
Bickel 2005 (7)	6 men and 3 women	• ES of the vastus lateralis with knee secured at 70^0 of flexion. • Bx were taken pre Ex and 12 and 24 hours after one bout, and 24 and 48 hours after a second bout.	First bout of ES	MGF mRNA 12 hours	No Change
				IGF-1 mRNA 12 hours	-46%
				MGF mRNA 24 hours	No Change
				IGF-1 mRNA 24 hours	No Change
			Second bout of ES	MGF mRNA 24 hours	No Change
				IGF-1 mRNA 24 hours	No Change
				MGF mRNA 48 hours	No Change
				IGF-1 mRNA 48 hours	No Change
Bamman 2001 (3)	7 men and 3 women	• 8 x 8 reps • 85% of 1RM for CE • 110% of 1RM for Ecc Ex • Bx were taken 48 before familiarization trials, 48 hours post Ecc Ex and 48 hours post CE	Ecc Ex	Locally produced IGF-1 mRNA in the VL	Increase
			CE		No Change
Matsakas 2005 (43)	Male Rats	• End by swimming • Myo and IGF-1 mRNA was assessed 24h after acute bout • 24h after 3 days of End • 24h after 5 days of End	Acute swimming	IGF-1 mRNA	No Change
				Myo mRNA	Decrease at 7 but not 24 hours
			3 days swimming	IGF-1 mRNA	No Change
				Myo mRNA	Decrease
			5 days swimming	IGF-1 mRNA	No Change
				Myo mRNA	Decrease

RE = resistance exercise, AE = Aerobic exercise, SE = sprints, ES = electirical stimulation, LE = leg extensions, LP = leg press, 1RM = 1 repetition maximum, Ex = exercise, Ecc = eccentric, CE = concentric exercise, IC = isokinetic contractions, VL = vastus lateralis, Bx = muscle biopsies, Reps = repetitions, Myo = myostatin, End = Endurance training, h = hours

Satellite Cell Responses to Acute Exercise

Acute resistance exercise can activate and stimulate the proliferation of satellite cells. Satellite cell activation involves the initiation of mitosis, whereas proliferation describes the division of the cells and a consequential increase in satellite cell number. Significant proliferation has been observed as early as 6 hours following eccentric cycling (42) and as late as 8 days in the instance of resistance exercise (17, 18, 48). Activated satellite cells have been observed 48 hours following electrical stimulation (40) as well as 8 days following exercise (18, 48). Because the activation of satellite cells must precede proliferaton, it can be infered that in studies only showing increases in satellite cell numbers, the activation of satellite cells coincided.

Typically, eccentric resistance exercise is used to elicit a satellite cell response to acute exercise (17, 18, 21, 48, 53). However endurance running (36km), inherently associated with a large eccentric mechanical loading component, can also initiate satellite cell proliferation (41). Activated satellite cells, or those that have entered the proliferative phase, respond to eccentric resistance exercise (18) as well as electrical stimulation (40). The response has also been shown to vary with age. Dreyer et al. noted a 141% increase in NCAM stained satellite cells in young adults in response to eccentric exercise of the vastus lateralis, with only a 51% increase for old individuals (21).

Satellite cells appear to respond to acute exercise in a manner that follows the hypertrophic tendencies of training with respect to the type of exercise and the age of the individual. In general, the response is analogous to the strength and hypertrophic tendencies of exercise; the response greatest with high intensity resistance compared to endurance exercise, and greater in young individuals compared to old.

Table 2.7: Satellite Cell Responses to Acute Exercise

Author	Subjects	Study Design	Variables	Results
O'Reilly 2008 (53)	8 healthy, recreationally active males	• 300 Ecc Con at 180^0/s	NCAM stained satellite cells	Increase at 24 and 72h post exercise
Crameri 2007 (16)	8 healthy sedentary males	• Max Ecc Con • 10x10 reps at 30^0/s • 11x10 reps at 180^0/s	NCAM stained satellite cells	Increase at 4 and 8 days post Ex
Crameri 2004 (17)	8 healthy sedentary males	• 50x one-leg "drop-down jumps" • 8x10 at 30^0/s • 8x10 at 180^0/s • all Ecc Con	NCAM and FA1 stained satellite cells	Increase at 4 and 8 days post Ex
Dreyer 2006 (21)	10 young and 9 old healthy males	• 6x16 Ecc reps at 60^0/s	NCAM stained satellite cells in young and old	Increase 24h post Ex for both young (141%) and old (51%)
Mackey 2007 (41)	14 endurance trained males	• 36 km run	NCAM stained satellite cells	27% increase 8 days post Ex
Mikkelsen 2009 (48)	8 healthy males	• 200 max Ecc Con • 100 at 30^0/sec • 100 at 120^0/s	Pax7 stained satellite cells	96% increase at day 8 post Ex
Mackey 2009 (40)	7 healthy males	• ES	Ki-67 Stained satellite cells	Increase 48h post ES
Malm 2000 (42)	13 healthy males 19-32 Years old	• 30 minutes of Ecc cycling	NCAM stained satellite cells	Increase at 6, 24 and 48h post Ex

Reps = repetitions, km = kilometers, Ecc = eccentric, Con = contractions, ES = electrical stimulation, h = hours, Ex = exercise

Satellite Cell Response to Acute Exercise - Animal Models

The satellite cell response to acute exercise appears to be similar between humans and animals. Tanaka et al. reported that satellite cell proliferation results only from high intensity exercise, as satellite increased following downhill running but not walking, presumablly a result of the exaggerated eccentric mechanical loading of downhill running (63). This suggests that the magnitude of the satellite cell response, or the presence of a response at all, may be dependant on the intensity of the exercise. In a study assessing the role of estrogen in female rats, it was found that regardless of estrogen the number of total, activated and proliferating satellite cells increased 72 hours after 90 minutes of downhill running (23). Like human models, satellite cells respond to acute exercise, and the response appears to be dependant on the intensity.

Table 2.8: Satellite Cell Response to Acute Exercise - Animal Models

Author	Subjects	Design	Groups	Variable	Results
Tanaka 2009 (63)	4 week old Male Sprague-Dawley Rats	• SC assessed in the soleus of rats • Acute DR (n=4) • Acute DW (n=4)	DR	SC per 1000 fibers	Increase
			DW		No change
			Control		No change
Enns 2007 (23)	44 ovariectomized femal rats	Effects of estrogen on satellite cells 72 hours after 90 minutes of DR	No Estrogen Controls	Total	No Change
				Activated	No Change
				Proliferating	No Change
			Exercised Controls	Total	Increase
				Activated	Increase
				Proliferating	Increase
			Estorgen supplimented controls	Total	Increase
				Activated	No Change
				Proliferating	No Change
			Estrogen supplimented with exercise	Total	Increase
				Activated	Increase
				Proliferating	Increase

SC = satellite cells, DR = downhill running, DW = downhill walking, Ex = Exercise

Satellite Cell Adaptations to Exercise Training

As previously mentioned, satellite cell adaptations to exercise training coincide with hypertrophy, most likely in an attempt to maintain myonuclear domain. Chronic training increases the number of satellite cells per muscle fiber, the total number of activated satellite cells, as well as the number of myonuclei per fiber. Kadi et al. documented this in the trapezius muscles of women during a 10-week resistance training study, where myonuculear number increased by 70% (33). The authors reported that muscle fiber cross sectional area increased by 36% along with a 46% increase in the

number of satellite cells per fiber. In a subsequent study, Kadi assessed satellite cell number and myonuclear domain in response to training followed by detraining, and found that satellite cells increased in response to training and were largely maintained following 30 days of detraining (32). In this study however, Kadi observed that myonuclei per fiber did not increase following training, while myonuclear domain did. This implies that myonuclear domain is not completely maintained during hypertrophy.

In agreement with Kadi, Charifi et al. (11) and Roth et al. (61) also reported no change in myonuclei per fiber following training despite increases in satellite cells density, meaning that there was proliferation with out differentiation. The same results were found in elderly men. However, increases in myonuclei density following resistance training have been reported in elderly women (39). Petrella et al. found that myonuclear domain expansion coincided with increases in myonuclei per fiber in extreme and moderate responders, while only extreme responders showed increases in satellite cells per fiber (55).

As previously mentioned, the exact role of satellite cells during hypertrophy is not well understood, but do appear to play an important role in the hypertrophic process. However, disproportional increases in myonuclear domain compared to myonuclei per fiber suggest that the maintenance of myonuclear domain may not be the most important function of satellite cells during hypertrophy.

Table 2.9: Satellite Cell Adaptations to Exercise Training

Author/Year	Subjects	Design	Groups	Variable	Results
Charifi 2003 (10)	11 aged men (70-80 y.o.)	14 wks of ET, 4 d/wk for 45 (4 min at 65-75% VO_{2max} followed by 1 min at 85-95%)	Satellite Cell frequency	Per fiber	Increase
				Per Myonuclei	Increase
			Myonuclei	Per Fiber	No Change
Kadi 2000 (33)	9 Women	• 10 wks of RT • Bx taken from trapezius before and after training	Physiological affects	Fiber Cross Sectional Area	+36%
				Number of Satellite Cells	+46%
				Myonuclear Number	+70%
Kadi 2004 (32)	15 Young Men	• 90 days of RT, followed by 30 days of detraining • Bx taken pre training, at 30 days, 90 days and 30 days post training	Satellite Cells	After 30 days training	+19.3%
				After 90 days training	+31.4%
				After 30 days detraining	Non significant decrease (p=0.07)
			Myonuclei	After 30 days training	No Change
				After 90 days training	No Change
				After 30 days detraining	No change
			Myonuclear domain	After 90 days training	Increase
				After 30 days detraining	Return to pre-training values
Mackey 2007 (39)	13 healthy elderly men and 16 healthy elderly women	12 wks of lower body RT, 3x/wk. Bx collected from VL pre and post training	Elderly Men	SC per fiber	Increase
				Myonuclei per fiber	No Change
			Elderly Women	SC per fiber	Increase
				Myonuclei per fiber	Increase

Table 2.9: Satellite Cell Adaptations to Exercise Training (Continued)

Author/Year	Subjects	Design	Groups	Variable	Results
Petrella 2008 (55)	66 Humans (no discrimination between age and sex)	16 wks of RT, Subjects were clustered as extreme responders, moderate responders and non responders	Extreme Responders	Myonuclei per fiber	+26%
				Myonuclear domain	Increase
				Satellite Cell per Fiber	+117%
			Moderate Responders	Myonuclei per fiber	+9%
				Myonuclear domain	Increase
				Satellite Cell per Fiber	No Change
			Non Responders	Myonuclei per fiber	No Change
				Myonuclear domain	No Change
				Satellite Cell per Fiber	No Change
Roth 2001 (61)	7 young men 7 young women 8 old men 7 old women	9 wks of heavy RT of the VL, with non exercising leg serving as control	Young Men	SC Proportions	Increase
			Young Women		Increase
			Old Men		Increase
			Old Women		Increase
			Average	Activated SCs	+31%
				Myonuclei Per Fiber	No Change

Wk = week, d/wk = days per week, RT = resistance training, ET = endurance training, VL = vastus lateralis, SC = satellite cell, Bx = muscle biopsies

Fiber-Type Specific Satellite Cell Activity With Training

Fiber type specific satellite cell activity levels have been reported following chronic resistance and concurrent training. Verney et al. examined the impact of upper body resistance exercise combined with lower body endurance training in elderly men on deltoid and vastus lateralis fiber type specific satellite cell activity, myonuclear domain and myonuclei per fiber (67). They found a 38% increase in total satellite cell number around MHC IIa muscle fibers of both the deltoids and vastus lateralis, an increase in cross sectional area only for the MHC IIa fibers in the VL, and no change in MHC I fiber satellite cell numbers, myonuclear domain or myonuclei per fiber. These data suggest that satellite cells proliferate to a greater extent around MHC IIa fibers. This concept is complimented by a recent resistance training study also showing increases in satellite cells around MHC IIa but not MHC I fibers (66). No change in myonuclear domain or myonuclei per fiber for either fiber type was reported in either study, however Verdijk et al. did report a non-significant increase for both myonuclear domain and myonuclei per fiber in MHC IIa fibers but not in MHC I.

Table 2.10: Fiber-Type Specific Satellite Cell Activity With Training

Author	Subjects	Design	Groups	Variable	Results
Verney 2008 (67)	10 active elderly men	14 wks of concurrent lower body endurance and upper body resistance. Bx of the VL and deltoid taken before and after training	Deltoids	SCs around MHC I Fibers	No Change
				SCs around MHC IIa	+38%
				MD	No Change
				M/F	No Change
			Vastus Lateralis	SCs around MHC I Fibers	No Change
				SCs around MHC IIa	+38%
				MD	No Change
				M/F	No Change
Verdijk 2009 (66)	13 healthy old men	12 wks RT. Bx taken pre and post training from the quadriceps.	MHC I	SC Content	No Change
				M/F	No Change
				MD	No Change
				CSA	No Change
			MHC II	SC Content	Increase
				M/F	No Change
				MD	No Change
				CSA	+24%

Wks = weeks, VL = vastus lateralis, Bx = muscle biopsies, SC = satellite cell, RT = resistance training, MHC = myosin heavy chain, MD = myonuclear domain, M/F = myonuclei per fiber, CSA = Cross sectional area

Summary

Concurrent training attenuates size and strength gains compared to resistance training alone, and the differential responses appear to be mediated according to fiber type. The proposed mechanism of molecular interference facilitating the attenuated responses is interference in the PI3K/Akt signaling pathway leading to muscle protein

synthesis. However, rates of muscle protein synthesis following acute resistance and concurrent exercise do not differ, nor does protein degradation and basal metabolic rate following resistance and concurrent training. This suggests that the interference effect is elicited by another mechanism, such as satellite cell dynamics. Their role during hypertrophy is not clearly defined. However their importance is well documented as the ablation of satellite cells completely blunts hypertrophy. The hormones that regulate satellite cell activity, as implied by their mRNA, behave in a manner that would suggest differential satellite cell activity in response to resistance and concurrent exercise, with satellite cell activity following the hypertrophic tendencies of resistance and concurrent exercise. Furthermore, satellite cells proliferate to a greater extent in MHC II fibers compared to MHC I fibers following resistance training, also suggesting that their activity follows hypertrophy as MHC II fibers hypertrophy more than MHC I fibers following resistance and concurrent training. Thus, the aim of this study is to quantify fiber-type specific satellite cell dynamics following acute resistance, aerobic and concurrent exercise to gain insight into the possible role the satellite cells have in the interference effect of concurrent exercise.

CHAPTER THREE - METHODS

Subjects

Following IRB approval, eight young, recreationally active males were recruited for participation; characteristics summarized in Table 3.1. Prior to any testing, subjects were informed of the experimental procedures and their requirements via an informed consent approved by the James Madison University Institutional Review Board.

Table 3.1: Subject Characteristics

	Age (yrs)	Height (cm)	Weight (kg)	VO_{2max} (ml/kg/min)
Mean ± SE	23 ± 1	181 ± 2	83.4 ± 3.6	48.5 ± 1.6

Experimental Design

VO_{2max} testing preceded the experimental trials by 7 to 14 days and was conducted to assess cardiorespiratory capacity in addition to the workload that was utilized during the cycling portion of the concurrent exercise protocol. Each experimental trial is noted in order of occurrence as T1, T2, T3 and T4. During T1, a pre-exercise skeletal muscle biopsy was obtained from the vastus lateralis prior to unilateral resistance exercise (RE). Four days following T1, a post-RE muscle biopsy was obtained from the same leg (T2). During T3, skeletal muscle biopsies were obtained from the vastus lateralis of both legs. Immediately following the biopsy procedure, the same unilateral resistance exercise protocol was performed on the opposing leg, which was then followed by 90 minutes of cycling. The non-RE leg served as the aerobic exercise leg (AE),

whereas the resistance exercise leg represented concurrent exercise (CE). Four days following T3 (T4), post-exercise biopsies were obtained from each leg.

Preliminary Testing (n=1 trial)

Subjects reported to the laboratory and completed an informed consent and health-screening questionnaire. Subjects then performed a cardiorespiratory (VO_{2max}) test on a cycle ergometer. Subjects completed a 5-minute warm up on an electronically braked cycle ergometer (Velotron RacerMate Inc, Seattle WA, USA) at a self-selected pace. Following the warm up, workload increased by 25 watts (W) every two minutes until a cycling cadence of ≥ 50 revolutions per minute could no longer be maintained. Expired respiratory gasses were measured via metabolic Sensor Medic cart (SensorMedics, San Diego, CA, USA). The peak workload subjects achieved during the VO_{2max} trial was used to assign workload during the concurrent exercise trial.

Experimental Trials (n=2 trials)

Subjects were divided into two groups of 4 subjects. One group performed the RE trial with their dominant leg only while the other group used their non-dominant leg. Subjects exercised the contralateral leg for the RE portion of the CE trial. The RE trial took place 7 to 14 days after preliminary VO_{2max} testing, and RE and CE trials were separated by 10 days to avoid and residual satellite cell responses to heavy resistance exercise. Prior each exercise trial, a one-repetition max (1RM) test was performed to determine resistance. The protocols for the 1RM tests, RE and CE trials are as follows:

One Repetition Maximum Test (1RM)

Immediately prior to each exercise protocol subjects performed unilateral 1RM tests for both leg extension and leg press. Subjects performed a 5-min warm up on a treadmill at a self-selected walking pace. Subjects then performed 10 repetitions at 20% of their body weight for a one-legged leg extension on a standard leg extension device (Cybex V3 Series, Medway MA, USA). Following 4 min of passive recovery subjects performed 2 repetitions at 50-70% of their perceived 1RM. This was again followed by 4 min of passive recovery, after which subjects attempted a resistance that was perceived as their 1RM. This was repeated with 4 minutes passive recovery in between attempts until failure. This protocol was then immediately followed by a unilateral one-legged leg press 1RM test. The protocol was identical with the exception of the warm up and with 30% of their body weight for the first set of 10 repetitions.

One-Legged Resistance Exercise Trial (RE)

Following 1RM testing, subjects performed 3 sets of 10 repetitions, with a 4^{th} set to ≥ 10 repetitions (to exhaustion) at 75% of their 1RM for the one-legged leg extension. The protocol was immediately repeated for unilateral leg press at the same intensity. Subjects were provided with 2 minutes of passive rest between each set and were given assistance when necessary to achieve all 10 repetitions. The subjects were provided with constant feedback in attempt to maintain a 2 second concentric phase and a 3 second eccentric phase for each repetition.

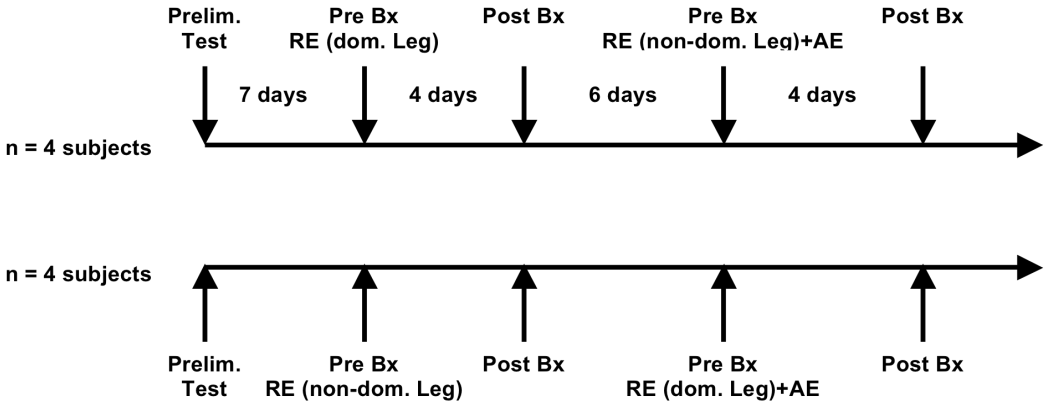
Concurrent Exercise Trial (CE)

Ten minutes following an RE protocol identical to the aforementioned protocol, subjects cycled for 90 min on an electronically braked cycle ergometer at 60% of their wattage maximum (W_{max}) that was established during preliminary testing.

Biopsies

Six skeletal muscle biopsies were obtained from the vastus lateralis at 4 different time points throughout the experimental procedure: immediately before both exercise trials (T1 and T3), and 4 days after each exercise trial (T2 and T4). Only one muscle biopsy was obtained from the exercised leg both pre and post RE (T1 and T2). Muscle biopsies from both legs were taken pre and post CE (T3 and T4)(Figure 3.2). Once samples were obtained, they were quickly immersed in isopentane at -20^0C, then frozen in liquid nitrogen and stored at -80C until cutting. 10µm serial cross sections were cut at a temperature of -25°C.

Figure 3.1: Study Design



Immunohistochemistry

Two serial cross sections were selected and stained for MHC I or fluorescent double staining for NCAM and Ki-67 with a DAPI counter stain. NCAM (Santa Cruz Biotechnology, Santa Cruz California, USA) was used to locate satellite cells, Ki-67 (Santa Cruz Biotechnology, Santa Cruz California, USA) was used to identify activated satellite cells, while DAPI (Invitrogen, Carlsbad CA, USA) was used to locate DNA content. All secondary antibodies were obtained from Jackson Immunoresearch (West Grove PA, USA). MHC I antibody (Sigma Aldrich, St Louis MO, USA) was used to identify MHC I muscle fibers to determine fiber type specific satellite cell dynamics. We did not use a MHC IIa antibody. Therefore, fibers that stained positive for MHC I will be referred to as MHC I fibers and fibers that were negative for MHC I will be referred to as non-MHC I fibers. Notably, muscle fibers that stained positive for MHC I does not infer that the fibers are pure MHC I fibers, as there was most definitely a subpopulation of the MHC I fibers that were comprised of both MHCI/MHCIIa proteins (i.e. hybrid fibers).

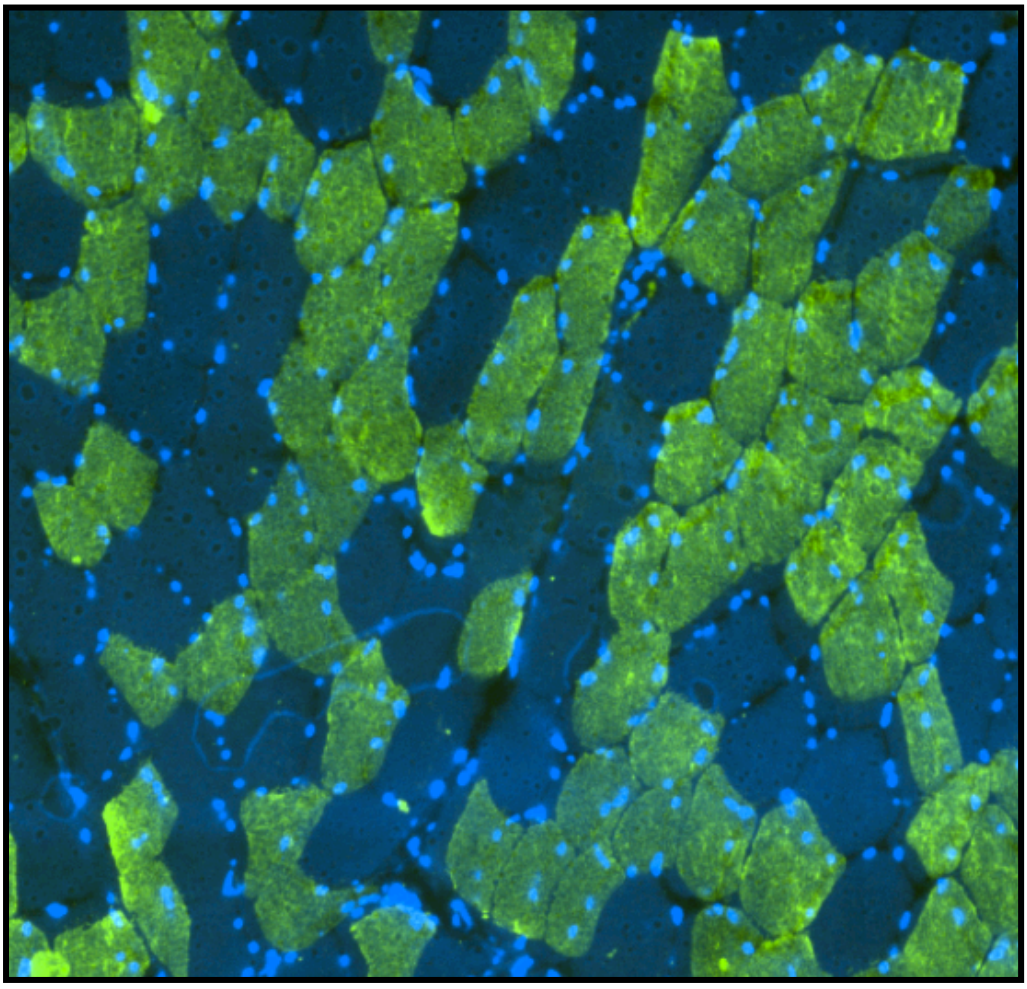
Following sectioning, samples were placed on slides and allowed to dry for 30 minutes. Each sample was circled with a PAP pen to localize incubation. Samples were then fixed in methanol, and rinsed with 0.05% tween-20 in PBS for 2 x 2 minutes. Samples were then incubated for one hour with 10% normal goat serum and again washed with PBS/Tween-20. The primary antibodies were first diluted to a 1:50 dilution in PBS with 2% normal goat serum and applied to the sample. Samples were then incubated for one hour at room temperature and washed in PBS/Tween-20 for 3 x 5 minutes. The secondary antibody (1:300 dilution in PBS with 2% normal goat serum) was then applied to the sample. Samples are incubated for one hour at room temperature

in a dark room to avoid photo bleaching and again washed in PBS/Tween-20. Samples stained for MHC I were then covered with an aqueous mounting medium and a cover slip and stored in the dark at 4C until viewing. For NCAM, Ki-67 and DAPI, this procedure is done twice: once for each primary antibody with it's respective secondary antibody, then counterstained with DAPI (1:300 dilution in PBS for 5 minutes in the dark), rinsed with PBS then mounted with a cover slip using the aqueous mounting medium and stored until viewing.

Imaging and Quantification

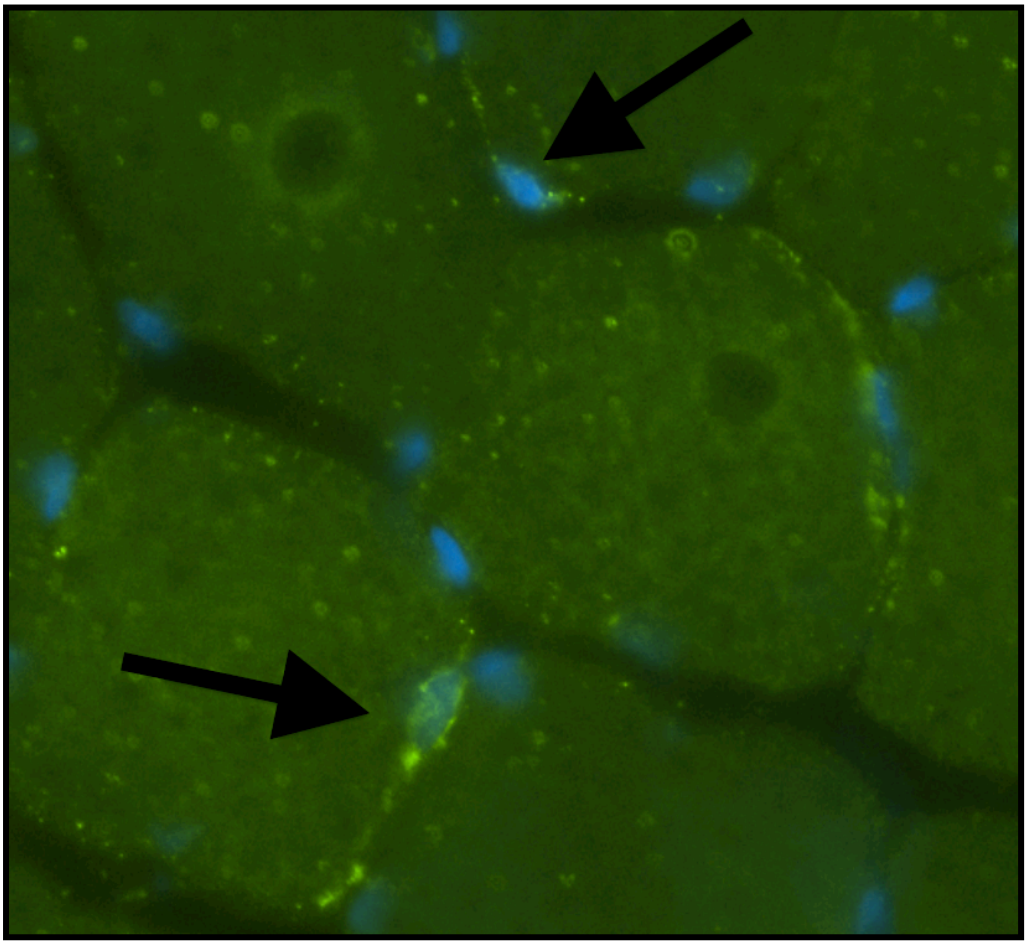
Imaging was conducted via fluorescent microscopy (Nikon Eclipse TE2000-E, Tokyo Japan). Within each sample, areas with the highest quality fibers and stains were selected for analysis. Images were initially captured at 4x magnification. The three serial cross sections were then superimposed. For determination of satellite cells and their location, samples were viewed at 40x magnification. The criteria for satellite cell determination was as follows: positive staining for both NCAM and DAPI, and located at the periphery of the muscle fiber. Activated satellite cells were determined by the same criteria with the addition of a positive stain for Ki-67. Within each cross section, an average of 359 ± 30 fibers were counted per sample, including 142 ± 22 MHC I fibers and 217 ± 24 non-MHC I fibers.

Figure 4.2 Superimposed Serial Cross Sections



Superimposed serial cross-sections; one cross section stained with DAPI and for NCAM, the other stained for MHC I. This represents the distribution of MHC I fibers throughout the whole cross section. Superimposed images were used for fiber-type specific analysis.

Figure 4.3 Satellite Cells That Meet the Criteria for Determination (40x)



Satellite cells double stained for NCAM and with DAPI. Satellite cell determination required the presence of both DAPI and NCAM staining. This image illustrates two cells that meet the satellite cell criteria, as indicated by the arrows. Note the presence of both blue and green staining

Dietary and Physical Activity Controls

Subjects were instructed to maintain normal dietary habits throughout the study and were also provided with a standardized breakfast that was consumed 2 hrs prior to each trial. Subjects were also instructed to refrain from physical activity outside of the exercise trials to ensure there was no residual satellite cell activity.

Statistical Analysis

A 2x3 (time x mode) repeated-measures ANOVA was used to analyze total number of satellite cells and the number of activated satellite cells before and after RE, AE and CE. This same approach was taken to assess MHC I and MHC II satellite cell numbers and activated satellite cells before and after RE, AE and CE. To specifically address potential differences in the satellite cell *response* between each mode, percent changes from pre- to post-exercise, for each parameter, were analyzed with a One-Way ANOVA. During the CE trial, the AE leg also served as 10 days post RE. Thus, a repeated measures ANOVA was utilized to assess the timecourse of satellite cell activation and proliferation following a single bout of RE. Statistical significance was set at $p \leq 0.05$ and a Bonferonni *post-hoc* test was performed where appropriate.

CHAPTER FOUR - MANUSCRIPT

Concurrent Aerobic Exercise Interferes With the Satellite Cell Response to Acute Resistance Exercise in MHC I Muscle Fibers

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Running Head: Concurrent Exercise and Satellite Cell Dynamics

Key Words: Concurrent training, muscle stem cells

ABSTRACT

Purpose: Concurrent training attenuates hypertrophy compared to resistance training alone, and does so in a fiber-type specific manner. The mechanism responsible for this 'interference' is unclear, and satellite cell physiology, an important hypertrophic factor, has not been examined within this context. Therefore, the purpose of this investigation was to assess the fiber-type specific satellite cell response to acute resistance, aerobic and concurrent exercise. **Methods:** Eight recreationally active college-aged males (23±1 yrs, 83.4±3.6 kg, 181±2 cm, and 48.5±1.6 ml/kg/min) performed 3 sets of 10 repetitions with a fourth set ≥ 10 repetitions at 75% of 1RM for both unilateral leg extensions and unilateral leg press for acute RE. Ten days later subjects performed the same resistance exercise with the opposite leg followed by 90 minutes of cycling at 60% VO_{2max} to represent acute concurrent and aerobic exercise. Muscle biopsies were obtained immediately before and 4 days after each exercise session. Muscle samples were cross sectioned and stained with for NCAM, Ki-67, DAPI and MHC I via immunohistochemistry to assess satellite cells, activated satellite cells and fiber-type, respectively. **Results:** Total satellite cell number per fiber increased only in response to acute resistance exercise (+38±10%, $p < 0.05$), with no change following acute aerobic or concurrent exercise. Changes in total satellite cell number per fiber between resistance, aerobic and concurrent exercise differed only in MHC I fibers ($p < 0.05$), with no satellite cell number per fiber time by mode interaction observed in non-MHC I muscle fibers. No changes in activated satellite cells were observed under any condition. **Conclusion:** Acute concurrent exercise blunts the satellite cell response of resistance exercise alone, and does so in a fiber-type specific manner by negating the satellite cell response in MHC

I, but not non-MHC I fibers. These results suggest that the interference effect of concurrent resistance training on MHC I hypertrophy may be regulated at the satellite cell level.

INTRODUCTION

Concurrent resistance and aerobic training is popular among athletes, the elderly, individuals in weight loss programs, and astronauts attempting to counter the detrimental affects of microgravity in space. However, concurrent training has been shown to attenuate the size (7, 24, 29, 35, 45) and strength gains (20, 26, 27, 29) that can be achieved with resistance training alone. Interestingly, this appears to be manifested in a fiber-type specific manner. Following sufficient resistance training, both MHC I (slow-twitch) and MHC IIa (fast-twitch) muscle fibers hypertrophy (7, 24, 35), with minimal hypertrophy in MHC I fibers following aerobic training (35). Concurrent training however largely negates MHC I fiber hypertrophy, attenuates MHC IIa fiber hypertrophy, which consequently attenuate whole muscle growth (7, 12, 24, 35, 45). The underlying biological mechanism for this interference effect is unknown.

Muscle fiber hypertrophy most often results from the accumulation of muscle contractile proteins. One proposed mechanism for the effects of concurrent training is interference of the molecular signaling pathways leading to muscle protein synthesis. In response to aerobic exercise (AE), AMPK is upregulated, which inhibits the activity of protein kinase B (PKB), a critical step in the molecular pathway leading to muscle protein synthesis. This mechanism could hypothetically attenuate size gains in response to concurrent training compared to resistance training alone. However, this hypothesis has been tested and there are no difference in the rates of mixed muscle (65) or myofibrillar (10) protein synthesis following acute resistance (RE) and concurrent exercise (CE), at least in the early hours following a single session. This implies that

other mechanisms are responsible for the attenuated size and strength grains of concurrent training.

Satellite cells, undifferentiated muscle stem cells, have gained considerable attention over the past several years for their influence on skeletal muscle growth. The specific role of satellite cells in the growth process may lie in their capacity to maintain myonuclear density. Specifically, each myonuclei is responsible for a given amount of physical space with in the muscle fiber – termed myonuclear domain. As muscle fibers hypertrophy, the myonuclear domain expands, ultimately to an extent that is thought to limit any firther growth. Thus, to facilitate greater hypertrophy, satellite cells proliferate, or divide by mitosis, and differentiate into the myofiber as a new nucleus (1). This process does appear to be critical for skeletal muscle growth, as evidence by reports that hypertrophy is inhibited following satellite cell ablation (4, 37, 60)

Many of the hormones that regulate muscle hypertrophy also regulate satellite cell activity, namely mechano-growth factor (MGF) and myostatin. MGF stimulates both hypertrophy and satellite cell proliferation (70) while myostatin is a negative regulator of both hypertrophy and satellite cell activity by the inhibition of MGF's signaling pathway (71). The behavior of these hormones, as implied by their mRNA, creates an anabolic environment following RE (25, 38, 47, 59), and an attenuated response following CE (13, 14). Although measurements of mRNA reflect transcription and not necessarily the presence of a physiologically relevant protein, it at least reflects cellular intent, and suggests that satellite cell dynamics could mimic the patterns of hypertrophy seen in response to RE, AE and CE.

Satellite cell population has been shown to increase as early as 6 hours post (42) and as late as 8 days following acute resistance exercise (17, 18, 48, 53). Activated satellite cells have been observed 48 hours- (40) and 8 days following resistance exercise (18). Eccentric resistance exercise is the most prevalent model used to study satellite cell physiology (17, 18, 21, 53). However long distance running (36km), which is inherently eccentric in nature, can also stimulate satellite cell proliferation (41). In general, the response of satellite cells to acute exercise mimics the hypertrophic tendencies of training. To date, satellite cell proliferation and activation following acute concurrent exercise has not been examined. The training response of satellite cells to resistance and concurrent training is similar to the fiber-type specific hypertrophic response. Increases in satellite cell number per fiber are observed in MHC IIa fibers following both resistance and concurrent training, whereas MHC I fibers display no response to either but still hypertrophy in response to resistance training (66, 67).

To provide insight into the possible mechanism(s) responsible for attenuated size and strength gains with CE training, the primary aim of this investigation was to examine satellite cell physiology before and after acute resistance, aerobic, and concurrent exercise. Specifically, we tested the hypothesis that satellite cell activation and proliferation will reflect the hypertrophic and hormonal tendencies of resistance, aerobic and concurrent exercise - specifically that, both satellite cell numbers and the number of activated satellite cells will increase to a greater extent following resistance compared to aerobic and concurrent exercise, and will increase to a greater extent in MHC IIa fibers compared to MHC I fibers.

METHODS

Subjects

Following IRB approval, eight young, recreationally active males were recruited for participation; characteristics summarized in Table 3.1. Prior to any testing, subjects were informed of the experimental procedures and their requirements via an informed consent approved by the James Madison University Institutional Review Board.

Table 4.1: Subject Characteristics

	Age (yrs)	Height (cm)	Weight (kg)	VO_{2max} (ml/kg/min)
Mean ± SE	23 ± 1	181 ± 2	83.4 ± 3.6	48.5 ± 1.6

Experimental Design

VO_{2max} testing preceded the experimental trials by 7 to 14 days and was conducted to assess cardiorespiratory capacity in addition to the workload that was utilized during the cycling portion of the concurrent exercise protocol. Each experimental trial is noted in order of occurrence as T1, T2, T3, T4. During T1, a pre-exercise skeletal muscle biopsy was obtained from the vastus lateralis prior to unilateral resistance exercise (RE). Four days following T1, a post-RE muscle biopsy was obtained from the same leg (T2). During T3, skeletal muscle biopsies were obtained from the vastus lateralis of both legs. Immediately following the biopsy procedure, the same unilateral resistance exercise protocol was performed on the opposing leg, which was then followed by 90 minutes of cycling. The non-RE leg served as the aerobic exercise leg (AE),

whereas the resistance exercise leg represented concurrent exercise (CE). Four days following T3 (T4), post-exercise biopsies were obtained from each leg.

Preliminary Testing (n=1 trial)

Subjects reported to the laboratory and completed an informed consent and health-screening questionnaire. Subjects then performed a cardiorespiratory (VO_{2max}) test on a cycle ergometer. Subjects completed a 5-minute warm up on an electronically braked cycle ergometer (Velotron RacerMate Inc, Seattle WA, USA) at a self-selected pace. Following the warm up, workload increased by 25 watts (W) every two minutes until a cycling cadence of ≥ 50 revolutions per minute could no longer be maintained. Expired respiratory gasses were measured via a Sensor Medics metabolic cart (SensorMedics, San Diego, CA, USA). The peak workload subjects achieved during the VO_{2max} trial was used to assign workload during the concurrent exercise trial.

Experimental Trials (n=2 trials)

Subjects were divided into two groups of 4 subjects. One group performed the RE trial with their dominant leg only while the other group used their non-dominant leg. Subjects exercised the contralateral leg for the RE portion of the CE trial. The RE trial took place 7 to 14 days after preliminary VO_{2max} testing, and RE and CE trials were separated by 10 days to avoid any residual satellite cell responses to heavy resistance exercise. Prior each exercise trial, a one-repetition max (1RM) test was performed to determine resistance. The protocols for the 1RM tests, RE and CE trials were as follows:

One Repetition Maximum Test (1RM)

Immediately prior to each exercise protocol subjects performed unilateral 1RM tests for both leg extension and leg press. Subjects performed a 5-min warm up on a treadmill at a self-selected walking pace. Subjects then performed 10 repetitions at 20% of their body weight for a one-legged leg extension on a standard leg extension device (Cybex V3 Series, Medway MA, USA). Following 4 min of passive recovery subjects performed 2 repetitions at 50-70% of what they perceived their 1RM to be. This was again followed by 4 min of passive recovery, after which subjects attempted a resistance that was perceived as their 1RM. This was repeated with 4 minutes passive recover between attempts until failure. This protocol was then immediately followed by a unilateral one-legged leg press 1RM test. The protocol was identical to the leg extension protocol with the exception of the warm up and with 30% of their body weight for the first set of 10 repetitions.

One-Legged Resistance Exercise Trial (RE)

Following 1RM testing, subjects performed 3 sets of 10 repetitions, with a 4^{th} set to ≥ 10 repetitions (to fatigue) at 75% of their 1RM for the one-legged leg extension. The protocol was immediately repeated for unilateral leg press at the same intensity. Subjects were provided with 2 minutes of passive rest between each set and were given assistance when necessary to achieve all 10 repetitions. The subjects were provided with constant feedback in attempt to maintain a 2 second concentric phase and a 3 second eccentric phase for each repetition.

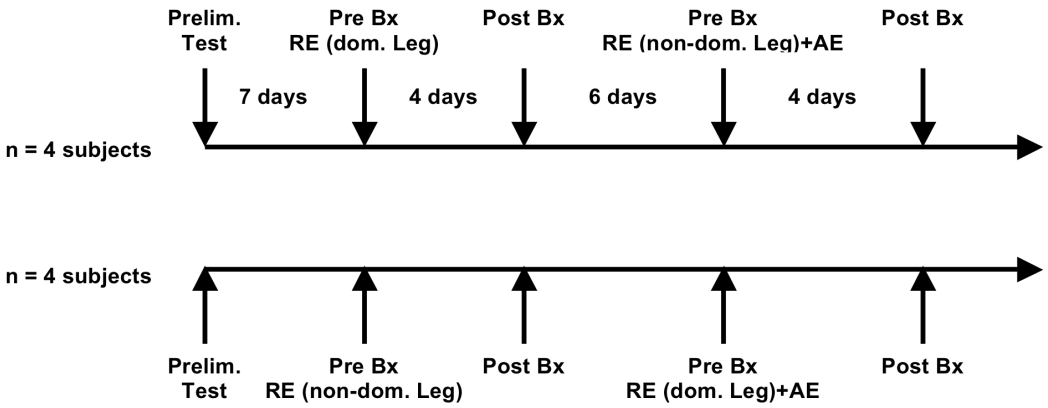
Concurrent Exercise Trial (CE)

Ten minutes following an RE protocol identical to the aforementioned protocol, subjects cycled for 90 min on an electronically braked cycle ergometer at 60% of their W_{max} that was established during preliminary testing.

Biopsies

Six skeletal muscle biopsies were obtained from the vastus lateralis at 4 different time points throughout the experimental procedure: immediately before both exercise trials (T1 and T3), and 4 days after each exercise trial (T2 and T4). Only one muscle biopsy was obtained from the exercised leg both pre and post RE (T1 and T2). Muscle biopsies from both legs were taken pre and post CE (T3 and T4)(Figure 3.2). Once samples were obtained, they were quickly immersed in isopentane at -20^0C, then frozen in liquid nitrogen and stored at -80C until cutting. 10µm serial cross sections were cut at a temperature of -25°C.

Figure 4.1: Study Design



Immunohistochemistry

Two serial cross sections were selected and stained for MHC I or fluorescent double staining for NCAM and Ki-67 with a DAPI counter stain. NCAM (Santa Cruz Biotechnology, Santa Cruz California, USA) was used to locate satellite cells, Ki-67 (Santa Cruz Biotechnology, Santa Cruz California, USA) was used to identify activated satellite cells, while DAPI (Invitrogen, Carlsbad CA, USA) was used to locate DNA content. All secondary antibodies were obtained from Jackson Immunoresearch (West Grove PA, USA). MHC I antibody (Sigma Aldrich, St Louis MO, USA) was used to identify MHC I muscle fibers to determine fiber type specific satellite cell dynamics. We did not use a MHC IIa antibody. Therefore, fibers that stained positive for MHC I were referred to as MHC I fibers and fibers that were negative for MHC I were referred to as non-MHC I fibers. Notably, muscle fibers that stained positive for MHC I does not infer that the fibers are pure MHC I fibers, as there was most definitely a subpopulation of the MHC I fibers that were comprised of both MHCI/MHCIIa proteins (i.e. hybrid fibers).

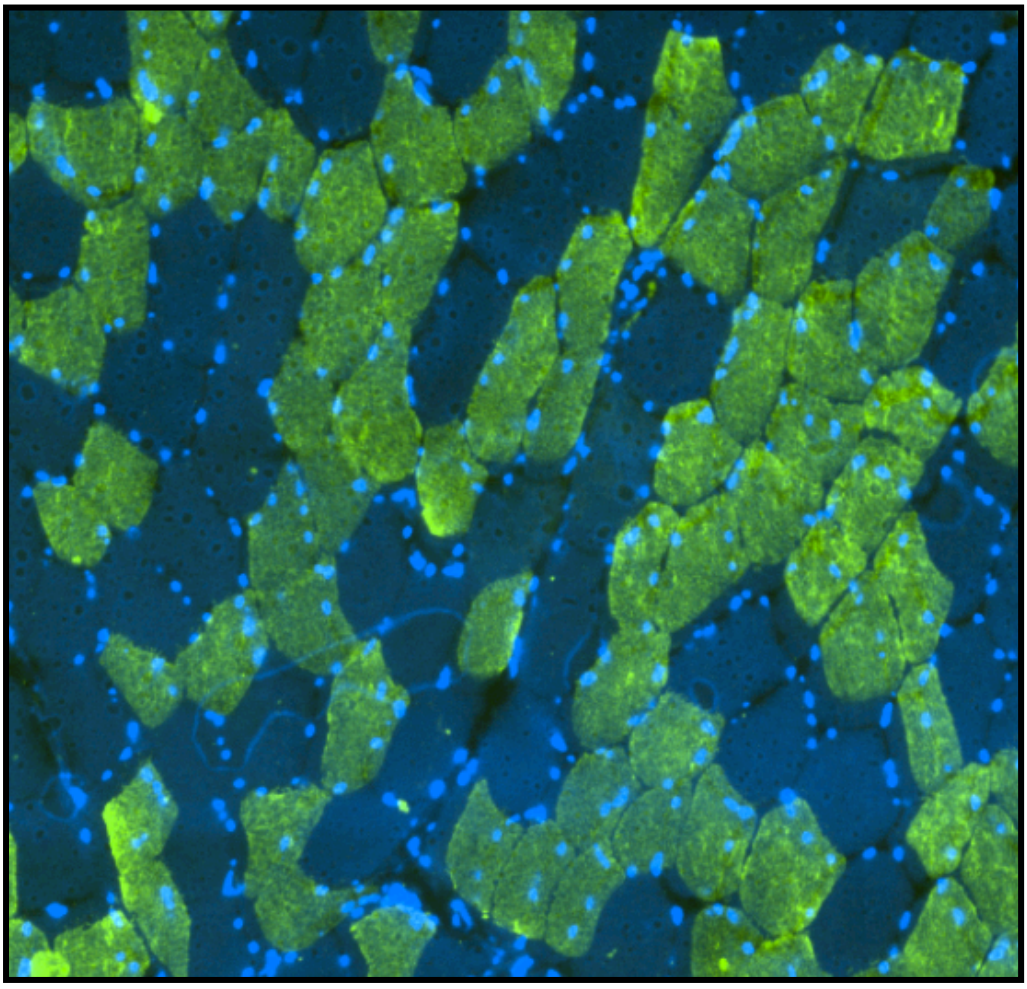
Following sectioning, samples were placed on slides and allowed to dry for 30 minutes. Each sample was circled with a PAP pen to localize incubation. Samples were then fixed in methanol, and rinsed with 0.05% tween-20 in PBS for 2 x 2 minutes. Samples were then incubated for one hour with 10% normal goat serum and again washed with PBS/Tween-20. The primary antibodies were first diluted to a 1:50 dilution in PBS with 2% normal goat serum and applied to the sample. Samples were then incubated for one hour at room temperature and washed in PBS/Tween-20 for 3 x 5 minutes. The secondary antibody (1:300 dilution in PBS with 2% normal goat serum) was then applied to the sample. Samples were incubated for one hour at room

temperature in a dark room to avoid photo bleaching and again washed in PBS/Tween-20. Samples stained for MHC I were then covered with an aqueous mounting medium and a cover slip and stored in the dark at 4C until viewing. For NCAM, Ki-67 and DAPI, this procedure is done twice: once for each primary antibody with it's respective secondary antibody, then counterstained with DAPI (1:300 dilution in PBS for 5 minutes in the dark), rinsed with PBS then mounted with a cover slip using the aqueous mounting medium and stored until viewing.

Imaging and Quantification

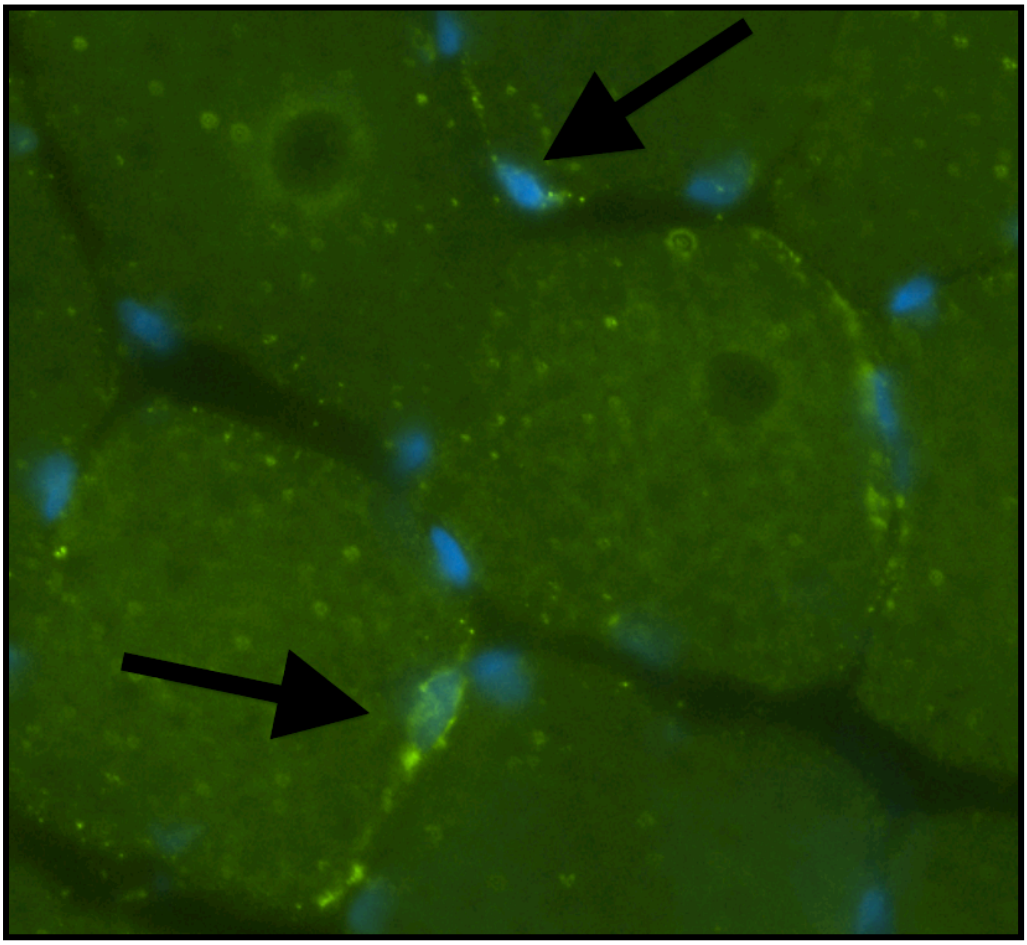
Imaging was conducted via fluorescent microscopy (Nikon Eclipse TE2000-E, Tokyo Japan). Within each sample, areas with the highest quality fibers and stains were selected for analysis. Images were initially captured at 4x magnification. The three serial cross sections were then superimposed. For determination of satellite cells and their location, samples were viewed at 40x magnification. The criteria for satellite cell determination was as follows: positive staining for both NCAM and DAPI, and located at the periphery of the muscle fiber. Activated satellite cells were determined by the same criteria with the addition of a positive stain for Ki-67. Within each cross section, an average of 359 ± 30 fibers were counted per sample, including 142 ± 22 MHC I fibers and 217 ± 24 non-MHC I fibers.

Figure 4.2 Superimposed Serial Cross Sections



Superimposed serial cross-sections; one cross section stained with DAPI and for NCAM, the other stained for MHC I. This represents the distribution of MHC I fibers throughout the whole cross section. Superimposed images were used for fiber-type specific analysis.

Figure 4.3 Satellite Cells That Meet the Criteria for Determination (40x)



Satellite cells double stained for NCAM and with DAPI. Satellite cell determination required the presence of both DAPI and NCAM staining. This image illustrates two cells that meet the satellite cell criteria, as indicated by the arrows. These samples were identified as satellite cells because they were double stained with both DAPI and NCAM, and located on the periphery of the muscle fiber

Dietary and Physical Activity Controls

Subjects were instructed to maintain normal dietary habits throughout the study and were also provided with a standardized breakfast that was consumed 2 hrs prior to each trial. Subjects were also instructed to refrain from physical activity outside of the exercise trials to ensure there was no residual satellite cell activity.

Statistical Analysis

A 2x3 (time x mode) repeated-measures ANOVA was used to analyze total number of satellite cells and the number of activated satellite cells before and after RE, AE and CE. This same approach was taken to assess MHC I and MHC II satellite cell numbers and activated satellite cells before and after RE, AE and CE. To specifically address potential differences in the satellite cell *response* between each mode, percent changes from pre- to post-exercise, for each parameter, were analyzed with a One-Way ANOVA. During the CE trial, the AE leg also served as 10 days post RE. Thus, a repeated measures ANOVA was utilized to assess the time course of satellite cell activation and proliferation following a single bout of RE. Statistical significance was set at $p \leq 0.05$ and a Bonferonni *post-hoc* test was performed where appropriate.

RESULTS

Table 4.2 Workloads

		RE	RE(CE)
Leg Extension	1RM (lbs)	156 ± 9	144 ± 9
	Workload (lbs)	119 ± 7	110 ± 7
	Notes	All subjects needed assistance by the second set. All subjects performed 10 reps on the final set	
Leg Press	1RM (lbs)	210 ± 10	194 ± 14
	Workload (lbs)	159 ± 10	148 ± 10
	Reps on Final Set	11 ± 1	14 ± 2
Cycling Workload (W)		152 ± 6	
Notes	4 subjects reduced cycling workload to complete the protocol		

Satellite Cell Activation

The number of active satellite cells was unaffected by exercise. Satellite cell activation data are displayed in table 4.2

Mixed Fiber Satellite Cell Proliferation

A 2x3 ANOVA revealed an overall effect of $p = 0.065$. The percent change in the number of satellite cells per fiber from pre- to post-exercise significantly varied between modes ($p = 0.01$). Specifically, exercise modified total satellite cells per fiber by 38 ± 9, -

10 ± 10, and 9 ± 10% for RE, AE and CE, respectively. Post-hoc analyses revealed a significant difference in satellite cell proliferation between RE and CE ($p = 0.008$). Total satellite cells per fiber and percent changes in satellite cells are displayed in Table 4.3.

Fiber-Type Specific Satellite Cell Proliferation

A 2x3 ANOVA of satellite cell proliferation in MHC I fibers revealed an overall effect between exercise modes ($p = 0.046$). The percent change in satellite cell numbers in MHC I fibers from pre- to post-exercise also revealed a significant overall effect ($p = 0.006$). Specifically, RE increased satellite cells in MHC I fibers by 46 ± 14%, while AE and CE decreased satellite cells in MHC I fibers by 7 ± 17% and 22 ± 10%, respectively. Post-hoc analysis revealed a difference in satellite cell proliferation between RE and CE ($p = 0.006$) and between RE and AE ($p = 0.035$). The number of satellite cells and percent changes in MHC I fibers are displayed in Table 4.3. Satellite cell proliferation in non-MHC I fibers were unaffected by exercise modality. Satellite cell number per fiber and percent changes in non-MHC I fibers are listed in Table 4.3.

Table 4.3 Active Satellite Cells

	Pre RE	Post RE	% Change	Pre AE	Post AE	% Change	Pre CE	Post CE	% Change
Mixed ASC/Fiber	0.011 ± 0.001	0.011 ± 0.002	+5 ± 17%	0.01 ± 0.002	0.01 ± 0.02	+11 ± 19%	0.011 ± 0.001	0.013 ± 0.003	+9 ± 15%
MHC I ASC/Fiber	0.011 ± 0.002	0.008 ± 0.003	-7 ± 27%	0.014 ± 0.003	0.009 ± 0.004	-24 ± 25%	0.01 ± 0.002	0.016 ± 0.005	+62 ± 45%
non-MHC I ASC/Fiber	0.011 ± 0.002	0.014 ± 0.004	+36 ± 31%	0.007 ± 0.002	0.010 ± 0.002	+27 ± 22%	0.012 ± 0.002	0.014 ± 0.004	+7 ± 25%

Table 4.4 Satellite Cell Proliferation

	PreRE	PostRE	%Change	PreAE	PostAE	%Change	PreCE	PostCE	%Change
Total SC/Fiber	0.061 ± 0.005	0.085 ± 0.01	+38 ± 9%*	0.062 ± 0.013	0.061 ± 0.009	+10 ± 10%	0.093 ± 0.013	0.093 ± 0.025	-9 ± 10%
MHC I SC/Fiber	0.067 ± 0.013	0.089 ± 0.008	+46 ± 14%*	0.068 + 0.016	0.05 ± 0.009	-7 ± 17%	0.101 ± 0.021	0.079 ± 0.022	-22 ± 10%
non-MHC I SC/Fiber	0.061 ± 0.006	0.086 ± 0.014	+39 ± 14%	0.056 ± 0.01	0.067 ± 0.011	+27 ± 12%	0.096 ± 0.017	0.107 ± 0.027	+3 ± 13%

* $p \leq 0.01$, RE vs CE

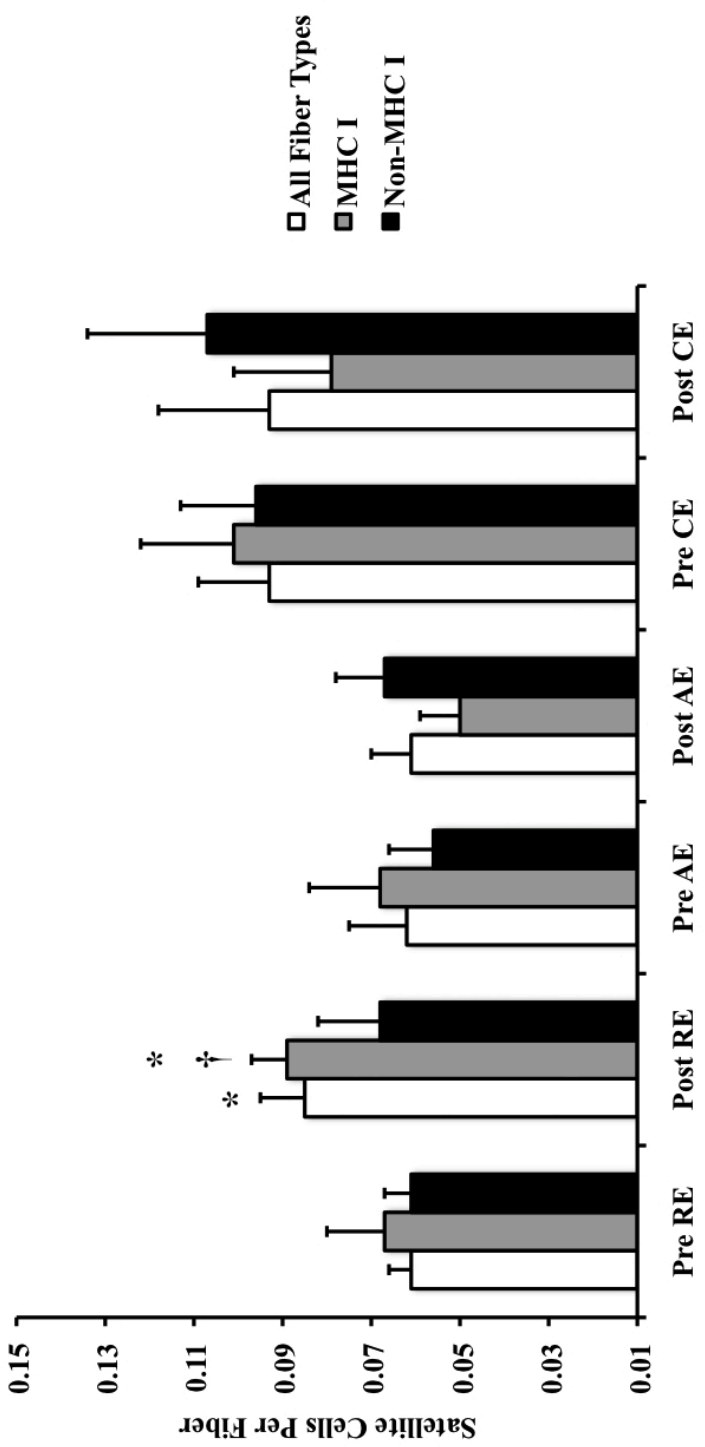


Figure 4.4 Satellite Cell Proliferation

* $p \leq 0.01$, RE vs CE, † $p \leq 0.05$, RE vs AE

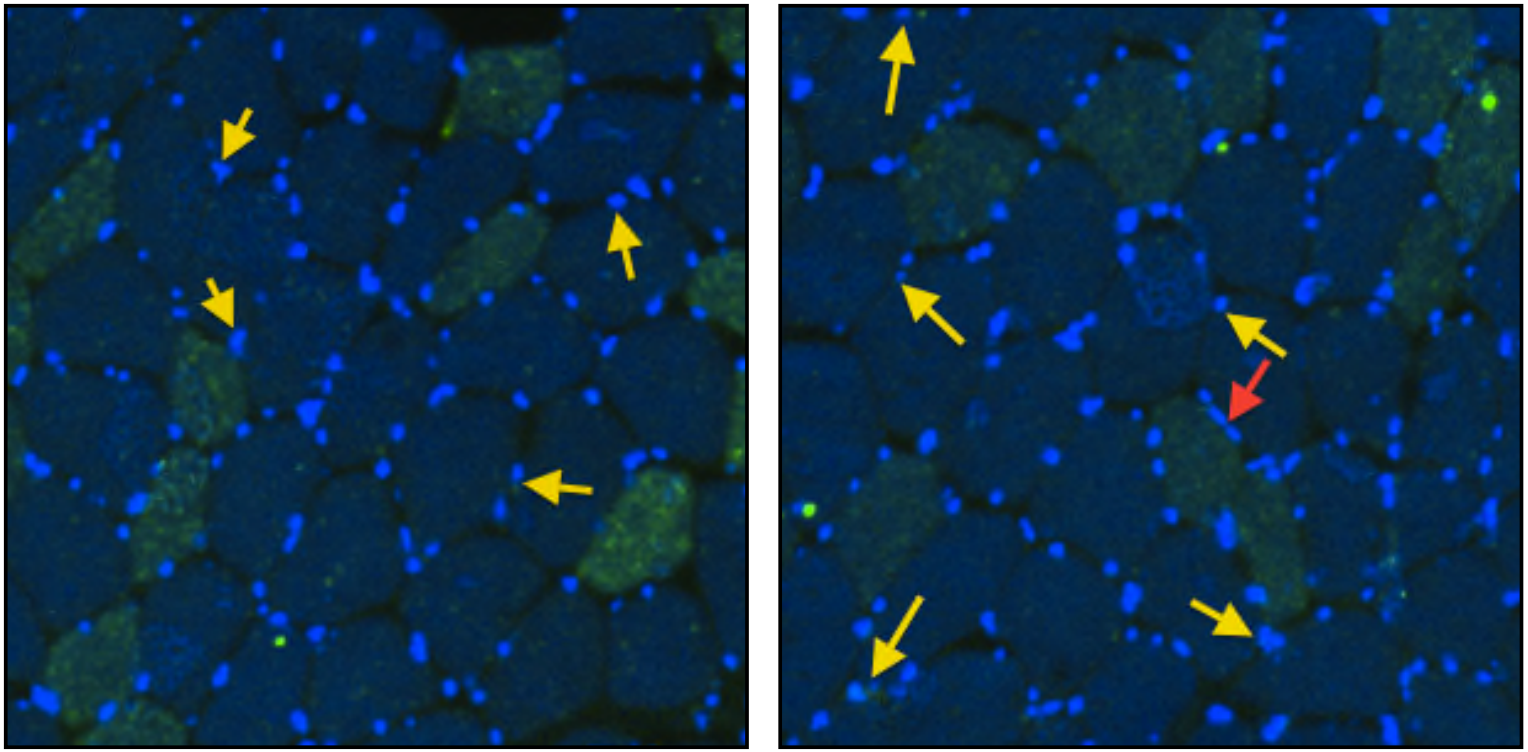
Time Course of Satellite Cell Proliferation Following Acute Resistance Exercise

During the concurrent exercise trial, pre-AE also represented 10 days post-RE, allowing a comparison between pre RE, 4 days post RE and 10 days post RE. The number of satellite cells per fiber was different across the three time points ($p = 0.017$). Post-hoc analysis revealed differences between pre-RE and 4 days post-RE ($p = 0.007$), and between 4 days post RE and 10 days post RE ($p = 0.047$). Non-MHC I proliferation was also different across the three time points ($p = 0.021$). Post-hoc analysis revealed significant differences between pre RE and 4 days post RE ($p = 0.033$), and between 4 days post RE and 10 days post RE ($p = 0.043$). A trend towards significance was detected in MHC I fibers ($p = 0.073$). The time course of the satellite cell proliferative response to RE is displayed in Table 4.4

Time Course of Satellite Cell Activation Following Acute Resistance Exercise

Satellite cell activation was not different between the RE time points, with the exception of trend among non-MHC I muscle fiber ($p = 0.078$). The time course of the satellite cell activation response to RE is displayed in Table 4.4.

Figure 4.5 Quantification of Pre and Post RE Samples



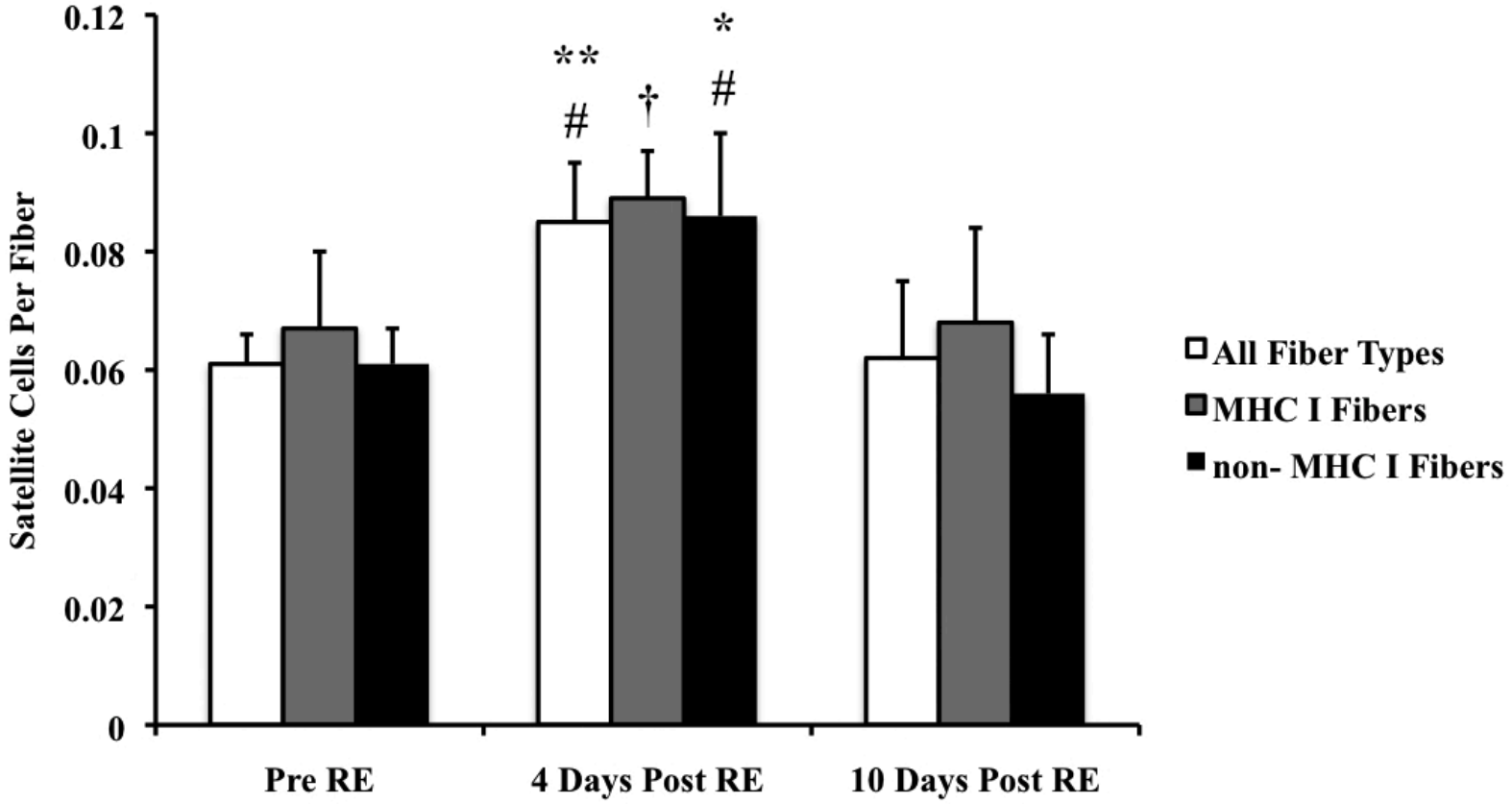
Representative images from before and after resistance exercise in the same subject. Yellow and red arrows indicate satellite cells and active satellite cells, respectively. Left: Pre RE. Right: Post RE

Table 4.5 Time Course of Satellite Cell Activity Following Acute Resistance Exercise

	Pre-RE	4 Days Post-RE	10 Days Post-RE	Main Time Effect
Mixed SC/Fiber	0.061 ± 0.005	0.085 ± 0.010**$^{\#}$	0.062 ± 0.013	p = 0.017
MHC I SC/Fiber	0.067 ± 0.013	0.089 ± 0.008	0.068 + 0.016	p = 0.262
Non-MHC I SC/Fiber	0.061 ± 0.006	0.086 ± 0.014*$^{\#}$	0.056 ± 0.010	p = 0.021
Mixed ASC/Fiber	0.011 ± 0.001	0.011 ± 0.002	0.010 ± 0.002	p = 0.685
MHC I ASC/Fiber	0.011 ± 0.002	0.008 ± 0.003	0.014 ± 0.003	p = 0.363
Non-MHC I ASC/Fiber	0.011 ± 0.002	0.014 ± 0.004	0.007 ± 0.002	p = 0.078

* $p \leq 0.05$ vs pre RE, ** $p \leq 0.01$ vs pre RE, $^{\#}$ $p \leq 0.05$ vs 10 days post RE, $^{\#\#}$ $p \leq 0.01$ vs 10 days post RE

Figure 4.6 Time Course of Satellite Cell Proliferation



* $p \leq 0.05$ vs Pre RE, ** $p \leq 0.01$ vs Pre RE, $^{\#}$ $p \leq 0.05$ vs 10 Days Post RE, † $p = 0.073$ vs Pre RE

DISCUSION

The primary objective of the current investigation was to assess satellite cell dynamics following acute RE, AE and CE. Specifically, we quantified the total number of satellite cells and active satellite cells per muscle fiber in a mixed and fiber type specific fashion. The most novel finding from this study is that acute concurrent exercise blunts the satellite cell response to acute resistance exercise and that this effect is preferentially manifested in MHC I muscle fibers. These findings suggest that satellite cell physiology, particularly among MHC I muscle fibers, may partially explain why concurrent training can interfere with the whole muscle and fiber type specific hypertrophy observed with RE alone (24, 35, 45). Further, we observed for the first time that a single non-injurious session of RE results in transient proliferation of satellite cells – more abundant satellite cells at 4 days but returns back to pre-exercise levels 10 days following exercise.

The satellite cell response to acute RE was comparable to previous reports (17, 18, 21, 53). The resistance exercise employed in the current study elicited a 38% increase in satellite cell numbers per fiber. This value is lower than the >80% increase in satellite cells found in previous studies (96–141%) (17, 18, 21, 53). However, this is consistent with the less demanding exercise stimuli implemented in the present study. Although there is a paucity of dose-response satellite cell data, the >80% gains in satellite cell numbers have followed > 90 maximum eccentric contractions (96-300 repetitions) (17, 18, 21, 48, 53). This is in contrast to the 80 total repetitions at 75% 1RM used in the current study. Although we provided assistance when needed, our model was less intense and closer to a conventional resistance exercise session.

The response of satellite cells to acute AE in the current study does not agree with the one other study that has assessed satellite cell proliferation following acute AE. Here, 90 min of cycling at 60% of W_{max} did not influence satellite cells population, however a single 36km run increased satellite cells by 27% in endurance trained males (41). This is not necessarily surprising as running inherently involves a substantial eccentric component. Also, aerobic training is not typically associated with muscle fiber hypertrophy, and thus would not be expected to elicit a satellite cell response.

Satellite cell proliferation following RE was negated when an identical RE protocol was followed by 90 min of cycling (CE). Notably, for unknown reasons, the baseline CE values were high compared to pre RE and pre AE values. Although unlikely, it is conceivable that RE performed on one leg affected proliferation in the other leg, with differentiation occurring only in the RE leg which brought satellite cell numbers back to baseline. Regardless, the response following CE is in agreement with the hypertrophic adaptations to resistance and concurrent training. Although speculative and beyond the scope of the current study, the similarities in these responses may be explained by the behavior of mechano-growth factor (MGF), an isoform of insulin-like growth factor (IGF-1) that stimulates both hypertrophy and satellite cell proliferation, and insulin-like growth factor binding protein (IGFBP-3), a binding protein that inhibits the effects of IGF-1. IGFBP-3 is present in both circulation (36) and skeletal muscle (54), binds to IGF-1 isoforms, preventing it from binding to it's receptor and ultimately, prevents it from eliciting a cellular response (5, 54). IGFBP-3 is upregulated in response to aerobic exercise, but not resistance exercise (49), thereby decreasing the bioavailability of IGF-1 (15).

To our knowledge, this is the first information gathered on the fiber-type specific response to acute resistance, concurrent and aerobic exercise. The attenuated satellite cell response in the current study appears to have been driven by MHC I fibers. The MHC I fiber satellite cell response to RE (46%), and AE (-7%) and CE (-22%) were clearly divergent, as the number of satellite cells per MHC I fiber increased with RE but not AE or CE. In contrast, the non-MHC I fiber satellite cells responded similarly following each form of exercise, although was only statistically elevated from baseline following RE. These findings support the fiber-type specific effects of concurrent training on hypertrophy. Specifically, the interference effect is facilitated by the attenuation of MHC I fiber growth. MHC IIa fibers appear to be fairly responsive to both resistance and concurrent training (7, 12, 24, 35, 45), whereas MHC I growth observed with resistance training is virtually negated with the addition of aerobic exercise. When combined with the current data, it appears that the MHC I satellite cell response to concurrent exercise may partially explain the reduction in whole muscle and cellular hypertrophy with concurrent training compared to resistance training alone.

The fiber-type specific results generally agree with a recent study conducted by Verney and colleagues. The authors found that MHC IIa satellite cells, but not MHC I, proliferate with resistance and concurrent training in old men (67). Following resistance training only, Verdijk et al. also observed adaptations in MHC IIa satellite cells, and not in MHC I fibers of old men (66). Our data follows a similar pattern, with significant satellite cell proliferation observed in MHC IIa fibers following acute resistance exercise, although a very strong trend towards significance was observed in MHC I fibers ($p = 0.073$). The satellite cell response to acute exercise is markedly different between young

and old individuals (21), and the fiber-type specific adaptations to training in young men are currently unknown.

Notwithstanding a trend within MHC II muscle fibers, there were no changes in the number of activated satellite cells observed at any time point during this study. Because activation must precede proliferation, it can only be assumed that the satellite cells were activated at an earlier time point. Although significant satellite cell activation has been observed up to 8 days post exercise (17, 18, 48), activation has been reported as early as 48 hours post electrical stimulation (40). Because the magnitude of the satellite cell response may be dependant on intensity (63), future studies assessing satellite cell dynamics should consider the use of multiple post exercise time points when using more conventional modes of resistance exercise.

As previously mentioned, increases in satellite cell numbers have been observed 8 days post RE (17, 18), but no study has documented the acute satellite cell response past 8 days. When assessing the time course of satellite cell dynamics in response to acute RE, we observed an increase in mixed, MHC I (0.073), and non-MHC I fibers after 4 days, with a return to pre exercise values at 10 days. Although this is the first assessment of the satellite cell response 10 days post acute RE, the return to baseline could have occurred at any time following day 4. With the use of a less intense, and more conventional mode of resistance exercise, the increase that we observed may have peaked and returned to pre exercise values much earlier than 10 days post, as opposed to a continual increase through 8 days post RE as seen with high intensity eccentric contractions (17, 18). This information is useful for future studies and suggests that physical activity control for 10

days prior to a ‘baseline’ measure is suitable, at least in recreationally active, college-aged males.

Our results provide the first evidence that acute concurrent exercise attenuates the satellite cell response to resistance exercise alone, and does so by preferentially blunting MHC I satellite cell proliferation, albeit for unknown reasons. The satellite cell response to acute resistance and concurrent exercise mimic the fiber-type specific adaptations to training. Whether or not the MHC I fiber satellite cell response to concurrent exercise can be manipulated to respond more similarly to RE is unknown. Future insight into how altering the order of exercise (aerobic followed by resistance), mode, duration, and intensity of the aerobic exercise component are warranted. Also of note is that a more conventional resistance exercise stimulus, as opposed to eccentric only, is sufficient to stimulate a significant satellite cell response. Most importantly, the current data provide a compelling biological mechanism for the interference effect of concurrent exercise.

CHAPTER FIVE – SUMMARY

The primary aim of this study was to assess the fiber-type specific satellite cell response to acute resistance, aerobic and concurrent exercise to provide insight into possible mechanisms for why concurrent training can attenuate whole muscle size and strength adaptations compared to those elicited by resistance exercise alone.

In response to RE, satellite cell number per fiber increased 38%, with no increase following AE or CE. Although the number of activated satellite cells per fiber did not change with exercise, CE markedly interfered with satellite cell proliferation, and did so by preferentially blunting the response in MHC I fibers. This response coincides with fiber-type specific patterns of hypertrophy and satellite cell dynamics elicited by resistance and concurrent training.

When assessing the time course of satellite cell dynamics following RE our findings show an increase in total, MHC I (0.073) and MHC II fibers, with a return to baseline by 10 days. This in particular is useful for future studies when determining a period of inactivity before baseline satellite cell assessments.

In conclusion, our findings demonstrate that acute concurrent exercise interferes with the satellite cell response of acute resistance exercise by preferentially blunting the response in MHC I fibers. This follows the fiber-type specific patterns of hypertrophy resulting from resistance and concurrent training. We also conclude that increases in satellite cells from an acute bout of conventional resistance exercise will return to baseline by 10 days. Most importantly, the current data provide a compelling biological mechanism for the interference effect of concurrent exercise.

SUBJECTS WANTED FOR

AN EXERCISE STUDY

The Human Performance Laboratory at JMU will be conducting a study examining the muscle fiber response to resistance and aerobic exercise

Who are we looking for?

- Males
- 18-25 years old
- Recreationally active

What you will be asked to do:

- Complete preliminary fitness testing (1 visit ~ total 60 minutes)
- Participate in 2 exercise protocols (60 and 150 minutes), one of which includes a resistance exercise protocol. The other trial will consist of an identical resistance exercise protocol, followed by 90 minutes of moderate intensity cycling
- Receive a total of 6 muscle biopsies from the thigh muscle (vastus lateralis)
- Subjects will be asked to avoid exercise for the 10 days between preliminary testing and exercise protocols

What are the benefits of participation?

- Free evaluation of aerobic capacity (VO_{2max}) and muscle physiological data
- $75 for study completion

For more information, please contact Lyle Babcock at babcoclw@dukes.jmu.edu

Department of Kinesiology, James Madison University

James Madison University
Department of Kinesiology
Informed Consent

Purpose

You are being asked to volunteer for a research project conducted by Dr. Nick Luden and Lyle Babcock from James Madison University titled "Fiber Type Specific Satellite Cell Proliferation: Does Concurrent Aerobic Exercise Interfere with the Intentions of Resistance Exercise?"

The primary goal of this study is to examine how the 'muscle building' machinery in the thigh muscle responds to resistance exercise and how this response changes when you perform concurrent resistance and aerobic exercise.

Experimental Procedures

You will be asked to visit the Human Performance Laboratory (Godwin 209) a total of 5 times. Specifically, you will be asked to report to the laboratory for one preliminary trial, two experimental trials, and two follow up/biopsy trials 4 days after the experimental trials. The preliminary test will each require approximately 45 minutes, the experimental trials will require approximately 60-150 minutes, (RE = 60 minutes and AE + RE 150 minutes) and the two follow up/biopsy trials will require approximately 30 minutes, for a total time commitment of approximately 6 hours. Detailed information for each of these trials is provided below:

Preliminary Test 1 (n = 1 visit)

Before any physical evaluation is given, you will be asked to complete screening forms and an informed consent, to ensure that you meet the study criteria, that you do not have any risk factors for heavy exercise, and that you do not have any known allergies to local anesthesia. In the process of filling out these forms, you will be asked to share information regarding your general health and lifestyle with the researchers. If you meet the criteria for the study, the researchers will measure your height and weight and you will perform a cardiorespiratory fitness test. During this assessment, an exercise test will be conducted to determine your maximal oxygen uptake (VO_{2max}). To do this, you will ride a stationary cycle ergometer at an initial workload that is 'fairly easy'. Workload will be increased by 25 watts every 2 minutes during the test. You will be encouraged to continue to cycle until you request to stop due to fatigue or are unable to continue at a cadence >50 revolutions per minute.

Experimental Trials (n = 2 visits) + Post-Exercise Biopsy Trials (n = 2 visits) (total n = 4 visits)

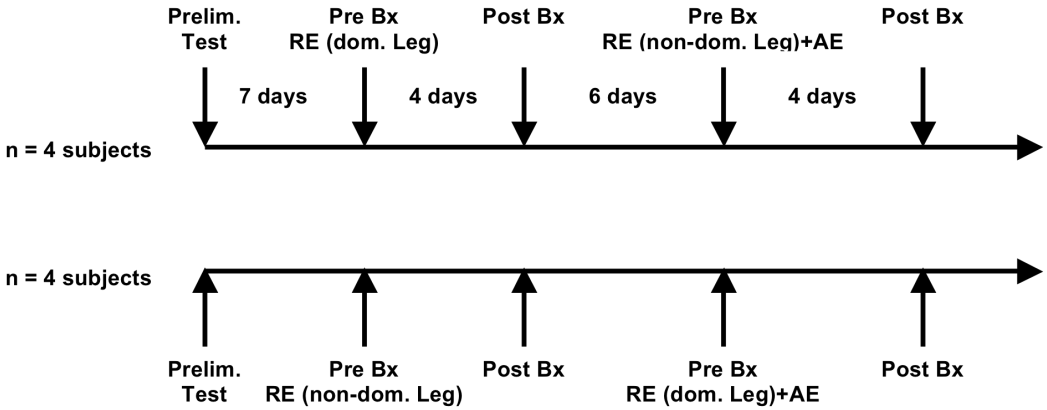
Experimental trial - RE: You will be asked to perform a one-legged one-repetition maximum strength test for both a leg press and leg extension. Following a 5-minute self selected warm-up on a treadmill, you will complete a warm-up set of 10 to 12 repetitions followed by 4 minutes of rest. This will be followed by another set of 2 repetitions at 50-70% of your perceived one repetition maximum. The following trials with be one repetition until a one-repetition maximum is achieved. This protocol will be used for both a one-legged leg extension then a one-legged leg press. You will rest for 4 minutes in between each set. The one-repetition maximum will be used to prescribe the resistance used for the following RE protocol. You will then be asked to perform 3 sets of 10 one-legged leg extension repetitions of a weight corresponding to 75% of your one-repetition maximum. This will be followed by a fourth set of repetitions until you are unable to complete a full repetition (~8 to 15 repetitions). Each of the four sets will be separated by 2 minutes. Following the one-legged leg extension protocol, you will perform, with the same leg, 4 sets of one-legged leg presses as previously described (RE-only leg).

Experimental trial - AE+RE: You will first perform RE identical to the RE trial outlined above (1RM followed by 4 sets of one-legged leg extensions followed by 4 sets of one-legged leg presses). The RE protocol will then be followed by 10 minutes of rest and 90 minutes of cycling at 60% of peak power at VO_{2max} (determined during preliminary test 1). Thus, one leg will be exposed to 8 total sets of resistance exercise followed by 90-minutes of cycling (AE + RE leg), while the other leg will be exposed to only 90 minutes of cycling (AE-only leg).

Skeletal muscle biopsies: Muscle biopsies of the exercised vastus lateralis will be obtained immediately prior to- and 4 days following exercise. Thus, for the RE trial, one biopsy will be obtained from the exercised leg before and 4 days after RE (n = 2 biopsies). For the AE + RE trial, biopsies will be obtained from each leg before and 4 days after exercise (4 biopsies), for a grand total of 6 skeletal muscle biopsies.

Five minutes following the injection of local anesthesia (Xylocaine – a common local anesthetic), using sterile procedures (towels, gloves, gauze, needles, scalpels, autoclaved biopsy needles, etc.), a scalpel is used to make a small incision (~1/4 inch length) through the skin, subcutaneous fat layer, and epimysium (fascia or connective tissue wrapping around the whole muscle). The biopsy needle is then inserted into the belly of the muscle for 2-3 seconds for muscle tissue sampling. Each biopsy sample weighs approximately 50-100 milligrams - the size of a small pea. Immediately following the procedure, light manual pressure is applied to the biopsy site using sterile gauze. Once bleeding from the incision has subsided (typically 3-5 minutes), a band-aid is applied over the incision and covered with an elastic pressure bandage. Approximately 10 minutes following the biopsy, you will begin the respective exercise protocol with 5 minutes of self-selected warm-up on a treadmill as stated above.

Treatment Periods:



Bx = vastus lateralis muscle biopsy; RE = resistance exercise only; AE = aerobic exercise only; RE+AE = aerobic exercise combined with resistance exercise; Prel. Test = preliminary testing including VO_{2max} and 1RM testing; dom. = dominant; non-dom. = non-dominant.

Dietary and Exercise Controls

You will be asked to maintain consistent dietary habits for 3 days prior to each experimental trial and for the 4 days following each experimental trial. You are to complete a diet record for the 24 hrs preceding each trial each biopsy. You will also be asked to avoid physical activity for 10 days prior to each experimental trial, and to record all physical activity performed during the 72 hrs preceding each trial. You will be asked to consume your final 'self-selected' meal no less than 10 hrs prior to the start of the treatment trials (i.e. dinner on the evening prior to testing). After this time, you are to consume only the standardized meal (provided by the investigators) 2 hours prior to the start of the trial and water *ad libitum* until the end of each treatment trial.

Risks

You are expected to be honest about disclosing all known risk factors to the researcher. According to the American College of Sports Medicine, the risks associated with maximal exercise/testing for healthy individuals are very minimal. If you do not meet the criteria for "low risk", you will not be allowed to participate in the study. In the unlikely event of cardiac or other complications during exercise, an emergency plan is in place. This includes immediate access to a phone to call emergency personnel. In addition, each of the investigators is CPR certified.

The exercise protocol may result in minor-moderate levels of muscle soreness and fatigue for 1-2 days following each exercise session. However, the level of muscle soreness is expected to be lower than levels normally experienced when people perform other 'normal' activities that are not part of their regular exercise routine (i.e. if a cyclist played a game of basketball with friends for 2 hours).

The risks associated with the muscle biopsy technique include a possible dull pain during the administration of the anesthetic and the biopsy procedure, and delayed soreness for one to two days following the biopsy. Sterile procedures will be used during the biopsy procedure to minimize these risks. There is a small risk of bleeding, infection, and scarring of the skin. Temporary numbness of the skin near the biopsy site occurs rarely. You may feel lightheaded and there is a slight risk of fainting. Following the biopsy you will be provided with a 'biopsy care package' that will include instructions for care, band-aids, and alcohol pads. You are also encouraged to contact a member of our research team if you have any concerns about your recovery. There is a small risk of an allergic reaction to the local anesthetic used during the muscle biopsy procedure. Symptoms may include an itching sensation of the skin, difficulty breathing, fainting, and shock. Allergic reactions to the local anesthetic used are extremely rare. You will be pre-screened, as part of the medical history document, for any known allergic reaction to local anesthetics.

You will be contacted via email with in 48 hours after each biopsy to check on recovery and to answer any questions you may have.

Benefits

The benefits associated with this project include a free VO_{2max} assessment, and a $75 payment for study completion, or $12.50 per biopsy

Inquiries

If you have any questions or concerns please contact Dr. Nicholas Luden at ludennd@jmu.edu or (540) 568-4069. In the case of any immediate concerns or adverse reactions during the study, contact Dr. Luden on his cell phone (540) 746-6134.

Questions about Your Rights as a Research Subject
Dr. David Cockley
Chair, Institutional Review Board
James Madison University
(540) 568-2834
cocklede@jmu.edu

Confidentiality

All data and results will be kept confidential. You will be assigned an identification code. At no time will your name be identified with your individual data. The researcher retains the right to use and publish non-identifiable data. All data will be kept secured in a locked cabinet. Final aggregate results will be made available to participants upon request.

Freedom of Consent

Your participation is entirely voluntary. You are free to choose not to participate. Should you choose to participate, you can withdraw at any time without consequences of any kind.

I have read this consent form and I understand what is being requested of me as a participant in this study. I freely consent to participate. I have been given satisfactory answers to my questions. The investigator provided me with a copy of this form. I certify that I am at least 18 years of age.

Name of Subject (Printed)	Name of Researcher (Printed)
Name of Subject (Signed)	Name of Researcher (Signed)
Date	Date

AHA/ACSM Health/Fitness Facility Pre-participation Screening Questionnaire

Assess your health status by marking all *true* statements

History

You have had:

_____ a heart attack
_____ heart surgery
_____ cardiac catheterization
_____ coronary angioplasty (PTCA)
_____ pacemaker/implantable cardiac
_____ defibrillator/rhythm disturbance
_____ heart valve disease
_____ heart failure
_____ heart transplantation
_____ congenital heart disease

If you marked any of these statements in this section, consult your physician or other appropriate health care provider before engaging in exercise. You may need to use a facility with a ***medically qualified staff***.

Symptoms

_____ You experience chest discomfort with exertion
_____ You experience unreasonable breathlessness
_____ You experience dizziness, fainting, or blackouts
_____ You take heart medications

Other Health Issues

_____ You have diabetes
_____ You have asthma or other lung disease
_____ You have burning or cramping sensation in your lower legs when walking short distances
_____ You have musculoskeletal problems that limit your physical activity
_____ You have concerns about the safety of exercise
_____ You take prescription medication(s)

Cardiovascular risk factors

_____ You are a man older than 45 years
_____ You are a woman older than 55 years, have had a hysterectomy, or are postmenopausal
_____ You smoke, or quit smoking within the previous 6 months
_____ Your blood pressure is > 140/90 mmHg
_____ You do not know your blood pressure
_____ You take blood pressure medication
_____ Your blood cholesterol level is > 200 mg/dl
_____ You do not know your cholesterol level
_____ You have a close blood relative who had a heart attack or heart surgery before age 55 (father or brother) or age 65 (mother or sister)
_____ You are physically inactive (i.e. you get < 30 minutes of physical activity on at least 3 days of the week)
_____ You are > 20 pounds overweight

If you marked two or more of the statements in this section, you should consult your physician or other appropriate health care provider before engaging in exercise. You might benefit from using a facility with a ***professionally qualified exercise staff*** to guide your exercise program.

_____ None of the above

You should be able to exercise safely without consulting your physician or other appropriate health care provider in a self-guided program or almost any facility that meets your exercise program needs.

Subject Prescreening Information

Please Complete the Following:

Age (yrs):

Height (inches):

Weight (lbs):

Average Exercise Habits over the Past 2 Months:

Avg. # days of exercise per week:

Avg. # of days of aerobic exercise per week:

Avg. # of days of resistance exercise per week:

Avg. # of days of cycling per week:

Do you have a muscle or joint injury that precludes the completion of the exercise protocol?

Do you currently use cardiac medications (Digoxin, Digitalis, etc)?

Are you allergic to local anesthetics (numbing agents) such as Lidocaine (Xylocaine, Novocain, etc)?

Have you had Novocain administered at the dentist?

Time	Food and/or Drink	Method of Preparation	Quantity Consumed	Brand Name

24-HOUR DIET RECORD

*Subject number*_____ *Date*_____ *Day of Week*_____

Adapted From: Lee RD, Nieman DC. *Nutritional Assessment.* 2nd ed. United States of America: Mosby; 1996

INSTRUCTIONS FOR KEEPING YOUR 24-HOUR FOOD RECORD

Keep your record for three days per trial. You will include the day before, the day of, and the day after each trial. Include all meals, snacks, nibbling, and beverages including water and cocktails

1. Fill out the date and day of the week at the top of food record sheet

2. Record the time you consumed your food and/or drink. To be most accurate, fill out the food record as soon as you finish eating.

3. List the first food and/or drink you consumed when you began your day and continue to record until you consume your last food and/or drink of your day (usually before bedtime)

4. List each food and/or drink on a separate line
   Example: cereal with milk, cereal and milk should each be on separate lines
   spaghetti, noodles and sauce should each be on separate lines

   Combination foods:
   List parts of food on separate lines
   Include preparation method, quantity, and brand name of each food
   Example: Sandwich (4 oz healthy choice turkey, 2 slices Sara Lee wheat bread, 1 tbsp Hellman's light mayo, 2 oz Kraft American cheese, 1 slice of red fresh tomato)

5. Record the method of preparation
   Example: fried, baked, grilled
   salt, oil (olive, canola, corn, other) butter or margarine, spices, etc.

6. Record quantity consumed
   Do not record any food not eaten
   Example: made two cups of vegetables but ate half so you would record one cup

   Quantity of food and/or drink
   Example: cups, ounces, liters, grams, each, or other unit of measure
   Example: 1 cup of vegetables, 4 ounces of meat, one medium apple

7. Record brand name
   Example: fast food chain name and/or package name
   Example: Wendy's, Betty Crocker, Lean Cuisine, Gatorade, Thomas Bagel
8. Place any helpful food labels in manila envelope that is attached to folder

Use the following to help determine portion sized and types of foods

	PLEASE SPECIFY
Beverages	Sugar or creamer? Regular or sugar-free? Alcohol content? Name of drink and ingredients (if mixed drink)
Breads	Butter or margarine added?
Cereal/Milk	Milk, sugar, or fruit added? The type of milk? (skim, 1%, 2%, whole) Cereal: dry or cooked measure?
Dairy	Is yogurt fruited or plain? % fat of milk or yogurt? Indicate brand name of cheese substitute and/or nondairy creamer.
Desserts	Whipped topping added? Frosting? Fat modified (i.e., reduced)? Sugar-free?
Eggs	Preparation method (scrambled, hard-boiled, etc)? Fat used in cooking?
Fast Food	What restaurant? If not a national fast food chain, describe food in detail Size order of fries? Super-size? ExtRa toppings on sandwich?
Fats/Oils	Regular or salt-free? Stick, tub, or liquid margarine? Reduced calorie or diet product?
Fish	Water or oil packed (fresh or canned)? Baked or fried (With batter or without)? Type of fat added? Raw or cooked weight?
Fruit	Sweetened or unsweetened? Fresh, canned, or frozen? With or without skin?
Meats	Visible fat removed? Light or dark meat? Raw or cooked?
Sugars and Sweets	Regular or reduced-calorie? Don't forget hard candy as well as chocolate.
Vegetables	Raw or cooked? Fresh, frozen, or canned? Low-sodium or regular? Added fat or sauce?

Helpful Hints with Portion Sizes

- 1 teaspoon (5 ml)
  - about the size of the top half / tip of your thumb
- 1oz (28g)
  - approximately inch cube of cheese
  - volume of four stacked dice
  - slice of cheese is about the size of a 3 1/2 inch computer disk
  - chunk of cheese is about as thick as 2 dominoes
  - 1 handful (palm) of nuts
- 2 ounces (57 g)
  - 1 small chicken leg or thigh
  - 1/2 cup of cottage cheese or tuna
- 3 ounces (85 g)
  - serving of meat is about the size of a deck of playing cards (3 exchanges)
  - the size of the palm of your hand
  - 1/2 of whole chicken breast
  - 1 medium pork chop
  - 1 small hamburger
  - unbreaded fish fillet
- 1/2 cup (118 ml)
  - fruit or vegetables can fit in the palm of your hand
  - about the volume of a tennis ball
- 1 cup (236 ml)
  - about the size of a woman's fist
  - breakfast cereal goes halfway up the side of a standard cereal bowl
  - broccoli is about the size of a light bulb
- 1 medium apple = A tennis ball

Daily Activity Records
Subject #_____ Trial #_____ Date:_____

Date	Type of Exercise Performed	Duration of Exercise (minutes)	Intensity of Exercise (use scale below)

Intensity Scale

6
7 Very, very light
8
9 Very light
10
11 Fairly light
12
13 Somewhat hard
14
15 Hard
16
17 Very hard
18
19 Very, very hard
20

Inventory of Supplies Necessary to Complete this Project

Muscle Biopsies

Supplies Needed	**Brand and Item Number**
Biopsy Needle	Stille: 119-29187
Crosstex self-sealing sterilization pouch	Fisher Brand: 01-312-51
Lidocaine HCl 0.1%	Hospira: NDL 0409-4276-02
BD 3 mL Syringe, Luer-Lok Tip	Becton Dickinson: 309585
23G TW Needles, Precision Glide	Becton Dickinson: 305193
Monoject Safety Needles, 20G x 1”	Tyco Healthcare: 8881850010
Safety Lock carbon steel surgical blades	Bard-Parker: 371151
1” Durapore Tape	3M: 1538-1
Kendal Curity Gauze Sponges 4 x 4	Tyco Healthcare: 2187
Kendal Versalon All-Purpose Sponges, 2x2	Tyco Healthcare Group: 9022
Betadine Swab Stick	Purdue Products: NDC 67618-153-01
Poly lined sterile field	Basse: 696
Elastikon Tape	Johnson and Johnson: 005171
Coban	3M: NDC 8333-1582-01
Maxizyme	Henry Schein: 101-9031
Kenal 140 mL Luer-Lok Syringe	Tyco Healthcare: 8881114063
33” Tubing, latex free	Smiths Medical: 2009-12

Tissue Processing

Supplies Needed	**Brand and Item Number**
Disposable Scalpel, #10	Feather: 2975
Cryo Tube Vials	Nunc: 375418
Petri Dishes for 47 mm cultures	Fisher Brand: 09-720-500
Kendal Curity gauze Sponges 4 x 4	Tyco Healthcare: 2187
Dulmont Medial Tweezers, 110mm, #5	Ted Pella, Inc.: 38125
Liquid Nitrogen	JMU Chemistry

Tissue Staining

Supplies Needed	Brand and Item Number
NCAM Primary Antibody	Santa Cruz Biotechnology sc-7326
Ki-67 Primary Antibody	Santa Cruz Biotechnology sc-15402
MYC I Primary Antibody	Santa Cruz Biotechnology m8421
Dylight 488 Secondary Antibody	Jackson ImmunoResearch 115-485-062
Dylight 488 Secondary Antibody	Jackson ImmunoResearch 115-485-146
Cy5 Secondary Antibody	Jackson ImmunoResearch 111-165-144
Normal Goat Serum	Jackson ImmunoResearch 005-000-121
Methanol	JMU Biology
DAPI	Invitrogen D3571
Glass Slides	Fisher Scientific 99-910-01
Cover Slides	Fisher Scientific 12-542B
Tragacanth Gum	BakeDeco CC500-2
Isopentane	Fisher Chemical O35514
PBS	Invitrogen AM9624
Tween 20	Invitrogen 00-3005
Mounting Solution	Invitrogen 8030
Gel Mount	Fisher Scientific NC9034735
Pap Pen	Fisher Scientific 12-542B

REFERENCES

1. **Adams GR**. Satellite cell proliferation and skeletal muscle hypertrophy. *Appl Physiol Nutr Metab* 31: 782-790, 2006.
2. **Baar K**. Training for endurance and strength: lessons from cell signaling. *Med Sci Sports Exerc* 38: 1939-1944, 2006.
3. **Bamman MM, Shipp JR, Jiang J, Gower BA, Hunter GR, Goodman A, McLafferty CL, Jr., and Urban RJ**. Mechanical load increases muscle IGF-I and androgen receptor mRNA concentrations in humans. *Am J Physiol Endocrinol Metab* 280: E383-390, 2001.
4. **Barton-Davis ER, Shoturma DI, and Sweeney HL**. Contribution of satellite cells to IGF-I induced hypertrophy of skeletal muscle. *Acta Physiol Scand* 167: 301-305, 1999.
5. **Baxter RC**. Insulin-like growth factor (IGF)-binding proteins: interactions with IGFs and intrinsic bioactivities. *Am J Physiol Endocrinol Metab* 278: E967-976, 2000.
6. **Bell GJ, Petersen SR, Wessel J, Bagnall K, and Quinney HA**. Physiological adaptations to concurrent endurance training and low velocity resistance training. *Int J Sports Med* 12: 384-390, 1991.
7. **Bell GJ, Syrotuik D, Martin TP, Burnham R, and Quinney HA**. Effect of concurrent strength and endurance training on skeletal muscle properties and hormone concentrations in humans. *Eur J Appl Physiol* 81: 418-427, 2000.
8. **Bickel CS, Slade J, Mahoney E, Haddad F, Dudley GA, and Adams GR**. Time course of molecular responses of human skeletal muscle to acute bouts of resistance exercise. *J Appl Physiol* 98: 482-488, 2005.
9. **Carlson CJ, Booth FW, and Gordon SE**. Skeletal muscle myostatin mRNA expression is fiber-type specific and increases during hindlimb unloading. *Am J Physiol* 277: R601-606, 1999.
10. **Carrithers JA, Carroll CC, Coker RH, Sullivan DH, and Trappe TA**. Concurrent exercise and muscle protein synthesis: implications for exercise countermeasures in space. *Aviat Space Environ Med* 78: 457-462, 2007.
11. **Charifi N, Kadi F, Feasson L, and Denis C**. Effects of endurance training on satellite cell frequency in skeletal muscle of old men. *Muscle Nerve* 28: 87-92, 2003.
12. **Chilibeck PD, Syrotuik DG, and Bell GJ**. The effect of concurrent endurance and strength training on quantitative estimates of subsarcolemmal and intermyofibrillar mitochondria. *Int J Sports Med* 23: 33-39, 2002.
13. **Coffey VG, Jemiolo B, Edge J, Garnham AP, Trappe SW, and Hawley JA**. Effect of consecutive repeated sprint and resistance exercise bouts on acute adaptive responses in human skeletal muscle. *Am J Physiol Regul Integr Comp Physiol* 297: R1441-1451, 2009.
14. **Coffey VG, Pilegaard H, Garnham AP, O'Brien BJ, and Hawley JA**. Consecutive bouts of diverse contractile activity alter acute responses in human skeletal muscle. *J Appl Physiol* 106: 1187-1197, 2009.
15. **Copeland JL, and Heggie L**. IGF-I and IGFBP-3 during continuous and interval exercise. *Int J Sports Med* 29: 182-187, 2008.

16. **Cortes E, te Fong LF, Hameed M, Harridge S, Maclean A, Yang SY, Reid WM, and Goldspink G**. Insulin-like growth factor-1 gene splice variants as markers of muscle damage in levator ani muscle after the first vaginal delivery. *Am J Obstet Gynecol* 193: 64-70, 2005.
17. **Crameri RM, Aagaard P, Qvortrup K, Langberg H, Olesen J, and Kjaer M**. Myofibre damage in human skeletal muscle: effects of electrical stimulation versus voluntary contraction. *J Physiol* 583: 365-380, 2007.
18. **Crameri RM, Langberg H, Magnusson P, Jensen CH, Schroder HD, Olesen JL, Suetta C, Teisner B, and Kjaer M**. Changes in satellite cells in human skeletal muscle after a single bout of high intensity exercise. *J Physiol* 558: 333-340, 2004.
19. **Dennis RA, Przybyla B, Gurley C, Kortebein PM, Simpson P, Sullivan DH, and Peterson CA**. Aging alters gene expression of growth and remodeling factors in human skeletal muscle both at rest and in response to acute resistance exercise. *Physiol Genomics* 32: 393-400, 2008.
20. **Dolezal BA, and Potteiger JA**. Concurrent resistance and endurance training influence basal metabolic rate in nondieting individuals. *J Appl Physiol* 85: 695-700, 1998.
21. **Dreyer HC, Blanco CE, Sattler FR, Schroeder ET, and Wiswell RA**. Satellite cell numbers in young and older men 24 hours after eccentric exercise. *Muscle Nerve* 33: 242-253, 2006.
22. **Dudley GA, and Djamil R**. Incompatibility of endurance- and strength-training modes of exercise. *J Appl Physiol* 59: 1446-1451, 1985.
23. **Enns DL, and Tiidus PM**. Estrogen influences satellite cell activation and proliferation following downhill running in rats. *J Appl Physiol* 104: 347-353, 2008.
24. **Hakkinen K, Alen M, Kraemer WJ, Gorostiaga E, Izquierdo M, Rusko H, Mikkola J, Hakkinen A, Valkeinen H, Kaarakainen E, Romu S, Erola V, Ahtiainen J, and Paavolainen L**. Neuromuscular adaptations during concurrent strength and endurance training versus strength training. *Eur J Appl Physiol* 89: 42-52, 2003.
25. **Hameed M, Orrell RW, Cobbold M, Goldspink G, and Harridge SD**. Expression of IGF-I splice variants in young and old human skeletal muscle after high resistance exercise. *J Physiol* 547: 247-254, 2003.
26. **Hennessey LC, and AWS W**. The interference effects of training for strength and endurance simultaneously. *J Strength Conditioning Research* 8: 12-19, 1994.
27. **Hickson RC**. Interference of strength development by simultaneously training for strength and endurance. *Eur J Appl Physiol Occup Physiol* 45: 255-263, 1980.
28. **Hill M, and Goldspink G**. Expression and splicing of the insulin-like growth factor gene in rodent muscle is associated with muscle satellite (stem) cell activation following local tissue damage. *J Physiol* 549: 409-418, 2003.
29. **Izquierdo M, Hakkinen K, Ibanez J, Kraemer WJ, and Gorostiaga EM**. Effects of combined resistance and cardiovascular training on strength, power, muscle cross-sectional area, and endurance markers in middle-aged men. *Eur J Appl Physiol* 94: 70-75, 2005.

30. **Janssen I, Shepard DS, Katzmarzyk PT, and Roubenoff R**. The healthcare costs of sarcopenia in the United States. *J Am Geriatr Soc* 52: 80-85, 2004.
31. **Kadi F, Charifi N, and Henriksson J**. The number of satellite cells in slow and fast fibres from human vastus lateralis muscle. *Histochem Cell Biol* 126: 83-87, 2006.
32. **Kadi F, Schjerling P, Andersen LL, Charifi N, Madsen JL, Christensen LR, and Andersen JL**. The effects of heavy resistance training and detraining on satellite cells in human skeletal muscles. *J Physiol* 558: 1005-1012, 2004.
33. **Kadi F, and Thornell LE**. Concomitant increases in myonuclear and satellite cell content in female trapezius muscle following strength training. *Histochem Cell Biol* 113: 99-103, 2000.
34. **Kim JS, Cross JM, and Bamman MM**. Impact of resistance loading on myostatin expression and cell cycle regulation in young and older men and women. *Am J Physiol Endocrinol Metab* 288: E1110-1119, 2005.
35. **Kraemer WJ, Patton JF, Gordon SE, Harman EA, Deschenes MR, Reynolds K, Newton RU, Triplett NT, and Dziados JE**. Compatibility of high-intensity strength and endurance training on hormonal and skeletal muscle adaptations. *J Appl Physiol* 78: 976-989, 1995.
36. **Kraemer WJ, and Ratamess NA**. Hormonal responses and adaptations to resistance exercise and training. *Sports Med* 35: 339-361, 2005.
37. **Li P, Akimoto T, Zhang M, Williams RS, and Yan Z**. Resident stem cells are not required for exercise-induced fiber-type switching and angiogenesis but are necessary for activity-dependent muscle growth. *Am J Physiol Cell Physiol* 290: C1461-1468, 2006.
38. **Louis E, Raue U, Yang Y, Jemiolo B, and Trappe S**. Time course of proteolytic, cytokine, and myostatin gene expression after acute exercise in human skeletal muscle. *J Appl Physiol* 103: 1744-1751, 2007.
39. **Mackey AL, Esmarck B, Kadi F, Koskinen SO, Kongsgaard M, Sylvestersen A, Hansen JJ, Larsen G, and Kjaer M**. Enhanced satellite cell proliferation with resistance training in elderly men and women. *Scand J Med Sci Sports* 17: 34-42, 2007.
40. **Mackey AL, Kjaer M, Charifi N, Henriksson J, Bojsen-Moller J, Holm L, and Kadi F**. Assessment of satellite cell number and activity status in human skeletal muscle biopsies. *Muscle Nerve* 40: 455-465, 2009.
41. **Mackey AL, Kjaer M, Dandanell S, Mikkelsen KH, Holm L, Dossing S, Kadi F, Koskinen SO, Jensen CH, Schroder HD, and Langberg H**. The influence of anti-inflammatory medication on exercise-induced myogenic precursor cell responses in humans. *J Appl Physiol* 103: 425-431, 2007.
42. **Malm C, Nyberg P, Engstrom M, Sjodin B, Lenkei R, Ekblom B, and Lundberg I**. Immunological changes in human skeletal muscle and blood after eccentric exercise and multiple biopsies. *J Physiol* 529 Pt 1: 243-262, 2000.
43. **Matsakas A, Friedel A, Hertrampf T, and Diel P**. Short-term endurance training results in a muscle-specific decrease of myostatin mRNA content in the rat. *Acta Physiol Scand* 183: 299-307, 2005.

44. **McCarthy JP, Agre JC, Graf BK, Pozniak MA, and Vailas AC**. Compatibility of adaptive responses with combining strength and endurance training. *Med Sci Sports Exerc* 27: 429-436, 1995.
45. **McCarthy JP, Pozniak MA, and Agre JC**. Neuromuscular adaptations to concurrent strength and endurance training. *Med Sci Sports Exerc* 34: 511-519, 2002.
46. **McCroskery S, Thomas M, Maxwell L, Sharma M, and Kambadur R**. Myostatin negatively regulates satellite cell activation and self-renewal. *J Cell Biol* 162: 1135-1147, 2003.
47. **McKay BR, O'Reilly CE, Phillips SM, Tarnopolsky MA, and Parise G**. Co-expression of IGF-1 family members with myogenic regulatory factors following acute damaging muscle-lengthening contractions in humans. *J Physiol* 586: 5549-5560, 2008.
48. **Mikkelsen UR, Langberg H, Helmark IC, Skovgaard D, Andersen LL, Kjaer M, and Mackey AL**. Local NSAID infusion inhibits satellite cell proliferation in human skeletal muscle after eccentric exercise. *J Appl Physiol* 107: 1600-1611, 2009.
49. **Miller BF, Olesen JL, Hansen M, Dossing S, Crameri RM, Welling RJ, Langberg H, Flyvbjerg A, Kjaer M, Babraj JA, Smith K, and Rennie MJ**. Coordinated collagen and muscle protein synthesis in human patella tendon and quadriceps muscle after exercise. *J Physiol* 567: 1021-1033, 2005.
50. **Nader GA**. Concurrent strength and endurance training: from molecules to man. *Med Sci Sports Exerc* 38: 1965-1970, 2006.
51. **Nelson AG, Arnall DA, Loy SF, Silvester LJ, and Conlee RK**. Consequences of combining strength and endurance training regimens. *Phys Ther* 70: 287-294, 1990.
52. **O'Connor RS, and Pavlath GK**. Point:Counterpoint: Satellite cell addition is/is not obligatory for skeletal muscle hypertrophy. *J Appl Physiol* 103: 1099-1100, 2007.
53. **O'Reilly C, McKay B, Phillips S, Tarnopolsky M, and Parise G**. Hepatocyte growth factor (HGF) and the satellite cell response following muscle lengthening contractions in humans. *Muscle Nerve* 38: 1434-1442, 2008.
54. **Oliver WT, Rosenberger J, Lopez R, Gomez A, Cummings KK, and Fiorotto ML**. The local expression and abundance of insulin-like growth factor (IGF) binding proteins in skeletal muscle are regulated by age and gender but not local IGF-I in vivo. *Endocrinology* 146: 5455-5462, 2005.
55. **Petrella JK, Kim JS, Mayhew DL, Cross JM, and Bamman MM**. Potent myofiber hypertrophy during resistance training in humans is associated with satellite cell-mediated myonuclear addition: a cluster analysis. *J Appl Physiol* 104: 1736-1742, 2008.
56. **Psilander N, Damsgaard R, and Pilegaard H**. Resistance exercise alters MRF and IGF-I mRNA content in human skeletal muscle. *J Appl Physiol* 95: 1038-1044, 2003.
57. **Putman CT, Sultan KR, Wassmer T, Bamford JA, Skorjanc D, and Pette D**. Fiber-type transitions and satellite cell activation in low-frequency-stimulated

muscles of young and aging rats. *J Gerontol A Biol Sci Med Sci* 56: B510-519, 2001.

58. **Putman CT, Xu X, Gillies E, MacLean IM, and Bell GJ**. Effects of strength, endurance and combined training on myosin heavy chain content and fibre-type distribution in humans. *Eur J Appl Physiol* 92: 376-384, 2004.
59. **Raue U, Slivka D, Jemiolo B, Hollon C, and Trappe S**. Myogenic gene expression at rest and after a bout of resistance exercise in young (18-30 yr) and old (80-89 yr) women. *J Appl Physiol* 101: 53-59, 2006.
60. **Rosenblatt JD, Yong D, and Parry DJ**. Satellite cell activity is required for hypertrophy of overloaded adult rat muscle. *Muscle Nerve* 17: 608-613, 1994.
61. **Roth SM, Martel GF, Ivey FM, Lemmer JT, Tracy BL, Metter EJ, Hurley BF, and Rogers MA**. Skeletal muscle satellite cell characteristics in young and older men and women after heavy resistance strength training. *J Gerontol A Biol Sci Med Sci* 56: B240-247, 2001.
62. **Sale DG, MacDougall JD, Jacobs I, and Garner S**. Interaction between concurrent strength and endurance training. *J Appl Physiol* 68: 260-270, 1990.
63. **Tanaka S, Takuya M, T. F, E. K, T. K, H. F, and T. N**. Differing Responses of Satellite Cell Activity to Exercise Training in Rat Skeletal Muscle. *Journal of Physical Therapy and Science* 21: 141-145, 2009.
64. **Thomson DM, and Gordon SE**. Diminished overload-induced hypertrophy in aged fast-twitch skeletal muscle is associated with AMPK hyperphosphorylation. *J Appl Physiol* 98: 557-564, 2005.
65. **Tipton KD, Ferrando AA, Williams BD, and Wolfe RR**. Muscle protein metabolism in female swimmers after a combination of resistance and endurance exercise. *J Appl Physiol* 81: 2034-2038, 1996.
66. **Verdijk LB, Gleeson BG, Jonkers RA, Meijer K, Savelberg HH, Dendale P, and van Loon LJ**. Skeletal muscle hypertrophy following resistance training is accompanied by a fiber type-specific increase in satellite cell content in elderly men. *J Gerontol A Biol Sci Med Sci* 64: 332-339, 2009.
67. **Verney J, Kadi F, Charifi N, Feasson L, Saafi MA, Castells J, Piehl-Aulin K, and Denis C**. Effects of combined lower body endurance and upper body resistance training on the satellite cell pool in elderly subjects. *Muscle Nerve* 38: 1147-1154, 2008.
68. **Wood RH, Reyes R, Welsch MA, Favaloro-Sabatier J, Sabatier M, Matthew Lee C, Johnson LG, and Hooper PF**. Concurrent cardiovascular and resistance training in healthy older adults. *Med Sci Sports Exerc* 33: 1751-1758, 2001.
69. **Wozniak AC, Kong J, Bock E, Pilipowicz O, and Anderson JE**. Signaling satellite-cell activation in skeletal muscle: markers, models, stretch, and potential alternate pathways. *Muscle Nerve* 31: 283-300, 2005.
70. **Yang SY, and Goldspink G**. Different roles of the IGF-I Ec peptide (MGF) and mature IGF-I in myoblast proliferation and differentiation. *FEBS Lett* 522: 156-160, 2002.
71. **Yang W, Zhang Y, Li Y, Wu Z, and Zhu D**. Myostatin induces cyclin D1 degradation to cause cell cycle arrest through a phosphatidylinositol 3-kinase/AKT/GSK-3 beta pathway and is antagonized by insulin-like growth factor 1. *J Biol Chem* 282: 3799-3808, 2007.