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Running Economy: Improvements In Physiological Efficiency Attained Through Changes In Muscle Structural Morphology

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RUNNING ECONOMY: IMPROVEMENTS IN PHYSIOLOGICAL EFFICIENCY ATTAINED THROUGH CHANGES IN MUSCLE STRUCTURAL MORPHOLOGY

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Running Economy (RE) is a crucial determinant for running performance. While strategies for improving RE have been determined, the mechanisms governing this phenomenon have eluded the scientific community. My objective was to determine what adaptations, physiological, morphological, or otherwise, occur to bring about the altered RE associated with plyometric training. Specifically this project was designed to examine whether measureable transformations in muscle protein isoform makeup brought on through specific training will result in better RE in moderately trained runners. Participants (n=25) were placed into either a plyometrics-training or control group. All participants underwent similar testing before and after the 6-week training intervention: hydrostatic-weighing, vertical-jump, sit-and-reach, muscle stiffness, Vo_{2MAX}, RE, lactate-threshold, biomechanics, plus titin-protein isoform identification via gel electrophoresis from *vastus lateralis* biopsies. Post-testing revealed faster running performance for the plyometrics group without concomitant improvements in fitness data. While RE was not altered, anaerobic energy production was curtailed in the plyometrics group, and this correlated significantly to performance gains and titin isoform shifts, with greater proportions of T1:T2 linking to a blunted lactate response and better 3km time trial results.

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INTRODUCTION

Seeking ways to improve physical performance is part of the mission of sports scientists. Running is essentially the most fundamental physical activity a human can partake in, and it has a long history of being studied both specifically, and as the "go to" default exercise amongst generalizable exercise studies. Because of this, the physiological parameters of running performance have been recognized and identified beginning with A. V. Hill, and H. Lupton (1922). In the last 90 years an advanced understanding of oxygen uptake kinetics, enzyme function, muscle structure & function, as well as how they relate to and change with acute and chronic bouts of exercise has been investigated. Still, there remains a superfluity of mysteries to keep exercise scientists well-occupied, even in this most rudimentary of modalities, running. Perhaps chief amongst these is the concept of running economy (RE).

Quantifying RE is straightforward. It equates simply to the oxygen consumption at a given speed. Often in the literature the terminology "energy cost of running" or "EC" is used in assigning a value to RE at each given workload or speed tested. In this way, a higher value for RE implies a greater user of oxygen and thus equates to poorer economy.

A larger VO_{2max} will lead to performance gains (Conley & Krahenbuhl, 1980; Costill, 1967; Costill, Thomason, & Roberts, 1973; Saltin & Astrand, 1967; Schabort, Killian, St Clair Gibson, Hawley, & Noakes, 2000), but VO_{2max} has a genetic ceiling. VO_{2max} is better thought of as a theoretical pinnacle for performance if and when all other performance factors are maximized. RE in fact, is derived from the fundamental concept of oxygen utilization for obtaining energy (aerobically) during physical work i.e. running. It may seem logical then to simply measure one's capacity for maximizing oxygen utilization or their VO_{2max}. While this is routinely done, and can provide information on a person's fitness, research has shown that when compared to RE, VO_{2max} has a weaker correlation to endurance running performance (Conley & Krahenbuhl, 1980; Di Prampero, Capelli, Pagliaro, et al., 1993; Morgan, Baldini, Martin, Kohrt, 1989; Pollock, 1977).

Lactate threshold is trainable, and relates well to performance (Billat, Flechet, Petit, Muriaux, & Koralsztein, 1999; Farrell, Wilmore, Coyle, Billing, & Costill, 1979; Nicholson & Sleivert, 2001), but is not well-understood by the public at large, and it's ambiguously defined (Davis, 1985) with researchers using various methods to determine LT (Beaver, Wasserman, & Whipp, 1985; Cheng, Kuipers, & Snyder, et al., 1992; Heck, Mader, Hess, Mücke, Müller, & Hollmann, 1985; Wassermannm & McIlroy, 1964). In addition to this, LT is an "all-or-none" measure where you are either above or below a particular definition of threshold. In this regard, it is more attuned to ability to compete in events raced just at or above LT pace (typically about an hour, or 10K-half marathon distance depending on level of the athlete). That said, LT is not as comprehensive an indicator for overall running performance as RE.

RE is a trainable, straightforward laboratory measure, and it correlates strongly with running performance (Conley & Krahenbuhl, 1980; Morgan, et al., 1989). While the base of research identifying RE as a mutable characteristic is more plentiful than that illustrating exactly how to modify RE itself (Bailey & Pate, 1991; Saunders, Cox, Hopkins, & Pyne, 2010), there does exist a multitude of known ways

to produce this outcome: intervals (Denadai, Ortiz, Greco, & de Mello, 2006; Thomas, Fernhall, & Blanpied, et al., 1995), altitude training (Katayama, Matsuo, & Ishida, et al., 2003; Katayama, Sato, & Matuso, et al. 2004; Saunders, et al., 2009; Saunders, 2004), weight lifting (Beaver, et al., 1985; Guglielmo, Greco, & Denadai, 2009; Johnston, Quinn, Kertzer, & Vroman, 1997; Millet, Jaouen, Borrani, & Candau, 2002; Storen, Helgerud, Stoa, & Hoff, 2008; Yamamoto, Lopez, & Klau, et al. 2008), and plyometrics (Paavolainen, Hakkinen, Hamalainen, Nummela, & Rusko, 1999; Saunders, Telford, Pyne, & Peltola, et al., 2006; Spurrs, Murphy, & Watsford, 2003; Turner, Owings, & Schwane, 2003). Other training has failed to result in superior RE: Swiss ball training (Stanton, Reaburn, & Humphries, 2004) and isometric training (Fletcher, Esan, & MacIntosh, 2010). To date, how these training programs have improved RE has eluded detection, but the success and failures of the aforementioned protocols helps identify likely suspects. A search through the literature produces a surfeit of cross-sectional data on runners with higher and lower RE, but this only provides a snapshot of individual variation. Longitudinal studies exist that confirm the use of the above training modalities to improve RE, but no study to date had examined the mechanistic adaptations leading to these adaptations in terms of cellular structure of the myofibril.

Parameters to consider for bolstering running ability also may include biomechanical (biomechanics is defined here as the distinct movement patterns associated with the act of running) alterations (Cavanagh & Williams, 1982; Collins, Perasall, & Zavorsky et al., 2000; Fredrick, 1983), maximal volume of oxygen uptake (VO_{2max}), and lactate threshold (LT). Biomechanical inefficiencies are difficult to identify and coach without qualified individuals and expensive equipment. Moreover, attributing improvements in RE to biomechanical changes has proven problematic (Kyrolainen, Belli, & Komi, 2001; Morgan, Martin, & Baldini et al., 1990; Nichol, Komi, & Marconnet, 1991; Saunders, Telford, Pyne, Hahn, & Gore, 2009), though it has been suggested that diminished performance toward the end of long-duration events is in part due to inefficiency from deteriorating running form (Hausswirth, Bigard, & Guezennec, 1997). While specific biomechanical aspects of the running gait are intuitively linked to efficiency (Anderson, 1996), transforming running form as a means of altering RE has proven unsuccessful (Arendse, Noakes, & Azevedo, et al., 2004). Similarly, kinematic breakdown of a runners stride has proven useful in identifying runners with better or worse RE, yet a course of action for modifying kinematics has yet to be shown.

Plyometric training is thought to imprve RE through specific neural adaptations (Hakkinen 1994; Sale, 1991) and/or increased elastic recoil (Aura & Komi, 1986; Komi, 1984; Komi, 1986). The first of these possibilities is thought to be accomplished through two possible pathways: 1.) Muscle Spindle (intrafusal fibers) reflexive action, facilitating or amplifying the contractile strength of a given muscle and 2.) A dampening effect on the Golgi Tendon Organs inhibition of opposing muscle groups. Considering the former, elevated force production is achieved via intrafusal fiber contractions, and therefore would require more use of ATP, and hence no improvement in economy would be observed. Similar suppositions regarding neuromuscular changes resulting in altered motor unit recruitment would yield an equivalent result; greater force, greater energy use. As per the latter explanation it is possible that less ATP turnover and hence oxygen consumption could be related to the minimized muscle action,

though it is a dubious assumption that the effect would be large enough to surface in the gross measurement of whole body oxygen utilization associated with RE.

An intriguing hypothesis for improved RE with plyometric trainins is through the elastic properties of the musculotendinous unit (MTU). When a contraction is preceded by a pre-stretch, greater force is generated leading to more propulsion and a faster running speed. Energy from the pre-stretch would be momentarily stored as potential energy analogous to that of a spring during the loading phase of a running stride, and then released during the power phase. This notion is supported in the earlier works done by Cavagna et al. (1968, 1970, 1971, & 1977). Naturally this leads to the questions of "Can & How is it that the elastic characteristics of muscle can be modified to exploit this property?"

Plyometric training mimics the stretch-shortening-cycle associated with a running stride. In this way it could be considered as specific-resistance training for the action required during (high speed) running. Whereas, the major effect of weight training is likely to be increased strength, plyometrics allow for this effect in conjunction with increased total joint recoil. This is important in that a less compliant muscle/tendon complex is akin to a stiffer spring, and can generate more energy resulting from a given distance stretched in its return to "resting" state. Plyometrics have proven effective for bettering RE in untrained (Turner, et al., 2003), moderately trained (Paavolainen, et al., 1999; Saunders, 2004; Spurrs, et al., 2003), and highly trained individuals (Saunders, 2004; Saunders, et al., 2006). The ease of integrating a plyometric routine into a runners program, and the association with changes in stiffness made this an ideal intervention for use in studying RE.

It is well established that muscle stiffness is related to force production in the heart, and this phenomenon has been studied extensively in cardiac tissue. Replicating this finding is problematic in the multifarious system of *in vivo* skeletal muscle with tendinous aponeuroses, various joint angles, and an array of muscle arrangements on bony skeletal anchors. Ettema (2001) failed to identify any link between elasticity and force production in skeletal muscle, while Kubo and colleagues (2001) found passive stiffness does not correlate to tendon elasticity. More recently, this same group failed to see a difference in muscle stiffness, but did observe less extensibility of the tendons in knee extensors, though not plantar flexors in runners with better economy (Kubo, Tabata, & Ikebukuro, et al., 2010). Lastly, Fletcher and others concluded, "RE and *triceps surae* stiffness change together" (Fletcher, et al., 2010). Counter to this, investigations have found that in the *triceps surae* MTU during counter movement jumps -similar to knee extensor groups during running (including the *vastus lateralis* (VL)) - muscle worked most efficiently when contractions were of a more isometric nature due to extensibility of the tendon during muscular contraction (Kawakami, Muraoka, Ito, Kanehisa, & Fukunaga, 2002). Furthermore, a tendon can better absorb shock (rather than the muscle doing so with a concomitant energy cost) when the muscle is stiffer and the tendon is not. These findings lead to the conclusion that more economical runners may be so because of stiffer muscles and not necessarily tendons. Lower tendon, but greater muscle stiffness may aid in both shock absorption and force generation, both resulting in possible improved economy. This notion was upheld by the findings of Arampatzis et al. (2006) when they concluded that less muscle would be activated in the quadriceps with a more compliant knee extensor tendon. Along these same lines, a group looking at distal limb tendon stiffness

correlated a stiffer t*riceps surae* tendon with a worse RE (Fletcher, et al., 2010). Clearly, more information needs to be obtained before any conclusions can be drawn regarding RE and MTU stiffness. The juxtaposition of the role of tendon stiffness on opposite sides of the knee, along with the variety of passive stiffness measures, and the absence of a reliable active stiffness measure, contribute to the confusion of this relationship. Indeed Prado et al. (2005) found only a low correlation between active and passive tension of skeletal muscles examined in the rabbit model.

Non-energy-consuming ways of producing force during running are postulated to come from elastic recoil of the muscle utilizing stored energy from the pre-stretch of that same muscle during impact (Cavanagh & Kram, 1985). Probable alterations in muscle that could exist between individuals, and perhaps be induced through a (plyometric) training regime, are fiber type composition such as myosin heavy chain (MHC) (Kyrolainen, et al., 2003; Prado, Makarenko, & Andresen, et al., 2005) and titin isoforms (Funatsu & Tsukita, 1993; Horowits, Maruyama, & Podolsky, 1989; Kyrolainen, et al., 2001; Markovic, Dizdar, Jukic, & Cardinale, 2004; Marszalek, et al., 1999; Ottenheijm, Knottnerus, & Buck, et al., 2009; Prado, et al., 2005; Trinick, 1996; Turner, et al., 2003; Wang, McCarter, Wright, Beverly, & Ramirez-Mitchell, 1991). Differences in fiber types can affect RE in metabolic workings, contractile properties and structure. MHC and titin work in concert for structural stability during contraction, and can affect RE through strengthening of this stabilization and ability to return energy from shock absorption.

Searching within the muscle for proteins that are likely to influence RE results in a couple prime candidates: 1. The contractile proteins, MHC, being a major player in that action; 2. Titin, the aptly named “molecular spring”. The former is also commonly used in fiber typing (a process wherein the type of fiber (aerobic, anaerobic) is identified and a ratio of types is given). This in itself may have implications in efficiency of energy use and force production (see below for discussion). Titin on the other hand may be directly responsible for the force being produced by the muscle in replicating the supposed spring action during running. In this way, the specific isoforms of these molecules expressed (or their relative levels) within the muscle could serve as potential mediators or at least indicators of superior RE.

For the current study, consideration was given to altered RE resulting from increased muscle stiffness due to changes in titin and MHC composition (paralleling observations in rabbit skeletal muscle (Prado, et al., 2005)), brought on through plyometric training. Findings from Spurrs and coworkers (2003) allowed for identification of incurred musculotendinous stiffness increases following the plyometric training protocol in their subjects resulting in improved RE. In addition to these measures, the design employed here was able to reveal if in fact a morphological transformation of muscle proteins leads to these changes, thereby supplying a clearer picture and deeper understanding of the underlying determinants of RE.

Rationale, Significance and Problem/Sub-Problems

To model the changes that occur with improvement in RE, a group of habitual runners was stimulated with an intense plyometrics intervention over a six-week period with pre and post measures

taken. A second group was simultaneously put through the data collection process with no training intervention to serve as a control. Based on finding by others who also used a six-week plyometric intervention to successfully enhance RE (Spurrs, et al., 2003; Turner, et al., 2003) it was assumed that RE would improve in the experimental group. By adapting the training intervention and testing procedures employed in Spurrs et al. (2003), this assumption was justified. It has been hypothesized by numerous others that the action through which plyometric training improves RE is an increase in the energy-storage capacity in the leg muscles and tendons during the pre-stretch that occurs on impact during a running stride. This capacity indicates an alteration in the elasticity of the MTU through a change in the musculotendinous makeup (protein fibers). It is well reported in the literature that the titin and MHC isoforms found in a muscle, along with muscle fiber type, strongly influence muscle stiffness. There is a paucity of research involving active muscle stiffness due to complications in taking such a measurement (to date, there are no studies looking at protein changes and active muscle stiffness *in vivo*). However, it stands to reason that those same factors leading to improvements in passive muscle stiffness will also act to improve active muscle stiffness to some degree, and thereby energy return during the running stride, and RE as a result. By directly measuring several likely proteins along with muscle stiffness measures, while simultaneously considering other sources of improvement, we hoped to elucidate a potential mechanism behind changes seen in RE brought about through plyometric training.

Purpose Statement: With this study we will attempt to answer the question of "what adaptations, physiological, morphological, or otherwise, occur to in conjunction with the altered RE associated with plyometric training?" The underlying hypothesis directing this project is that measureable transformations in muscle protein isoform makeup brought on through specific training will result in improved RE in moderately trained runners after undergoing six weeks of plyometric training as compared with a control group.

Limitations

Regarding limitations of this study, the participants were recruited in and around the Missoula area. Volunteers came from 3 target populations being recruited: Run Wild Missoula running club, coed, all ages, ability, running background; general UM students/faculty, particularly from the HHP department, large age range and again coed; the local triathlon community same descriptive as general university population given. That said the ethnic makeup was quite homogeneous (Caucasian, 1 Native American). There was however, a wide age range (18-45), due mainly to monetary and temporal constraints inherent to the nature of using a longitudinal design within a Master's thesis. Though the diversity in this regard can be viewed as a positive aspect of the design in terms of breadth of applicability, it was not originally intended. It should also be considered that the time of year for the implementation of the protocol was late winter, which affected the timing of the intervention with regards to where in their training cycle the participants were (most building towards a spring or early summer marathon, or resuming training following a rest period in the early winter).

Furthermore, attention was focused on the upper anterior thigh, specifically the VL. Regarding protein concentrations, findings are restricted to pertinence for this muscle (group = quadriceps) only and not other major muscles of action during the running gait, (hamstrings, gluteals, and triceps *surae* group). While muscle stiffness changes at several measured sites was possible, only the VL was concurrently sampled for relation to titin and MHC. As such the plyometrics protocol was specifically arranged to target the quadriceps and the VL in particular. Similarly, expanding the findings of plyometric training effects on the measured variables cannot be assumed true for all resistance training or even explosive training for that matter.

Delimitations

It follows that there are several portions of the design that can be expanded upon, but have been curtailed in the interest of better research. Funding, time, man-power, and feasibility enact to police the scope of the proposed design. First, while a variety of biomechanical factors were assessed by trained experts, this component will receive only a truncated discussion here, in the interest of both fluidity and brevity. Enzyme activity affecting metabolic rates will also lead to a concomitant differentiation in energy use and therefore RE. Because of this, enzymatic variations were not measured and will not be discussed. Anaerobic strength will not be directly assessed, though a vertical jump measure will be taken to indicate power, and blood lactate values (Blac) will be tabulated throughout the treadmill protocol to indicate glycolytic flux (anaerobic activity). Though it could logically be inferred that it was the plyometrics that lead to changes in muscle proteins, and these in turn resulted in RE changes, as the first experiment of its kind, the design does not supply irrefutable evidence to support such a claim. It merely offers the opportunity to link cellular anatomy to whole body physiologic characteristics taken in conjunction with these micro-morphological measures.

Definition of terms

$\underline{VO_{2max}}$: Maximal aerobic capacity. The maximal volume of oxygen a person is capable of utilizing per unit time aerobically. Measured in the lab with open spirometry during a maximal graded exercise test. In absolute terms, units are L_O_2/min, for relative ml_O_2/min/kg_body_weight.

Steady State: Condition in which homeostasis is maintained. No excess by products/end products from reactions taking place are accumulated, and no substantial debts or surpluses of any kind are being created. In the lab, this is determined by HR, VO_2, respiratory measures, and/or blood markers (lactate).

Running Economy: The efficiency, in terms of aerobic energy use, of one's running at a set speed. Can be measured at various speeds to yield different RE values for the same person.

Lactate Threshold: Intensity at which blood lactate is seen to "increase" (accumulate faster than the rate it is being cleared). Somewhat ambiguous in that LT is measured traditionally as the intensity which causes a 1mmol increase in blood lactate above resting levels, but is also often determined other ways, numerically and graphically (i.e. point-slope, Onset of Blood Lactate Accumulation, D-Max). For this study we will use Onset of Blood Lactate Accumulation.

Plyometrics: Explosive movements taking advantage of the stretch-reflex and elastic recoil of muscles and tendons by pairing a quick eccentric movement with an explosive and strong concentric counter-movement.

Middle Distance: In running the race distances ranging above 400 and below 5000m (¼ to 3 miles). A middle distance runner is someone who typically competes in races of 800m (½ mile) and/or 1500m/1 mile. These events last roughly 2 and 4 minutes in elite runners (less for males).

Biomechanics: The kinesiological movement patterns associated with motion. Specifically, it will be in reference to running form within this paper.

REVIEW OF LITERATURE

Running performance is a multifaceted phenomenon that compounds many contributing factors yielding a spectrum of ability levels. What these factors are is fairly well-understood, however, identifying where/when/how they come to be, and mapping out their full mechanisms is still poorly understood. Currently the major contributing components to distance running performance are thought to be homeostatic regulation, $VO_{2max,}$ anaerobic threshold, muscle fiber composition, running economy, and psychological factors (which will not be discussed here). Other ingredients in creating a proficient endurance runner are strength, power, flexibility, and maximal running velocity. The interrelationships between these multifarious aspects of running performance are complex, consisting of both positive and negative interactions amongst the given elements. Understanding these components of running performance could lead to more efficient training systems to maximize an athlete's potential, and possibly identify prospective athletes with the highest natural aptitude.

IMPROVING RUNNING PERFROMANCE

Long established physiological measures of distance running performance include maximal aerobic capacity (VO_{2max}), anaerobic threshold (here taken to be equivalent to the lactate threshold), and efficiency of locomotion, or running economy. VO_{2max} is commonly considered the "gold standard" for measuring endurance capability, however, in events lasting longer than a few minutes (running events over a mile or 1500m in length), LT is thought to play a more prominent role. VO_{2max} reflects the individual's maximal ability to take in and utilize oxygen in a given amount of time. Typically, it is scaled to either body weight, or body weight to the ¾ power, in order to normalize it across individuals of different sizes –with running being a weight bearing exercise, this scaling enables numbers to be compared between individuals. Rarely though, is a runner in an event on the order of >10 minutes operating at a level equivalent to their $VO_{2max.}$ Instead, they are thought to be performing at a fractional percentage of this maximal aerobic capacity. Related to this ability is the anaerobic threshold. At the point when a person begins to glycolytically form lactate ions faster than they can clear, buffer, and/or use these molecules, Hydrogen ions and carbon dioxide both begin to accumulate in the body, and must be dealt with or expelled. This occurs at intensities near but still less than that of VO_{2max} (typically ~60-90% depending on the person's genetics and training background). The lowered pH and increased respiration are thought to be possible sources of fatigue during exercise (Bangsbo, Madsen, Kiens, & Richter, 1996). Training one or both of these physiological aspects is thought to lead to performance gains.

VO_{2max} typically increases with age (up to around 30-years) and with both total volume of training and volume at high aerobic intensity. That said, it is also a well-established concept that this number can be maximized in a mature athlete in a matter of months, and that there is a diminishing return on excessive time spent attempting to maximize this value due to a genetic ceiling. Moreover, there are popular studies attempting to reveal the "secret" of East-African dominance in distance running, that have looked at VO_{2max} values in elite African and non-African runners, revealing little to no

difference (Foster & Lucia, 2007; Lucia, Esteve-Lanao, & Oliván, et al., 2006; Noakes, 2000). Anaerobic threshold is another avenue sought to bring about faster running times. LT is much less impacted by genetics (other than that it must occur at an intensity below that of VO_{2max}) and it is widely contended that LT is highly trainable. Though LT is a superior predictor of endurance potential than VO_{2max} (Nicholson & Sleivert, 2001; Saltin, Kim, & Terrados, et al., 1995), the fact that it varies so much with training limits its use as a predictor measure. Certainly the best runners are those with high VO_{2max} values, and a still proportionately high LT. And the best way to produce this is to train at the precise intensities associated with VO_{2max} and LT respectively.

Repeated work bouts at corresponding levels of effort with minimal breaks to sustain the workload as a means of accumulating high volumes at said intensity are thought to be the best method for developing the above physiologic parameters (Daniels, J., 1998). More time will be spent in a well-designed program on whichever parameter best mimics that athlete's race pace. But such workouts are taxing sessions that wear down an athlete and heighten the risk for both overuse injury and burnout. Furthermore, these are not the only factors that dominate running ability. Professionals in the field have sought other training techniques to complement their already austere regimen: weight training, plyometrics, yoga, Pilates, meditation/visualization, core training… the list goes on and on, all in an attempt to gain a competitive edge without overtly escalating risk to the athlete.

Additionally, much aligned to both of these physiologic measures is the anatomical composition of the muscle fibers in an individual. Indeed, the 1997 benchmark paper from Johnston and colleague's ascribed the improvement in RE from a resistance training intervention in a collection of female athletes to a possible shift in muscle fiber types (though this was based solely on anecdotal evidence). Related to this muscle phenotype is the myosin heavy chain (MHC) constitution of the fibers, with greater abundance of the Type I isoform indicating aerobic potential and greater Type IIX isoform providing for anaerobic potential. Type I fibers both have a higher aerobic capacity, and are better suited for utilizing the lactate produced as an energy source in situations involving anaerobic glycolysis. Type II fibers are capable of greater glycolytic flux and faster energy/higher power outputs, but not as packed with mitochondrial networks for carrying out aerobic respiration. Because having greater numbers of Type I fibers leads to more efficient oxygen consumption and dampened lactate production as well as improved clearance rate, a high fiber composition of this type is thought to reflect greater potential for endurance type activities. Additionally, there exists a Type IIA isoform, the so-called "intermediate fiber" that has a moderate aptitude for both power production and aerobic capacity. High proportions of this fiber type are advantageous for middle distance, shorter endurance and mixed intensity events (basketball, soccer). Also, shifts in muscle type by cross-sectional area are common, and small adjustments by number may be possible into adulthood. It is thought that these changes are more likely due to hypertrophy of existing fibers and less indicative of hyperplasia (It should be noted this is a simplified discussion of the status, as the intermediate fibers with qualities of both Type I and Type IIX, can vary on the spectrum between these two).

Another possible avenue to success is improving RE: that is the amount of energy (measured in terms of oxygen consumption) required to perform at a given level. By becoming more economical, runners can decrease race times without concomitant gains in traditional "fitness". While VO_{2max} and LT

seem more applicable at set speeds, RE is a more comprehensive measure in terms of relating to various intensities. Running at the same speed for two runners requires less effort for the one able to maintain that pace with a lower VO_2 requirement than the other, even if all other factors are equal ($VO_{2max,}$ LT, psychological, etc.) regardless of the speed itself. So by improving RE an individual will be able to run the same pace as before with a lowered VO_2 or metabolic stress, thereby boosting endurance, delaying fatigue and maintaining said pace for a longer duration/distance. Equivalently at the same metabolic stress level (consuming equivalent volumes of oxygen) they would be able to run at a faster speed, thereby resulting in faster times for a given race distance simply by improving RE. These values can be altered within a person through training as well as differ from person to person.

CURRENT UNDERSTANDING AND IMPROVEMENT OF RUNNING ECONOMY

It has been speculated that gross morphology and mechanical changes could be the major players in determining one's RE (Anderson, 1996; Cavanagh & Williams, 1982; Collins, et al., 2000; Fredrick, 1983; Hausswirth, et al., 1997; Kyrolainen, et al., 2001). Although this cannot be a complete description, as it does not account for the training effects observed. More efficient motor unit recruitment is certainly a trainable possibility capable of variance between people as well. Several authors have postulated such a difference to explain RE (Nummela, Paavolainen, & Sharwood, et al., 2006; Paavolainen, et al., 1999; Williams & Cavanagh, 1987). Certainly, motor unit recruitment could manifest itself through biomechanical inefficiencies, leading to lower RE. Moreover, studies have shown different recruitment patterns via electromyography for individuals with low versus high RE (Kyrolainen, et al., 2001). Most research looking to neuronal recruitment, however, still must bridge the gap between motor unit activation and actual limb movement and force generation to show a real change in RE.

Optimized enzyme function is another hypothesis but it is suited more for performance based measures than RE per say. Basically, if you amplify the amount of aerobic enzyme activity, you enlarge aerobic capacity, though this would lead to a concurrent rise in oxygen use, and so VO_{2max} would go up, but not RE.

A final proposition, that MTU's are able to produce mechanical energy due to elastically stored potential energy and recoil without any use of chemical energy synthesized by the body (Aura & Komi, 1986; Komi, 1984, Komi, 1986) has garnered growing attention (Dumke, Pfaffenroth, McBride, & McCauley, 2010). The idea is that muscles act as springs during the running process, and the stiffer the spring the more energy it is capable of storing. Consequently stiffer muscles lead to a more efficient running stride. This, like motor recruitment would provide for both a genetic and trainable means of enhancing RE. It is this idea that was explored in the current study, with relevant literature to be discussed below.

Not surprisingly there is an abundance of research correlating RE to actual running performance. While it seems logical to say that better runners are more economical, and more economical runners are better, this does little to discern how to achieve either of these, nor does it enable any cause/effect relationship. This is not to say, however, that methods for developing RE have not been identified.

The literature shows measuring RE is a superior predictor of distance running performance when compared to other measureable variables (Conley & Krahenbuhl, 1980; Morgan, et al., 1989). Daniels, J., Krahenbuhl, & Foster, et al. (1977) and later Daniels, J. & Daniels, N. (1992) present data on elite athlete's RE, illustrating that while males tend to have greater RE for an absolute speed than females, they are not fundamentally different in terms of RE measures compared to race times or how RE can be manipulated with training. These concepts are important for justifying the use of a coed design. Additionally, there exists myriad modalities known to bring about altered RE including interval workouts (Denadai, et al., 2006; Thomas, et al., 1995), altitude exposure (Katayama, et al., 2003; Kawakami, et al., 2002; Saunders, Elford, & Pyne, et al. 2004; Saunders, et al., 2009), weight training (Beaver, et al., 1985; Guglielmo, et al. 2009; Johnston, et al. 1997; Millet, et al., 2002; Storen, et al., 2008; Yamamoto, et al. 2008), and more pertinent to this study, plyometrics (Paavolainen, et al., 1999; Saunders, et al., 2006; Spurrs, et al., 2003; Turner, et al., 2003). The idea behind all of these approaches is to create more energy with less effort, by enhancing an element such that power is generated without increased metabolic demand. This is the fundamental idea behind RE. Simply put: "more bang (running speed) for your (metabolic) buck". Still, as elementary as the physiology of RE may be, its exact mechanism(s) of change are a mystery. Recent work has begun to investigate prospective physiological differentiations in the form of key protein structure within the working muscle. Kyrolainen et al. (2003) took 10 well trained young middle-distance runners and measured RE along with *vastus lateralis* (VL) biopsies for determination of fiber type distribution, muscle fiber area, myosin heavy chain (MHC) composition, activities of a number of metabolic enzymes (citrate synthase, lactate dehydrogenase, phosphofructokinase, and 3-hydroxyacyl-CoA-dehydrogenase), and titin isoforms in each of them. It was found that the MHC isoform distribution in the national level runners was 67.0% MHC I, 31.5% MHC IIA, and 1.5% MHC IIX. They were also able to link a higher maximal isometric force of the knee extensors with greater Type II fiber distribution and a negative correlation with oxygen consumption near race speed (meaning runners with more Type II fibers were also more economical). Interestingly, there was no correlation for any of the measured enzymes and RE, supporting the idea that RE must be changed through non-metabolic parameters such as neural coordination, elastic recoil, or biomechanics. Finally, it was noted that sodium dodecyl sulfate poly acrylamide gel electrophoresis (SDS PAGE) revealed all subjects expressed only the lighter isoform of titin save one, the most economical runner tested. He expressed this as well as the heavier isoform.

A 2010 study by Dumke et al. looked at the connection between RE and MTU stiffness of the *triceps surae* using free-oscillation technique in highly trained runners. This group found a negative correlation between oxygen consumption and MTU stiffness (better RE accompanied by a stiffer MTU). The implication for this and the study above is that, the stiffness associated with RE may be determined via major structural proteins of the muscle cell. MHC, and titin, the latter in particular, are liable targets. These studies represent only a snapshot of RE and some of its facets in the groups examined (highly trained runners in both), and therefore do not say anything about how or why RE is different across individuals, or within a person when provided two explicitly varied training routines. Because of this, longitudinal research needs to be completed, illuminating how the body actually functions differently in response to stimuli known to better RE.

PLYOMETRIC TRAINING

In the past, the old adage of, "if you want to be better at some specific task, go do exactly that task!" firmly applied to running. Despite the partial truth to this maxim, contemporary training protocols see runners devoting proportionately more of their time to non-running exercise. One such strategy is that of employing explosive training in the form of plyometrics. There is research supporting the effectiveness of such practices (for a review of the training effects of plyometric programs see Markovic & Mikulic (2010); for a review of the practical use of plyometrics in training track and field athletes See Lundin (1985)). Specifically, researchers have looked at the effect of plyometric training on RE (Beaver, et al., 1985; Paavolainen, et al., 1999; Saunders, 2004; Saunders, et al., 2006; Turner, et al., 2003). Turner et al. (2003) were able to identify improved RE through plyometric training in a sample of distance runners (not highly trained) after a 6 week plyometric protocol was introduced to their schedule. No change in vertical jump height (anaerobic power) or VO_{2max} were found. While the former was somewhat unexpected, the latter is a common finding in resistance training interventions with aerobic populations. They were not able to unequivocally identify a mechanism explaining this phenomenon however. Allowing for some speculation, together these two findings indicate that neither the anaerobic contribution nor the metabolic capacity changed over the six weeks, and the improved RE must have derived from some other component. Spurrs et al. (2003) also observed a significant improvement in RE after just 6 weeks of plyometric training using average distance runners. There were no changes seen in the control for any variables, while the experimental group saw improvements in performance (3000m time trial), RE at all speeds tested, counter movement jump (CMJ) height, MTU stiffness of the *triceps surae* (using free oscillation), and no change in either VO_{2max} or LT. While no biopsies were taken, and no cause effect relationship established, the authors did offer their conjecture that it was the augmented MTU stiffness leading to an enhanced RE, that contributed principally to the superior performance. Similarly, a study using highly trained middle distance runners by Saunders et al. (2006) found improvements in RE without a concomitant change in VO_{2max} following administration of a nine week plyometric intercession. This last investigation of RE in highly trained runners represents a current inadequacy in the literature examining this population. Use of plyometric training is one of the proven interventions to bolster RE in runners or an assortment of abilities.

Plyometric training mimics the stretch-shortening-cycle associated with a running stride. In this way it is specific-resistance training for the action required during (high speed) running. Whereas, the major effect of weight training is likely to be increased strength, plyometrics allow for this effect in conjunction with increased total joint stiffness. This is important in that a less compliant muscle/tendon complex is akin to a stiffer spring, and can generate more energy resulting from a given distance stretched in its return to "resting" state. Plyometrics have proven effective for improving RE in untrained (Turner, et al., 2003), moderately trained (Paavolainen, et al., 1999; Spurrs, et al., 2003), and highly trained individuals (Saunders, 2004; Saunders, et al., 2006). The ease of integrating a plyometric routine into a runners program, and the association with changes in stiffness makes this an ideal intervention for use in studying RE. Johnston et al. (1997) were able to foster RE in regular (female) runners with the addition of a weight training program three times per week to a running regimen of 4-5days totaling 20-

30miles each week. Millet et al. (2002) followed heavy weight lifting protocol to produce parallel results in highly trained runners. However, plyometric training, when compared to traditional weight lifting, allows for analogous neuromuscular and power development, without as great a risk of slowing athletes down with excess bulk.

As listed before, there are many studies identifying employment of plyometrics as an effective technique to garner improved RE. The question, therefore, is not whether plyometrics training improves RE, but how does it accomplish this? One interpretation for this action gaining support in the literature is an increase in muscle stiffness.

MUSCLE STIFFNESS & MYOTONOMETER TESTING

Many papers have looked at MTU stiffness for a plethora of reasons. Among them is the relationship between contractile force production and stiffness. The idea was first made possible by a paper written by Morgan, Proske & Warren (1978) showing that kangaroos were able to store massive amounts of energy in their Achilles tendon, and in fact consumed less oxygen at higher hopping speeds than slower ones. The fundamental idea being that the leg acts as a spring, and energy can be stored during a run (or a hop) by stretching the spring a given distance. This energy is released upon the next stride (or hop). The stiffer the spring, the more energy it can store and release. Since then, scientists have been attempting to research where this energy is coming from, without success. Confounding the issue is the myriad different measures by which stiffness is quantified. Some measurements are done in the passive or relaxed state. The obvious conjecture to this is that during muscle activation, the properties of the MTU are altered dramatically. But even measurements in the activated condition vary: some researchers use isometric contractions, while others opt for fixed loads, and those with the equipment and savvy have made attempts at measuring stiffness during dynamic motion. While the latter sounds ideal, a multitude of complications arise during such a measurement, and controlling for extraneous error is cumbersome if not impossible at this time.

This discrepancy aside, the question of where to look for stiffness changes also plagues the confluence of the literature available. Many believe that the tendon specifically collagen fibers provide the genesis for MTU stiffness (M. Bundle, personal communication), while others have looked within the muscle itself (Kyrolainen, et al., 2003; Labeit & Kolmerer 1995). If skeletal muscle follows suit with what has been observed regarding stiffness in cardiac muscle, with muscle proteins (titin) playing the major role at physiological levels of stretch/relaxation and collagen dominating at greater loads (Granzier & Irving, 1995) than it is possible that both parties are correct.

Specific to MTU stiffness and its role in determination of RE, is in which muscle groups are these differences found? From a pure physics prospective, it stands to reason that the closer to the point of impact a muscle is (those more distal on the leg), the more impact its stiffness will have on force production during the stride. But while physiologically examining the system, it is within those muscles that provide the dominant forces during the running movement (i.e. those more proximally located) that stiffness would have the greatest upshot, as these are most relevant to muscle action.

Measuring active muscle stiffness, ass alluded to earlier, has proven nearly impossible *in vivo* for humans (Saunders, 2004). Researchers have thus been reliant on measures of passive muscle stiffness. Unfortunately, there does not exist, a single standard measure for comparisons, nor has there been uniformity in the data collected or unanimity in the asserted relationship between active and passive stiffness in general. Most researchers rely intuitively on the concept of passive muscle stiffness representing active muscle stiffness, (Godges, Macrae, Longdon, & Tinberg, 1989; Kyrolainen et al., 2001; Labeit & Kolmerer 1995; Millet, et al., 2002; Sahlin, 1986; Sale, 1991; Saltin & Astrand, 1967) yet Prado et al. (2005) determined active and passive stiffness were "not strongly correlated" in rabbit muscle samples *in vitro*, and Kubo et al. (2001) found tendinous elastic properties to be unrelated to passive stiffness in the human *triceps surae*. Furthermore in a study looking at *in situ* rat *gastrocnemius*, Ettema (2001) concluded that elastic energy played no appreciable role in muscle efficiency, and that biomechanics in general were less important than gross structural design. Use of ultrasonography, MRI, free oscillation, CMJ reaction time, and numerous other techniques further cloud the issue and segregate various findings in the literature.

Due in part to these issues, and in part of the intricacy of the muscles, tendons, ligaments and bones and their relative arrangements and interworking involved in any particular movement (i.e. running), only limited information can be applied from the prodigious investigations of cardiac muscle stiffness. This has lead scientists to posit about the role of compliance within this system. It has been hypothesized that 1.) Like actual springs, higher stiffness yields greater energy return; 2.) Compliant tendons absorb more of the impact stress, reducing the energy used for stabilization by the muscle, and increasing whole MTU efficiency; 3) Similar to a two spring in series system, the spring with lesser stiffness (the muscle) stores the energy, and therefore, it is muscular and not tendonous stiffness that is most influential in this system; 4) VL/thigh stiffness in the muscle and compliance in the tendon should be high to maximize force production, while the *triceps surae* ideally will be characterized by high stiffness in both muscle and tendon for optimal energy return. Kawakami et al. in 2002 found that stiffer muscles and relatively more compliant tendons (like the situation during isometric contractions) lead to greater contractile efficiency during vertical jumping with as opposed to without a countermovement. They concluded that it was the tendon and not the muscle that acts as the elastic spring. Furthermore, shock absorption should be maximized for stiffer muscles, and more compliant tendons (to a point). Fletcher et al. (2010) measured a mild, but significant negative correlation (r^2=0.43, p=0.02) between *triceps surae* tendon (TST) stiffness and RE, while Arampatzis et al. (2006) also observed higher TST stiffness, but with reduced VL tendon stiffness in more economical runners.

Moreover, there are multiple findings verifying force generated by a MTU is not a purely a function of its length and velocity as a whole. Rather, pennation angles and joint morphology play a role. This adds to the complexities in estimating muscle fiber behavior solely from evaluation of joint performance (Fukanaga, Ichinose, Ito, Kawakami, & Fukashiro, 1997; Fukanaga, Kubo, Kawakami, Fukashiro, Kaneshisa, & Maganaris, 2001; Kawakami, et al., 2002). Lastly, in a review by Alexander (2002), the thickness, and positioning of the tendon relative to the direction of force during movement is discussed in a range of vertebrate movement patterns. Depending on these variables, and the magnitude of the force applied, optimization for MTU stiffness can fluctuate. Contact time during the

stance phase of a stride acts to slow the runner down due to frictional forces. Contradictory to this notion, to achieve peak force production, muscle contraction time should be long and hence velocity minimal. The entire MTU must stretch and return to a "neutral" position during each foot strike. This requires a temporary reduction in thin and thick filament overlap preceding each muscular contraction. It is therefore ideal to have the tendon show more compliance with the muscle demonstrating higher stiffness. Regardless, elastic recoil of the tendon will be much faster, and so allow for the muscle's inherent stiffness to be the predominant factor in determining the entire system's efficiency. This leads to development of a system with a more compliant tendon and stiffer muscle to maximize recoil time in the former, and allow for unabated contraction in the latter. In this way, overall stiffness of the joint will be increased for greater efficiency.

As discussed above, there is evidence to support the traditional spring model of less compliance enabling for greater energy storage/release, as well as evidence to the contrary. This, it has been argued, may even be position dependent (how far down the leg is the measurement). Moreover, passive and active muscle stiffness's have both been used, and no standard measure for either exists. Finally, both muscles and tendons are possible sources for the spring-like function of the MTU. As such, a measure for which both passive and active stiffness could be ascertained in the muscle as well as the tendon and for different sites on the leg is desirable until stronger concurrence exists within the scientific community.

A device that would provide such diversity is the Myotonometer. In brief it uses a force transducer along with a displacement sensor to give the stiffness of a muscle/tendon etc. As the device is pushed on a muscle a distance measurement is recorded along with the resistance force provided by the muscle being tested. The stiffer the muscle, the less compliant it will be and the less displacement it will allow under the force provided. In this way it is possible to test different MTU groups and to isolate given muscles and tendons. Loading the muscles with a fixed resistance or isometric contraction is also allowable with the Myotonometer. While this device had not previously been used in relation to RE, it has been extensively used in both clinical and research settings to determine muscle stiffness (Ashina, Bendtsen, Jensen, Sakai, & Olesen, 1999; Ditto,Fischer, Fehrer, & Leonard, 2002; Bizzini & Mannion, 2003).

FLEXIBILITY & ANAEROBIC POWER

Plyometrics and muscle stiffness are each instinctively paired with anaerobic power and flexibility respectively. In the first case, this can be misleading. MTU stiffness is not identical to flexibility, which is more a measure of range of motion of a joint. The relationship between flexibility and RE has been investigated, but with contrary results. Godges et al. (1989) employed acute static hip flexibility stretches preceding RE measurements at 40, 60, and 80% of VO_{2max} in seven "moderately athletic" college males. RE was observed to be superior following this routine for these individuals. Conflicting evidence has come from Gleim, Stachenfeld & Nicholas (1990). They took a coed group of 100 untrained subjects and tested both flexibility measures and RE at very low speeds. In this sample, it was discerned,

that tighter, less flexible people indeed had better RE. It has been argued that an all-male or all-female subject pool would eliminate this result, as females tend towards both greater flexibility and lesser RE, and the two may vary gender-specifically. Correspondingly, Craib et al. (1996) was only able to link two of nine trunk and lower limb flexibility measurements to RE (ankle dorsiflexion & external hip rotation). It was found that both external hip rotation and ankle dorsiflexion flexibility were inversely correlated to RE. Subjects were 19 moderately trained male runners (all had completed a 10K in under 40:00 in the previous 12 months). Morse et al. (2008) further linked acute bouts of stretching to significantly reduced MTU stiffness in the calf, though no measure of force production or efficiency was done. In elite distance runners Jones and associates (2002) too, saw an inverse relationship between sit-and-reach flexibility and RE (at high, but a still relatively sub maximal pace of 16Km per hour for the runners tested). In light of these results, flexibility testing was limited to a sit-and-reach measurement for low-back and hamstrings flexibility for this study.

Another instinctive pairing is the use of plyometric training and the development of anaerobic power. There exists virtually no evidence to support the notion that plyometric training would not lead to an increase in anaerobic power, and there would be little reason to suppose this. Because of this fact, we are capable of demonstrating the effectiveness of our training intervention through use of testing anaerobic power. Specifically, vertical jump (VJ) height using a counter movement jump, thereby mimicking the plyometric training was selected as this has long been established as the standard for assessment, and verified as the best indicator when measured against similar measures (Markovic, 2004). Changes in CMJ height in our participant pool provided the evidence for us to speak on the "bottom line" of whether the plyometrics induced their purported effect or not. Use of a CMJ reflects activity of the actual muscles used for propulsion during action mimicking the ballistic nature of running. What's more is the biopsy site is located at the VL. This is precisely the major source of power during a CMJ. In this way it is possible to draw a parallel between anaerobic power and findings determined from muscle tissue data.

MUSCLE PROTEINS: FIBER TYPE, MHC & TITIN

The exact variables examined from the VL biopsy are muscle fiber type, myosin heavy chain (MHC) isoform, and titin isoform. Muscle fiber type comes in two general categories (Types I & II) with subdivisions under Type II. Type I fibers are also known as slow-oxidative, or aerobic fibers. These more descriptive names imply their pension for a slower albeit more enduring action through aerobic metabolism and requisite presence of oxidative enzymes and dense mitochondrial networks. Type I fibers tend to be of slightly smaller diameter, but are present in higher numbers than any of the Type II varieties, and thus make up the majority of skeletal muscle. Type I fibers also have slower contraction velocity, and nervous signaling, giving them a lower maximal force capacity. Type IIX fibers (fast-glycolytic) are just the opposite. They are larger, have faster conductance, highly branched innervations, faster contractile speed, greater force capacity, and more glycolytic enzymes, with fewer/less networked mitochondria and the associated aerobic enzymes. They also fatigue relatively quickly. Type IIA, intermediate, or fast oxidative fibers are a sort of hybrid muscle capable of adapting their capacity to

mimic the fiber type (I or IIX) that best suits their (chronic) activity through training, though little inter-conversion between Type IIX and type I has been observed (Schantz, Billeter, Henriksson, & Jansson, 1982).

A substantial number of studies have looked toward these fiber type changes to explain observed training adaptations. Not excluded from this list are several investigations of RE. Johnston and coworkers (1997) speculated that it was perhaps just such a shift that caused the enhanced RE in their subjects. As pointed out by Dumke et al. (2010), research done by others has given results taken to indicate fiber type is indeed related to RE, with both prevalence of Type I (Kyrolainen, et al., 2003) and II (Bosco, Montanari, & Ribacchi, et al., 1987) fibers being linked to better RE. Lastly data from their research indicates an inverse relationship between muscle strength and RE (Dumke, et al., 2010). This would lead one to believe that a greater proportion of Type I fibers would be linked to superior RE values. In short, it is thought that the enhanced mitochondrial networks observed within the Type-I fibers allow for more efficient use of oxygen as a final electron acceptor. This would provide for superior RE in those with higher proportions of Type I fibers within the working muscles during running. On the other hand, if there were an enlarged anaerobic contribution during running, due to greater proportions of Type II fibers, it is feasible that oxygen consumption would be reduced. Aerobic metabolism accounts for the majority of the workload during non-sprint running and anaerobic, lactic and alactic, the remainder. However, if the anaerobic contribution is increased, the aerobic by default would be decreased at a given workload. Normally this would be viewed as detrimental to cellular function, as the metabolic byproducts (namely lactate's dissociated H^+ ion) are thought to have a negative impact on enzyme function, and generally cause stress to the cell and organism as a whole. But in regards to RE, a greater anaerobic output/reduced aerobic load would be interpreted as an improvement. So it is possible that a shift in fiber type, or at least utility in either direction could act to augment RE.

Associated with fiber type is the MHC isoform expressed within the particular myofibril. MHC names are analogous to fiber types (I with I, IIA with IIA and IIX with IIX), and relate to energetics and economy in much the same fashion. The MHC includes a globular head which acts as the "working" portion of the contractile unit. It is here that the mechanical motion is put into action. It is the repetition of the movement of this globular head (the power stroke), or more accurately millions of them, that generates movement and at least the bulk of the force of muscular contractions. The MHC isoforms are set to match the function of the fiber in which they exist. Aerobic fibers will overwhelmingly express MHC I, and anaerobic will have higher levels of MHC IIA and IIX. As such, the differences between these isoforms equates to differences in contractile properties and energetic demands of a given muscle fiber. Kyrolainen et al. (2003) identified a direct relationship between MHC II content of the muscle and RE in trained middle distance runners. Coupled with the finding that enzyme activity showed no correlation with RE, it seems that structural rather than enzymatic variance accounted for the disparities seen in RE.

This same group also measured Titin constitution of the muscle in their subjects. Titin is a large structural protein thought to play a role in MHC thick filament structural stability and elastic properties of striated muscle (Maruyama, Matsubara, & Natori, et al., 1977; Trinick, 1996). The myofilament was first discovered by Maruyama et al. in 1977. Since then, it has been bestowed the nickname "molecular spring" by more than one author when describing its function within the striated muscle. It is thought to

achieve this elastic recoil through 2 properties. One is the simple entropic relationship with energy production gained and lost from the system respectively from the folding and straightening of the Titin itself (Florey, 1969; Trinick, 1996; Tskhovrebova, Trinick, Sleep, & Simmons, 1997). The second manner of producing "free energy" is through the spring-like compression and extension of the PEVK region (Proline, Glutamate, Valine &, Lysine rich portion) of the Titin molecule (Labeit & Kolmerer 1995). As alluded to above, the role of titin as a molecular spring is widely accepted and found throughout the literature (Fukuda, Wu, Nair, & Granzier, 2005; Fukushima, Chung, & Granzier, 2010; Granzier & Irving 1995; Improta, Politou, & Pastore, 1996; Kyrolainen, et al., 2003; Labeit, Gautel, Lakey, & Trinick, 1992; Linke, Rudy, Center, Gautel, & Witt, 1999; Linke, 2000; Ottenheijm, et al., 2009; Prado, et al., 2005; Tskhovrebova, et al., 1997; Wang, et al., 1991; Warren, Krzesinski, & Greaser, 2003), particularly for cardiac function. Titin is found in either the larger slow mobility isoform T1 or the smaller fast mobility isoform T2 in human skeletal muscle (Horowits, 1992; Kyrolainen, et al., 2003).

Interestingly, only the most economical runner of the eleven tested in the Kyrolainen et al. (2003) study showed the sole discrepancy in titin composition (He displayed both the T1 and T2 isoforms of titin, whereas all others only had T2 present in measureable quantities). T2 has shorter immunoglobulin and PEVK regions. It is this PEVK region that acts as the distensible region of the molecule giving it its spring-like properties. Though they did not speculate on this, the shorter span would contribute to greater stiffness and hence greater economy for this individual (as was observed). Prado et al. (2005) reported a tendency for T1 to be the prevailing isoform in exist in Type one fibers with presence of both isoforms in Type II fibers. However no correlation to active stiffness was found with either isoform (though it seemed the variability in isoform in the Type II isoforms was such as to make them significantly less compliant in the passive state than the Type I fibers expressing exclusively T1. In light of this, the aforementioned findings make sense from a functional standpoint, but not from a morphological one. T1 should have been found in greater quantities, as it exists in both fast and slow twitch fibers, yet the authors were only able to identify T1 in 1 of 11 subjects. This casts doubt on the findings from this study, as T1 should be the predominant isoform in any sampling of a mixed muscle (the VL was used in this study), though there is scant evidence to refute the finding for human skeletal muscle.

Still, in the limited literature examining both RE and protein makeup of the myofibril, Kyrolainen et al. (2003) was at least able to provide some evidence that there is a relationship between titin isoform and RE. Additionally, Fry et al. (1997) was able to demonstrate diversity in titin makeup between individuals, however did not conjecture as to what these variation may mean. In the rabbit model, Prado and colleagues (2005) found that slow twitch fibers contained longer titin isoforms than fast twitch, indicating a stiffer muscle for the more forceful contractions in the fast twitch fibers. A similar finding has yet to be duplicated in the human model. In fact, given Kyrolainen et al.'s results, (higher type II fiber composition correlated to better RE, and the runner with the best RE was the only found to express T1, are evidence to support the complete opposite trend. Should titin prove to be a major player in active muscle stiffness, it is the ratio of T1:T2 protein as well as the ratio of titin (T1+T2):MHC that will likely be indicative of the spring properties of the muscle, and not just the normalized titin value. All of the preceding were examined for this paper. Correlational data for runners

with better or worse RE exists for titin and MHC, but no longitudinal study to date has been done to see which, if either of these proteins (MHC, titin) is malleable through plyometric training. Nor has any within subjects design been utilized to account for the dissimilarities seen between individuals. Tracking of RE changes along with muscle protein data over time is imperative to furthering our knowledge of the interplay of structural muscle proteins and their contribution to force production. The available research on this topic is currently devoid of studies integrating this approach.

THE CURRENT STUDY

Despite the discrepancies found in the literature, it is generally accepted that force due to elastic recoil from stiffer muscles is a contributing factor to RE. In the study described we have examined several likely parameters, and attempted to identify their role in determining RE, namely several established physiological parameters (VO_{2max}, LT), anaerobic strength/power, flexibility, biomechanics/ kinesthetics, muscle stiffness (passive and loaded), fiber composition, MHC composition (Kyrolainen, et al., 2003; Prado, et al., 2005) and titin (Fuerst, Osborn, Nave, & Weber, 1988; Funatsu & Tsukita, 1993; Horowits, et al., 1989; Kyrolainen, et al., 2003; Marszalek, et al., 1999; Ottenheijm, et al., 2009; Prado, et al., 2005; Trinick, 1996; Wang, et al., 1991) isoforms present in the VL.

The aim of the current study was to relate improvements in RE seen with 6 weeks of plyometric training to changes in MHC and titin. We proposed that RE would improve after participation in the plyometric training. Additionally, we foresaw no change in VO_2max or LT during the study. In the case that either of these postulates were not to be observed, that in and of itself would be a novel finding, and contribute to the literature on plyometric training within a group of runners. We also predicted a significant reduction in 3K time; for this time to correlate positively with RE; an increase in anaerobic power with a negative correlation to RE and no change in flexibility. Muscle stiffness was expected to increase with the plyometric training, and this measure to negatively correlate with RE.

Moreover, we hypothesized that there would be a significant increase in expression of the larger titin isoform (T1) correlated to the improvement in RE anticipated. We posited that individuals with greater Type II fiber compositions would likely show better economies at faster running speeds, whereas those with greater Type I fiber types would show better RE at the lower running speeds. Since RE was measured for all stages, this enabled us to report on a wider range of running speeds than is typically found in the literature. It was also speculated that because of the selection process for this study (targeting adult endurance runners), the majority of participants would have very low MHC IIX abundance. As such the anticipation only small changes in these values, as muscle plasticity in adulthood is limited, and six weeks will likely not provide a great enough stimulus to endow measureable shifts in MHC, but perhaps significant altered titin isoform expression (as little is known of titin skeletal muscle titin plasticity) seemed fair.

METHODOLOGY

Participants. Twenty-five volunteers were recruited from local running clubs and races in Missoula Montana. All participants were screened for standard health (PARQ) as well as training history, current training level and injury status/history. [Appendix 1-2] Persons were only permitted to do the study given a clear medical and injury history, and adequate running experience (minimum of 1-year). Volunteers with significant histories of plyometric/resistance training were also not permitted to partake in the study. There were no set exclusion criteria for fitness determinants: performance/ability level, RE, VO_{2max}, LT, etc. Both male and female participants were permitted to partake in the study.

Once recruited, participants were separated by gender and randomly placed into 1 of 2 groups: Plyometric training (EXP: n=14) or non intervention to serve as a control (CTL: n=11). The disparity in numbers was intentional to account for higher anticipated injury/drop-out rate from EXP. In fact, 3 dropouts from EXP evened out these numbers to 11 and 11 (1, unable to maintain the time commitment, 1 from an unrelated fall resulting in injury, and the third was unable to finish due to development of muscular issues in his back –possibly related to the plyometric training). General fitness and descriptive for the participant pool are given in table 1, though it is worth stating that there were disproportionately more females than males involved in this study, the groups were split evenly by sex. All participants signed Informed Consents and all research was carried out with the approval of the

DESCRIPTIVES	Mean CTL	Mean EXP	Mean ALL
N value & male:female ratio	n = 11. 4:7	n = 11. 4:7	N = 25. 10:15
age (yrs)	32.45	34.15	33.76 ±7.2
Height (cm)	171.83	170.52	171.66±8.1
Weight (Kg)	68.21	70.98	70.0±11.7
BMI	24.03	24.32	24.1.0±4.2
% BF	21.3	22.13	21.2±7.5%
LBM (Kg)	52.46	55.23	56.1±12.9
FM (Kg)	14.75	15.75	15.5±8.2
Flexibility (cm)	33.31	38.53	37.1±7.5
VJ (cm)	44.68	48.84	47.4±13.5
VO_{2MAX} (ml/kg/min)	48.22	47.87	48.1±6.2
OBLA_speed (m/s)	3.834	3.484	3.66±0.46
OBLA_VO2 (ml/kg/min)	42.5	40.26	41.6±5.3
OBLA (as $\%VO_{2MAX}$)	89.36	84.58	86.8±6.1
3Km Time Trial (min:sec)	12:11	13:50	13:21±1:10
RE (% below estimated)	14.59	17.20	15.9±1.8
Training Volume (miles/wk)	21.55	25.37	24.0±12

Table 1. Descriptive characteristics of participants combined and by group, mean± SD. No differences between groups in any measures.

University of Montana Institutional Review Board.

Overview. For pre test measures participants came into the lab during the final week of January on two separate occasions and once to an indoor track for a time trial (TT). The first visit consisted of all measures save the Myotonometer testing and muscle biopsy (Hydrostatic weighing, Vertical Jump, Sit-and-Reach, all treadmill testing including VO_{2max}, onset of blood lactate accumulation (OBLA –taken to estimate LT), RE, EMG data and biomechanics). Within 6 days of this first visit all participants subsequently completed both of the other testing sessions. A minimum of 72 hours was provided separating the biopsy from the TT, to mitigate any soreness from the procedure. For the following six weeks participants in EXP partook in a progressive plyometrics routine adapted from Spurrs et al. (2003). Those in the CTL group continued with their normal training. Both groups were encouraged to abstain from adding novel stimuli to their regime, including, but not limited to high intensity workouts, any form of resistance training or large volume increases. After the training intervention concluded, post testing was carried out with time of day and order of testing matched for all tests within 1-2 hours of pre-testing. All measures except for body composition were repeated.

Body Composition. Hydrostatic weighing was carried out to assess body composition. In brief, participants were instructed how to carry out the test, and guided through it by the tester. A minimum of 3 underwater weights were collected, and averaged together. Residual lung volume was calculated based on standard estimation equations. Percent body fat, lean body mass and fat mass were calculated for each participant. Additionally Body Mass Index (BMI) was calculated from the height and weight measurements for each participant during both pre and post testing.

Anaerobic Power. A vertical jump test using a measuring device (Vertec, Grand Rapids MI) in conjunction with the Just Jump Mat (Probiotics, Huntsville, AL) automated system was implemented for assessment of anaerobic power. Participants were instructed to use a counter movement jump to attain the highest vertical displacement possible. Roughly 2 minute rest intervals between trials were allowed and individuals continued jumping until they no longer continued to increase height with a minimum of 3 trials.

Flexibility. Upon completion of the VJ trials, participants underwent flexibility testing via a Sit-and-Reach protocol. Each person was shown a demonstration and given verbal instructions. They sat on the floor with back (low back, scapulas and head) against the wall and legs out straight. A 12 inch block was placed at their feet with a measuring stick on top. They were instructed to reach their hands out toward the stick without coming “off” the wall. The measuring stick was zeroed to this position. They then slid their hands down the stick by bending forward at the waist, without bending their knees as far as they could. A minimum of 3 trials were repeated in succession until they were unable to improve upon their score.

Treadmill Protocol. A discontinuous protocol was used to allow for simultaneous collection of VO_{2MAX}/RE data, biomechanics/EMG, and Blac. Stages 1-9 were run at 1% grade to best mimic the metabolic cost of over-ground running. Speed on stage 1 was 7.5km/hr and progressively increased by 1.5km/hr each stage until a max speed of 19.5km/hr was reached. At that point speed was maintained,

and grade increased by 2% for each subsequent stage completed for safety. This yields sequential speeds of 129, 153, 177, 201, 225, 249, 274, 298, and 322m/min. Despite the relative ease of early stages, individuals were instructed to run and not walk for all stages. The same standard research-grade treadmill was used for all testing. Blood collection was carried out during the 1-minute rest interval along with collection of a Rating of Perceived Exertion score from the Borg Scale. Participants were permitted to remove the breathing apparatus during the rest interval, but most did not. Two of the participants were asthmatic, and both took their inhaler ~10-minutes prior to testing, as this is what they would normally do. One of them used her inhaler during a rest interval, but this was noted, and no alterations in gas data were observed following the treatment. (see appendix 3 for protocol)

Aerobic Capacity. Maximal aerobic capacity or VO_{2MAX} was measured using 15-second averaging with a Parvomedics open spirometry TrueOne metabolic cart (Sandy, UT). Measurements were continuously taken throughout the protocol. The test consisted of 3-minute running stages with a 1-minute rest interval. The highest 15-second average (while running) was used for VO_{2MAX}. All participants reached at least two of set criteria: RER $\geq$ 1.15, observance of a plateau in VO_2, attainment of $\geq$ 95% of age estimated max heart rate, RPE $\geq$ 18 for attaining a VO_{2MAX} during both pre and post testing. The metabolic cart was calibrated at the start of each day and every 2-3 tests thereafter to control for any drift or fluctuations in room conditions, which were also recorded for each test.

Anaerobic Threshold. During the 1-minute rest intervals, a unistick lancet, the Lactate PRO (Sports Resource Group, NY) and a 50μL glass capillary tube was used to collect blood for lactate concentration. Blood from the capillary tubes were immediately injected into a cell lysis buffer and kept on ice until being stored at -80. These samples were later analyzed using the YSI 1500 sport (Life Sciences, Yellow Springs, OH)). Lactate Pro values were used only to assess roughly when OBLA had been reached. An exponential curve was generated using all stages for speed v. Blac, VO_2 v. Blac, and $\%VO_{2MAX}$ v. Blac. OBLA was determined for each of these intensity parameters off of these curves for each individual.

Running Economy. Gas analysis from the Parvomedics cart was collected using 4-breath analysis and representative samples from 20-30 seconds in length were used from the final minute of each stage to give O_2 consumption for that speed (both absolute VO_2 in L/min and relative VO_2 in ml/min/kg).This was done for all stages during which a steady state was achieved. Other standard gas parameters were tabulated as well (minute ventilation (VE) in L/min, VE/VO_2, $\%VO_{2MAX}$, VCO_2 in L/min and ml/min/kg, RER (VCO_2/VO_2), and RE calculated as ml of O_2 per kilogram body weight per kilometer by taking the relative VO_2 and dividing by speed in Km/min. This allowed for comparison of RE from stage to stage, to examine relative efficiencies. It also permitted averaging of RE over several stages without weighting the latter stages more heavily due to their higher workloads. These values were calculated for all stages, and for all stages without exceeding an RER of 1.00, 1.05, and 1.10. Doing so made it possible to estimate both fuel utilization shifts as well as increased anaerobic contribution to energy supply. These values along with the VO_2 at each speed were used to assess RE. Testing commenced no earlier than 7:15AM and no later than 6:15PM for all individuals and this time was matched within 1 hour for post-testing. Participants were encouraged to keep a food log and repeat their 24-hour diet –though this was not required, most actually did do this. All participants had fasted for at least 2 hours, and refrained from

any exercise that day and any intense exercise the previous day. They also were instructed to wear typical race-day attire, and used the exact same clothing and shoes for both pre and post testing. All participants were given verbal encouragement during the testing and instructed to run with until volitional fatigue.

Running Performance. As a performance measure, a 3,000 meter TT was completed at Peak Fitness on the indoor 200m non-banked tartan (rubber) surface running track. Participants were placed in groups of 5-8 by ability based in part on self-reported running ability (recent 5K time) and in part on the results of the treadmill test. All runners were instructed to treat the TT as a race, and were instructed to cover the distance as fast as possible. They were encouraged to pace themselves evenly throughout, and allowed to warm up prior. Split times for each participant were recorded, but not supplied to the runners, as not to influence their pacing. Verbal encouragement was offered by the timers.

Muscle Stiffness. Myotonometer data was collected prior to the biopsy. As such participants were 2-hour fasted, had abstained from any intense exercise for 24 hours and were in a euhydrated state. Four sites were measured in both the relaxed (passive) and loaded (active) conditions: The VL, medial *gastrocnemius*, medial *soleus*, and *Achilles* tendon. Poses for the passive measures were set to optimize relaxation at the site of measurement. Individuals were instructed to assume the required position and the tester manually checked for and reaffirmed verbally the relaxation of the participant. For active values a hammer-strength squat machine was used to apply a load of 45kg for each stance. Positions were chosen to maximize muscle activation for the site of interest. Each measurement was collected a minimum of 3 times and averaged together for each site. Values were given for displacement in mm and force applied in kg. A curve was generated and area under the curve calculated for each measure (kg x mm, or work done in displacing the muscle). These values were then tabulated as both absolute numbers for the relaxed and loaded conditions. Calculations for the difference between passive and active stiffness were carried out to assess how far from baseline muscle force generation could alter stiffness. This difference was reported in absolute magnitude as well as percent stiffness increase within each of the 4 sites (See appendix 4 for site descriptions). The same tester was used for all PRE measures and by necessity, a second tester for all POST.

Muscle Biopsies. After completion of the muscle stiffness testing, participants were brought over to the biopsy lab. Once there they were prepped, a small biopsy of the VL was taken and subsequently flash frozen in liquid N_2. Samples were then stored in a -80 freezer until analysis. After the biopsy, each participant was supplied with written instructions for care of the biopsy site, and suture removal was offered within the following 36-60 hours. The diet log from this visit was kept on file until post testing so that the individual could replicate this for the post testing session. To ensure dissipation of residual soreness no biopsies were performed less than 3 days prior to any other testing. Order and spacing of testing was matched pre to post by no more than one day, and still fell within the 6-day window for all participants.

MHC Analysis. Total myosin heavy chain (relative) concentration was calculated from the titin gels (see below). Muscle is currently being stored in both raw and solubilized form for MHC isoform analysis. See appendix 6 for detailed protocol.

Titin Analysis. Muscle samples were homogenized and solubilized into solution. Solubilized samples were loaded onto a 16x18cm 1% agarose gel in 4.5 and 9 μL aliquots for vertical agarose gel electrophoresis (VAGE). Each gel was run at a constant 15mA/gel at 4°C for 3:20. See appendix 7 for detailed protocol. A previously analyzed human *soleus* sample was run on each gel for normalization. Protein quantification was performed using Image Gauge version 3.12 software, and relative concentrations were calculated for T1, T2, T1:T2 ratio, total titin, MHC, and total titin:MHC ratio.

Plyometrics Intervention. The plyometric intervention is a progressive six week program involving an increase in frequency of session, (from two per week over the first three weeks to three per week over the final three weeks), development of exercises (see appendix 5) and volume of jumps (as tallied by total contacts, 60 during week one, and 228 during week six) was modified from Spurrs et al. (2003). Contact with the authors in addition to knowing the biopsy site, led to the revised protocol. Most subjects were able to complete the routine without serious injury, but notably half of this group reported at least some form of acute injury, and 1 participant was forced to withdraw entirely due to plyometrics-related injury.

Sessions were led in an open wooden-floored gymnasium by an instructor familiar with the motions, and a log of each workout was kept. A standardized 15-minute warm-up consisting of easy jogging along with static and dynamic stretching was carried out at the onset of each session. The runners were consistently reminded to perform all movements for maximal height/distance and minimal ground-contact time. The training was to be completed in addition to their normal run training. Participants were asked not to change the level of intensity or volume of their normal training during the eight weeks of this study. Each person was asked to report on their training both for several weeks prior to pre testing as well as during the study (prior to post testing). It was asked that they simply continue their normal run schedule and do not add or subtract anything radically from their routine during the investigation. All participants needed to complete at least 15 of the 18 sessions.

Post Testing. As stated post testing data was obtained in the same manner and order as pre. All efforts were made to match subjects testing conditions. Post testing occurred immediately following the sixth week of plyometrics beginning mid-March. All testing session were completed within 11 days of the final plyometrics training session.

Statistics. 2x2 ANOVAs were run (group by time) for all measures using SPSS version 16.0. All pair wise comparisons reported are for group x time differences unless otherwise specified. Main effects were identified in all such cases, but because they add little to the discussion are not addressed. Additionally Pearson's Correlations were carried out for all delta measurements (post-pre) as well as all pre and post measures for a cross-sectional analysis of the data. Paired t-tests were also computed for the delta values and or percent delta values in all measures for which these deltas were calculated. Finally correlations were run between all pre and delta measures allowing for identification of

responder/non-responders to the training. Grouping of subjects was carried out by sex, age, fitness (VO2max, OBLA, TT, %BF) and other performance characteristics (VJ, flexibility) as well as change in all these values, to examine the possibility of responders/non-responders to training and all statistical procedures repeated. Finally, grouping by a change in RE (those who improved versus those who did not change/saw a worse RE post) was completed and statistical procedures repeated for this group. All results are reported as mean values, and significance was set at an alpha level of $p \leq 0.05$.

Further investigations were carried out on descriptive data and key measures to identify paired commonalities within groups of individuals. In short, different divisions were superimposed to divide the participants into groupings for different analyses (i.e. by sex or by age). Though not provided for all divisions, concomitant changes in starting values for some of the measures did occur, and will be discussed as it pertains to the results below. In brief, for cross sectional analysis n=25 for physiological measures; 18 for all relating to protein data. For the ANOVA's n= 22 (11 EXP; 11 CTL); 13 (6 EXP; 7 CTL) for data relating to proteins.

RESULTS

Refer to table 1 for a breakdown of descriptive statistics for the sample population. Though body composition was not retaken at the conclusion of the study (POST), BMI was calculated from height and weight data, and though the means for CTL were significantly different PRE/POST, the magnitude of change was small.

Experimental Differences

Physical performance testing (VJ, flexibility, 3Km TT) yielded several changes. No changes were seen for the EXP group by time in either the sit-and-reach or VJ height, yet a significant increase in flexibility and a decreased VJ was observed in the CTL. Furthermore, the CTL group did not significantly improve their 3Km time, while the EXP group was faster POST.

Performance Data. Standard physiological measures (postponing discussion of stage-by –stage treadmill test data collected) included VO_{2MAX}, OBLA (by speed, metabolic workload (VO_2), and as a % of max aerobic capacity (%VO_{2MAX}). In regards to this information, it was observed that VO_{2MAX} significantly improved in the EXP group PRE to POST, but not in the CTL, though there was a trend for improvement (p = 0.068). Additionally, though no changes by time were seen for OBLA, at POST, EXP reached OBLA at a significantly higher proportion of the VO_{2MAX}. No such significant disparity between groups were present PRE, though there existed a trend for OBLA to occur at a greater percentage of VO2MAX (p = 0.063). Refer to table 2 for tabulated records.

Measure	Group	CTL	EXP	*p value*
BMI	PRE	24.32	24.03	*0.882*
	POST	24.02	23.88	*0.94*
	p value	***0.033****	*0.265*	
Flexibility (cm)	PRE	38.54	33.31	*0.091*
	POST	42.36	35.60	***0.035****
	p value	***0.003****	*0.059*	
VJ (cm)	PRE	48.84	44.68	*0.485*
	POST	45.49	44.45	*0.866*
	p value	***0.011****	*0.848*	
VO2 Max (ml/kg/min)	PRE	47.89	47.87	*0.992*
	POST	49.75	50.25	*0.856*
	p value	*0.068*	***0.022****	
OBLA_speed (m/s)	PRE	3.48	3.83	*0.185*
	POST	4.50	3.86	*0.178*
	p value	*0.831*	*0.549*	
OBLA_VO2 (ml/kg/min)	PRE	40.27	42.80	*0.271*
	POST	41.06	44.20	*0.167*
	p value	*0.412*	*0.155*	
OBLA_%VO2max	PRE	84.58	89.36	*0.063*
	POST	82.74	88.22	***0.025****
	p value	*0.395*	*0.595*	
3Km Time Trial (min:sec)	PRE	13:50	13:01	*0.300*
	POST	13:37	12:41	*0.267*
	p value	*0.169*	***0.042****	

Table 2. Fitness/Performance measures PRE and POST in both groups. Differences between groups are read across the table; differences by time are read down. ***bold/underline** denotes a significant difference.

Metabolic Data. Gas analysis data from the treadmill testing were evaluated and are described in table 6 at the conclusion of this chapter. In general there were no sweeping differences within or between either group across stages for any of the measures. Nor were there reliable trends for the lower versus faster running speeds. There were scant disparities isolated within the statistics, but generally the metabolic assessment is unremarkable in terms of differences in or between groups.

Interestingly VE or total breathing volume decreased in all stages for EXP, but only reached significance on stages 2 and 6, while it increased or barely dropped (< 10ml/kg/min) for all stages, but only reached a significant elevation during stage 2 for CTL. A similar trend was seen in VCO_2 with higher VCO_2 values in the CTL reaching significance at the three slowest speeds, stages 1-3. To a lesser extent this trend was present in VE/VO_2 and RER, but differences were only occasionally of significance. Lastly, VO_2 values were remarkably identical PRE and POST in the CTL, and EXP. EXP values tended to be only slightly higher, and a significant, but unsubstantial increase was seen at stage 5.

Focusing on RE measures, both as the energy cost of running, or VO_2 consumption for a given speed and as VO_2 consumption calculated per unit distance covered, there was virtually zero group divergence. This held true whether or not RER values were used to remove data points where anaerobic energy contributions were apparent. With the calculation of RE for comparison of different speeds within the protocol, analysis of "aerobic" (respiratory exchange ratio (RER) < 1.00) and aerobic plus anaerobic (RER > 1.00) RE values yielded no repeatable significant differences within or between groups. When alternative RER cutoff values were applied (namely 1.05, 1.10, 1.15) to allow for some overlap of the aerobic/anaerobic energy continuum there was a trend for impairment (increase) of RE PRE to POST in the CTL -averaging all RE values for which RER < 1.10- and a significant elevation in EXP under this condition as well as an RER cutoff of 1.05. In light of these findings, analysis was carried out for differences identified between those individuals who saw unchanged/worse RE PRE to POST and those who saw enhancement of RE during the study. These are reported later in the chapter.

Muscle Stiffness. Myotonometer evaluations reflecting muscle stiffness in both the passive and active states for all four sites measured are summarized in table 8 in the final portion of this chapter. There were no significant differences between groups for any of the measures either PRE or POST. Main effects for time however existed for many of the measurements. For the only tendon measured (the Achilles), PRE to POST changes were not observed in either CTL or EXP. All measures of muscles within the *triceps surae* group, revealed a decrease in muscle stiffness: within the medial *gastrocnemius* in the passive state for both CTL and EXP; within the medial *soleus* in both the passive and active state for both CTL and EXP. Computations revealed a greater stiffness increase both in absolute and by percent increase for the *gastrocnemius* and the in absolute terms for the *soleus* in both groups. For the proximal lower limb similar data was collected from the VL. This muscle experienced a significant loss of stiffness in the passive but not active state, and a

Measure	Group	CTL	EXP	*p value*
Total Titin	PRE	30.71	27.06	*0.747*
	POST	37.65	35.98	*0.863*
	p value	*0.375*	*0.202*	
Total T1	PRE	26.12	23.95	*0.827*
	POST	31.62	30.21	*0.872*
	p value	*0.400*	*0.287*	
Total T2	PRE	4.687	3.115	*0.404*
	POST	6.034	5.767	*0.867*
	p value	*0.406*	*0.078*	
Total MHC	PRE	110.19	83.41	*0.395*
	POST	135.30	127.95	*0.524*
	p value	*0.203*	***0.018****	
T1:T2 ratio	PRE	3.71	3.52	*0.913*
	POST	4.10	4.35	*0.849*
	p value	*0.758*	*0.473*	
Titin:MHC ratio	PRE	0.483	0.405	*0.656*
	POST	0.567	0.583	*0.900*
	p value	*0.495*	*0.117*	

Table 3. Total protein in AU and T1:T2 & titin:MHC ratios. AU were set so that the standard muscle run on each gel yielded a value of 100 AU/4.5µL loaded. Only significant differene EXP PRE to POST.

concomitant increase in passive to active stiffness separation in absolute terms in both groups, plus a percent passive to active stiffness increase PRE to POST for CTL only. There were no significant differences between groups at either time point.

Muscle Protein Data. Examination of the muscle protein values, including total titin, total T1, total T2, T1:T2 ratio, total MHC, and total titin to MHC ratio (T:M) illustrates minimal shifts in protein content or isoform makeup (n = 12, 6 EXP, 6 CTL). In looking for differences between these two groups in protein makeup, paired t-tests showed no significant differences in the changes or percent changes seen PRE to POST between the 2 groups. No distinguishing trends were noted from the ANOVA's, but Pearson's correlations were carried out and are discussed at the end of this chapter. Quantifications are tabulated in table 3.

Sham Group Differences

Considering the lack of differences seen between groups, improvement of RE was set as the criteria to allow for apportioning of participants to one of two groups: those who underwent no alteration or a degeneration in RE (DRE: n = 9) and those who experienced improvements in RE PRE to POST (IRE: n = 13), regardless of EXP/CTL grouping. In doing this a change in RE becomes the independent variable, while the training modality is ignored. In essence it is not entirely dissimilar from the intended design wherein the plyometric training was to be used simply as a tool to induce changes in RE in one group versus another. In this regard, the participant grouping is simply being reshuffled to fit the intended model. While this disqualifies data aggregated from this perspective as being considered a true experimental design and loses the ability to determine without question a cause effect relationship for RE versus the other factors considered, it seems pertinent to the discussion of the determinants of RE, and allows for a more in depth examination than simply correlating the pooled records.

Measure	Group	DRE	IRE	*p value*
BMI	PRE	25.43	22.37	*0.114*
	POST	25.12	22.28	*0.125*
	p value	***0.016****	*0.494*	
Flexibility (cm)	PRE	36.56	35.01	*0.633*
	POST	40.12	37.33	*0.414*
	p value	***0.003****	*0.083*	
VJ (cm)	PRE	44.94	49.39	*0.462*
	POST	43.18	47.55	*0.483*
	p value	*0.154*	*0.213*	
VO2 Max (ml/kg/min)	PRE	45.55	51.24	***0.037****
	POST	48.75	51.80	*0.269*
	p value	***0.001****	*0.570*	
OBLA_speed (m/s)	PRE	3.51	3.88	*0.063*
	POST	3.53	3.92	***0.044****
	p value	*0.751*	*0.605*	
OBLA_VO2 (ml/kg/min)	PRE	39.24	44.85	***0.011****
	POST	41.51	44.23	*0.244*
	p value	***0.008****	*0.51*	
OBLA_%VO2max	PRE	86.51	87.64	*0.677*
	POST	85.48	85.47	*0.998*
	p value	*0.604*	*0.365*	
3Km Time Trial (min:sec)	PRE	14:09	12:22	***0.020****
	POST	13:56	12:02	***0.020****
	p value	*0.123*	*0.054*	

Table 4. Fitness/Performance measures PRE and POST in the runners who eie (IRE) and did not (DRE) see mprovement in RE during the 6-week study. Significant values are in **bold underline** and marked with an asterisk*. Again, differences between groups are read across and changes by time down.

Performance Data. VO_{2MAX} increased significantly in DRE but not IRE, and it was higher in IRE than DRE PRE; VO_2 at OBLA also significantly increased for DRE, but not IRE and it too was higher PRE in IRE than DRE; 3K TT performance was significantly faster for IRE both PRE and POST (by nearly 2:00), yet did not significantly come down in either group, though it showed a strong trend (p = 0.054) to do so in

IRE; Relative workload (% of VO_{2MAX}) displayed trends for improvement in both, but particularly the IRE group being significantly lower (indicating better fitness) POST for IRE in all the stages for which significant improvements of RE were seen; speed at OBLA was significantly greater for IRE as compared to DRE at time POST but not PRE. Additionally, flexibility increased PRE to POST for DRE, but not for IRE, VJ declined for DRE, but not for IRE. Nothing was seen for % of VO_{2MAX} at OBLA. Surprisingly both BMI and weight significantly decreased for DRE and not IRE PRE to POST, but by very small amounts. In summary, it seems that the better conditioned individuals were more likely to improve upon RE, while less conditioned runners improved fitness parameters before addressing economy. See table 4 for a summary.

Metabolic Data. Treadmill testing data displayed no changes in BLac response or RPE. Nor did it indicate any alterations in fitness from $\%VO_2$ use at any except the slowest of stages, but did so for both groups. No changes in minute ventilation (VE) were seen by group by time or group. The interesting changes were seen in VE/VO_2, or respiratory efficiency, and RER (indicative of substrate utilization and aerobic/anaerobic energy derivation). VE/VO_2 was lower PRE to POST in DRE on stage 4, while it was lower on stages 2 and 4, and higher on stage 3 for IRE. In addition, IRE started with a greater value PRE during stage 7, while all other stages displayed no disparities between groups PRE. POST VE/VO_2 values were greater for IRE on stages 2 and 3 only. RER for DRE was significantly reduced PRE to POST on stage 6 and trended towards decreasing on stage 4 ($p = 0.08$) Meanwhile IRE exhibited significantly higher POST RER values than PRE for the slowest 3 stages PRE. Despite being no different than DRE during any PRE stages, IRE had significantly higher RER on stages 1 and 2 along with trends towards greater RER's on stages 6 and 7 ($p = 0.087$ & 0.077 respectively). No such disparity was present for the more moderate speeds, only the more extreme speeds both high and low. All findings are provided along with economy information in table 7.

As expected, changes in RE were significantly different between groups. Unpredictably however, the difference was seen mainly from a worsening of RE in DRE rather than an improvement in IRE. VO_2 use became lower at low speeds (stages 1-3) for IRE PRE to POST, but it was increased at moderate and high speeds for DRE (stages 3-7). This same trend was seen when VO_2 values with concurrent RER values > 1.00 were omitted. The RE computation yielded worse RE for all RER stipulations for DRE, and only showed trends towards improvement when no RER ceiling ($p = 0.067$) or the most stringent, RER < 1.00 ($p = 0.060$) was set.

Measure	Group	DRE	IRE	*p value*
Total Titin	PRE	36.40	19.14	*0.398*
	POST	45.62	25.60	*0.181*
	p value	*0.187*	*0.400*	
Total T1	PRE	31.88	16.21	*0.102*
	POST	38.81	20.87	***0.026****
	p value	*0.239*	*0.473*	
Total T2	PRE	4.52	2.93	*0.398*
	POST	6.80	4.74	*0.181*
	p value	*0.129*	*0.272*	
Total MHC	PRE	31.88	16.21	*0.102*
	POST	38.81	20.87	***0.026****
	p value	*0.239*	*0.473*	
T1:T2 ratio	PRE	4.88	2.00	*0.086*
	POST	4.45	3.97	*0.708*
	p value	*0.69*	*0.115*	
Titin:MHC ratio	PRE	0.557	0.293	*0.161*
	POST	0.675	0.451	***0.0112****
	p value	*0.290*	*0.210*	

Table 5. Total protein and T1:T2 & titin:MHC ratios. All non-ratio values are given in A U. n = 12.

Muscle Stiffness Data. Unexpectedly, myotonometer data was virtually identical to that seen with the plyometrics grouping, with the only discrepancies being that the active VL measure was

nearly stiffer in IRE (5.976 kg*mm RE versus 6.068 kg*mm POST, p = 0.053) and the percent difference between active and passive stiffness in the VL was significantly greater in both groups (54.7% to 64.2% and 41.9% to 48.9% PRE to POST for IRE and DRE respectively p < 0.05).

Muscle Protein Data. Once again no significant differences in the changes or percent changes seen PRE to POST between the 2 groups using paired t-tests were revealed. Between groups differences were seen for total titin:MHC POST (p = 0.012). There was no significant difference in either PRE, and both were higher in DRE than IRE POST. Table 5 displays all protein quantifications for the IRE/DRE groupings.

Data Correlates

Correlations below will be discussed both in terms of correlating PRE to PRE values as well as changes PRE to POST in one value to changes PRE to POST in another. This allows for discussion of how these measures vary with respect to one another both between and within individuals. For a comprehensive listing of all Pearson's r and p values for significance, see tables 9 & 10 at the end of this chapter.

Performance Data. Limited significant associations were observed for performance data and running economy measures. As expected, VO_{2MAX} positively correlated with VO_2 use throughout the stages, particularly the latter/faster ones. VO_2 at OBLA and flexibility also positively correlated to VO_2 consumption. Those with high VO_{2Max} values, greater speed and VO_2's at OBLA and more flexibility had worse RE. Moreover the same trend was present for changes PRE to POST in these values, meaning runners tended not to concurrently improve maximal aerobic capacity, metabolic or physical workload at threshold, or flexibility with RE. All other performance measures displayed no interrelation with O_2 consumption.

Metabolic Data. Numbers from the metabolic assessments PRE show no unbalanced correlative values between VE and VO_2 use per stage, with r values around 0.4 at the slower and in excess of 0.7 the faster speeds. Counter intuitively, changes in VE were not seen to correlate with changes in RE or VO_2 when paired by stage. Somewhat counter-intuitively VE/VO_2 showed a positive moderate correlation with RE, but only at the some of the lower speeds (stage 1: r = 0.42, p = 0.037; stage 2: r = 0.38, p = 0.059; stage 3: r = 0.52, p = 0.008). Paradoxically, the opposite trend was observed for the changes PRE to POST for these two measures (significant correlations: stage 1 r = -0.54, p = 0.010; stage 2 r = -0.43, p = 0.044. Also expected were strong correlations for higher CO_2 production with VE increases both in terms of PRE data and changes in these measures within individuals. This is precisely what was observed (data not provided). Moreover CO_2 production linked strongly with RE (higher CO_2 levels with worsening RE, particularly at the lower speeds, and less so as intensity increases). Neither PRE nor delta correlations showed any relationship between RER and VO_2 consumption. In summary better RE associates with lower VE, lower CO_2 production, and possibly higher/lower VE/VO_2.

Muscle Stiffness. Several changes in muscle stiffness were moderate when correlated to changes in TT performance (changes in the *gastroc* just missed significance: passive *gastroc*: r = 0.41, p = 0.061; active *gastroc*: r = 0.41, p = 0.058; passive VL: r = 0.67, p < .001; no correlations were seen in the

tendon (A*chilles)* with 3Km time). Generally better RE correlated to a stiffer *gastroc* (particularly at higher speeds), a stiffer *soleus* (though more sporadically, and weaker), a stiffer VL, and not at all with the *Achilles* tendon.

Muscle Protein Data. With respect to the muscle protein data, table 9 at the end of the chapter includes an extensive listing of significant r and p values for the various factors paired with these numbers. An abridgement of that information is as follows: Less fit people (by %BF, %VO_2, speed and VO_2 at OBLA, VO_{2MAX}, and 3Km TT) tended to have more titin (particularly T1) and less MHC; flexibility was better for those with a greater abundance of T1; VJ was higher for those with less T2. There was mixed data for some of the gas analysis data, but generally RER went down with increasing total titin and T1:T2; VE/VO_2 came down as titin went up. Perhaps most interestingly T:M seemed to increase with VO_2 consumption. Other protein quantifications correlated less consistently with VO_2 and RE. Noteworthy is that VO_2 did seem to inversely vary with titin quantification changes within subjects as well as cross-sectionally PRE but not POST. Lastly, muscle stiffness findings revealed that increases in total titin, T1, T2 and MHC all were associated with decreased stiffness (no relationship for T1:T2) while T:M paralleled increases seen in active VL stiffness.

Stage		1			2			3			4			5			6			7		
Measure	Group	CTL	EXP	p value	CTL	EXP	p value	CTL	EXP	p value	CTL	EXP	p value	CTL	EXP	p value	CTL	EXP	p value	CTL	EXP	p value
VO_2 (mL_O2/Kg/min)	PRE	27.32	26.50	*0.412*	30.54	29.91	*0.511*	34.11	33.50	*0.568*	38.59	37.06	*0.132*	42.37	41.26	*0.390*	47.57	46.48	*0.452*	50.83	48.81	*0.355*
	POST	27.12	26.13	*0.308*	30.93	29.24	*0.130*	35.03	33.25	*0.091*	38.64	38.06	*0.591*	43.06	42.65	*0.744*	47.78	47.26	*0.738*	51.61	50.73	*0.688*
	p value	*0.677*	*0.440*		*0.403*	*0.457*		*0.166*	*0.710*		*0.938*	*0.143*		*0.312*	***0.042****		*0.802*	*0.299*		*0.408*	*0.080*	
VE (mL_Air/Kg/min)	PRE	479.15	494.87	*0.671*	564.07	607.82	*0.322*	658.18	691.09	*0.534*	808.63	822.70	*0.842*	909.97	975.94	*0.331*	1104.7	1165.80	*0.447*	1279.6	1279.3	*0.997*
	POST	493.45	493.38	*0.998*	586.89	581.31	*0.894*	686.18	671.25	*0.756*	799.38	787.04	*0.830*	936.11	959.20	*0.701*	1095.5	1100.09	*0.958*	1278.2	1241	*0.719*
	p value	*0.250*	*0.903*		***0.030****	***0.014****		*0.066*	*0.183*		*0.713*	*0.166*		*0.277*	*0.461*		*0.666*	***0.003****		*0.971*	*0.385*	
VE/VO_2 (L_air/L_O_2)	PRE	17.47	18.05	*0.442*	18.38	19.75	*0.159*	19.20	20.01	*0.418*	20.93	21.44	*0.720*	21.48	23.00	*0.226*	23.20	24.28	*0.341*	25.16	26.14	*0.558*
	POST	17.96	18.82	*0.397*	18.71	19.85	*0.256*	19.32	20.15	*0.420*	20.37	20.66	*0.794*	21.48	22.48	*0.387*	22.65	23.25	*0.687*	31.57	24.35	*0.388*
	p value	*0.223*	*0.063*		*0.478*	*0.843*		*0.798*	*0.743*		*0.279*	*0.136*		*0.994*	*0.210*		*0.256*	***0.027****		*0.250*	*0.762*	
VCO_2 (mL_CO_2/Kg/min)	PRE	22.74	22.82	*0.953*	26.93	27.84	*0.512*	31.37	31.65	*0.859*	37.44	36.72	*0.702*	42.00	42.66	*0.707*	49.12	52.45	*0.300*	55.96	55.43	*0.915*
	POST	24.43	22.86	*0.304*	28.94	26.45	*0.136*	33.48	30.93	*0.153*	37.32	34.69	*0.172*	44.31	41.65	*0.192*	48.24	44.61	*0.389*	52.76	48.45	*0.409*
	p value	***0.014****	*0.944*		***0.030****	*0.123*		***0.046****	*0.478*		*0.941*	*0.229*		*0.133*	*0.482*		*0.870*	*0.078*		*0.639*	*0.331*	
RER (VCO_2/VO_2)	PRE	0.83	0.83	*0.885*	0.88	0.91	*0.263*	0.92	0.92	*0.940*	0.97	0.96	*0.773*	0.99	1.01	*0.438*	1.07	1.06	*0.677*	1.09	1.09	*0.920*
	POST	0.87	0.84	*0.252*	0.91	0.90	*0.697*	0.94	0.92	*0.506*	0.98	0.94	*0.275*	1.02	1.00	*0.535*	1.06	1.02	*0.280*	1.10	1.05	*0.325*
	p value	***0.012****	*0.800*		*0.119*	*0.541*		*0.153*	*0.926*		*0.767*	*0.222*		*0.084*	*0.374*		*0.683*	***0.029****		*0.713*	*0.231*	
%VO_2 max (VO_2/VO_2_max)	PRE	57.99	55.78	*0.472*	64.91	63.16	*0.652*	72.39	70.59	*0.640*	81.96	77.99	*0.311*	88.06	86.72	*0.701*	91.89	93.30	*0.635*	97.29	96.14	*0.694*
	POST	55.16	52.49	*0.332*	63.07	58.82	*0.246*	71.39	67.06	*0.296*	78.81	76.66	*0.627*	86.33	85.69	*0.87*	90.71	90.78	*0.985*	96.08	94.67	*0.715*
	p value	***0.022****	***0.009****		*0.118*	***0.001****		*0.493*	***0.023****		***0.023****	*0.313*		*0.210*	*0.429*		*0.514*	*0.129*		*0.569*	*0.529*	
BLac (mM)	PRE	2.03	1.85	*0.332*	2.15	1.66	***0.030****	2.81	1.97	***0.019****	3.63	3.26	*0.704*	5.02	4.14	*0.205*	6.56	4.90	*0.097*	5.88	6.39	*0.986*
	POST	2.30	1.93	*0.207*	2.31	1.69	***0.026****	2.91	1.80	***0.022****	4.32	2.52	***0.042****	5.01	3.63	*0.169*	5.69	4.92	*0.172*	6.13	6.36	*0.446*
	p value	*0.232*	*0.713*		*0.387*	*0.600*		*0.542*	*0.653*		*0.212*	*0.469*		*0.950*	*0.954*		***0.041****	*0.945*		*0.646*	*0.691*	

Table 6. Metabolic data from gas collection during the discontinuous treadmill session. Measures are given for each stage and PRE/POST & EXP/CT means and p values are given as formatted in other tables. Values further than stage 7 (18Km/hr) were analyzed, but showed little additional data, and there was a substantial drop-off in the number of participants to reach these stages. **Bold underline** with an asterisk* denotes significance.

Stage		1			2			3			4			5			6			7		
Measure	Group	DRE	IRE	p value	DRE	IRE	p value	DRE	IRE	p value	DRE	IRE	p value	DRE	IRE	p value	DRE	IRE	p value	DRE	IRE	p value
VO_2 (mL_O2/Kg/min)	PRE	26.34	27.74	0.162	29.64	31.10	*0.130*	33.02	34.94	*0.068*	37.29	38.61	*0.205*	40.84	43.05	*0.080*	45.77	47.88	*0.132*	48.63	50.65	*0.372*
	POST	26.69	26.54	0.880	30.32	29.75	*0.625*	34.64	33.43	*0.267*	39.05	37.34	*0.110*	43.16	42.43	*0.568*	48.20	46.93	*0.403*	51.92	50.80	*0.617*
	p value	*0.361*	***0.014****		*0.060*	***0.004****		***0.001****	***0.007****		***0.001****	***0.026****		***0.000****	*0.232*		***0.000****	***0.043****		***0.002****	*0.800*	
VE (mL_Air/Kg/min)	PRE	468.38	513.93	*0.219*	574.67	602.23	*0.543*	659.80	696.06	*0.500*	822.18	806.26	*0.824*	940.09	950.44	*0.881*	1113.4	1159.05	*0.572*	1161.3	1347	*0.069*
	POST	473.93	521.56	*0.209*	570.07	604.37	*0.418*	673.35	686.45	*0.789*	807.69	772.30	*0.542*	960.28	932.10	*0.642*	1097.5	1098.51	*0.991*	1192.9	######	*0.301*
	p value	*0.630*	*0.582*		*0.693*	*0.878*		*0.364*	*0.59*		*0.535*	*0.233*		*0.360*	*0.469*		*0.482*	***0.007****		*0.483*	*0.187*	
VE/VO_2 (L_air/L_O_2)	PRE	17.31	18.42	*0.138*	18.91	19.28	*0.716*	19.44	19.84	*0.692*	21.42	20.84	*0.685*	22.47	22.02	*0.728*	23.32	24.18	*0.446*	23.84	26.61	*0.086*
	POST	17.55	19.61	***0.036****	18.56	20.32	*0.077*	19.19	20.51	*0.204*	20.41	20.67	*0.815*	22.04	21.96	*0.950*	22.40	23.43	*0.486*	22.41	31.65	*0.280*
	p value	*0.477*	***0.008****		*0.364*	***0.035****		*0.516*	*0.153*		***0.034****	*0.754*		*0.277*	*0.895*		*0.076*	*0.098*		*0.832*	*0.336*	
VCO_2 (mL_CO_2/Kg.min)	PRE	22.42	23.30	*0.512*	27.20	27.66	*0.747*	31.24	31.90	*0.687*	37.52	36.45	*0.579*	42.28	42.42	*0.937*	52.01	50.95	*0.768*	50.40	58.35	*0.054*
	POST	22.82	24.84	*0.190*	26.76	29.05	*0.180*	31.91	32.63	*0.699*	36.35	35.50	*0.671*	43.29	42.42	*0.680*	42.84	47.09	*0.350*	53.47	49.17	*0.441*
	p value	*0.510*	***0.046****		*0.627*	*0.210*		*0.510*	*0.549*		*0.455*	*0.611*		*0.491*	*0.999*		*0.175*	*0.337*		*0.642*	*0.101*	
RER (VCO_2/VO_2)	PRE	0.828	0.839	*0.547*	0.897	0.890	*0.777*	0.924	0.913	*0.640*	0.981	0.945	*0.184*	1.01	0.986	*0.270*	1.06	1.06	*0.767*	1.08	1.10	0.608
	POST	0.832	0.881	*0.055*	0.883	0.929	***0.039****	0.919	0.945	*0.306*	0.958	0.959	*0.982*	1.01	1.00	*0.893*	1.01	1.06	*0.169*	1.03	1.10	0.088
	p value	*0.729*	***0.007****		*0.314*	***0.024****		*0.695*	***0.029****		*0.119*	*0.403*		*0.817*	*0.272*		***0.009****	*0.742*		*0.167*	*0.710*	
%VO_2 max (VO_2/VO_2_max)	PRE	58.69	54.27	*0.149*	66.25	60.84	*0.161*	73.63	68.40	*0.172*	82.96	75.67	*0.059*	89.64	84.33	*0.119*	91.35	93.73	*0.420*	94.28	98.19	*0.173*
	POST	55.53	51.37	*0.131*	63.26	57.61	*0.124*	72.37	64.68	*0.06*	81.45	72.36	***0.033****	88.83	82.22	*0.08*	90.47	90.97	*0.903*	92.16	97.31	*0.176*
	p value	***0.007****	***0.032****		***0.013****	***0.024****		*0.351*	***0.029****		*0.218*	***0.031****		*0.511*	*0.148*		*0.624*	*0.096*		*0.418*	*0.651*	
BLac (mM)	PRE	1.85	2.08	*0.232*	1.96	1.84	*0.62*	2.64	1.92	*0.085*	3.61	2.46	*0.171*	4.86	3.62	*0.208*	4.85	5.91	*0.449*	4.38	6.93	*0.074*
	POST	2.35	1.77	***0.043****	2.11	1.91	*0.466*	2.63	1.96	*0.193*	3.94	2.41	*0.056*	4.76	3.71	*0.221*	4.97	5.24	*0.78*	5.75	6.24	*0.740*
	p value	***0.006****	*0.132*		*0.350*	*0.681*		*0.961*	*0.813*		*0.594*	*0.948*		*0.878*	*0.877*		*0.801*	*0.071*		*0.254*	*0.445*	

Table 7. Sham-grouping metabolic data. Data displayed similar to table 6, only IRE/DRE groupings are substituted for EXP/CTL. Aside from the difference in VO_2 consumption, most differences were seen at the slow-moderate speeds. Also of note is the use of non-traditional units for several measures VE and VCO_2 are typically reported in absolute terms (L/min), but due to the variance in body size, all non-ratio respiratory measurements were scaled to weight.

Measure	Group	CTL	EXP	*p value*
Active VL stiffness	PRE	7.21	6.59	*0.360*
	POST	7.71	7.26	*0.619*
	p value	*0.281*	*0.155*	
Passive VL stiffness	PRE	13.02	13.18	*0.765*
	POST	17.86	16.05	*0.051*
	p value	***<0.001****	***<0.001****	
Absolute Stiffness Difference	PRE	5.81	6.59	*0.344*
Passive to Active VL	POST	10.15	8.80	*0.326*
	p value	***0.000****	***0.002****	
%Stiffness % Difference	PRE	44.00	49.90	*0.272*
Passive to Active VL	POST	55.90	54.40	*0.808*
	p value	***0.001****	*0.141*	
Active *gastroc* stiffness	PRE	9.50	9.32	*0.874*
	POST	9.99	8.48	*0.212*
	p value	*0.571*	*0.338*	
Passive *gastroc* stiffness	PRE	15.02	15.04	*0.977*
	POST	21.27	19.64	*0.164*
	p value	***<0.001****	***<0.001****	
Absolute Stiffness Difference	PRE	5.52	5.71	*0.849*
Passive to Active *gastroc*	POST	11.27	11.15	*0.944*
	p value	***<0.001****	***<0.001****	
%Stiffness % Difference	PRE	36.60	38.60	*0.759*
Passive to Active *gastroc*	POST	51.90	56.20	*0.529*
	p value	***0.003****	***0.001****	
Active *soleus* stiffness	PRE	7.18	6.14	*0.216*
	POST	9.18	7.82	*0.0325*
	p value	***0.004****	***0.012****	
Passive *soleus* stiffness	PRE	11.89	11.95	*0.935*
	POST	16.24	15.33	*0.423*
	p value	***<0.001****	***<0.001****	
Absolute Stiffness Difference	PRE	4.71	5.80	*0.120*
Passive to Active *soleus*	POST	7.10	7.51	*0.728*
	p value	***0.004****	***0.030****	
%Stiffness % Difference	PRE	40.00	48.80	*0.117*
Passive to Active *soleus*	POST	43.60	49.50	*0.420*
	p value	*0.368*	*0.858*	
Active *Achilles* stiffness	PRE	2.22	2.33	*0.796*
	POST	2.27	2.22	*0.792*
	p value	*0.881*	*0.778*	
Passive *Achilles* stiffness	PRE	11.59	12.31	*0.411*
	POST	11.34	11.06	*0.870*
	p value	*0.831*	*0.295*	
Absolute Stiffness Difference	PRE	9.37	9.98	*0.578*
Passive to Active *Achilles*	POST	9.07	8.84	*0.893*
	p value	*0.796*	*0.343*	
%Stiffness % Difference	PRE	79.90	80.10	*0.960*
Passive to Active *Achilles*	POST	77.60	79.50	*0.488*
	p value	*0.533*	*0.852*	

Table 8. Muscle stiffness measures for CTL and EXP PRE/POST. Significance denoted as previously

		VO_{2MAX}		OBLA speed		OBLA VO_2		3 Km TT		$\%VO_2$		VE		VE/VO2		RER	
		r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Total Titin	pre	-0.44	0.037					0.44	0.036	(avg stage 2-4)		(stage 4)				0.44	0.038
	post	-0.52	0.027	-0.59	0.009	-0.61	0.007	0.70	0.001	0.57	0.013	0.51	0.03	(avg stage 2,3,6)		(stage 4)	
	delta													0.17	0.0243	(stage 2)	
	% diff													-0.72	0.023	-0.59	0.044
T1	pre							0.47	0.023	(avg stage 2-4)		(stage 4)				0.48	0.021
	post	-0.49	0.041	-0.56	0.016			0.66	0.003	0.52	0.028	0.48	0.043	(avg stage 2,3,6)		(stage4/ stage6)	
	delta													-0.67	0.0363	-0.80	0.043
	% diff													-0.71	0.0217	-0.82	0.026
T2	pre									(avg stage 2-5)		(stage 4)				(stage 5)	
	post	-0.50	0.033	-0.56	0.016	-0.60	0.009	0.69	0.001	0.62	0.0125	0.48	0.044	(stage 3)		0.49	0.047
	delta													-0.69	0.031		
	% diff													-0.80	0.002		
MHC	pre									(avg stage 6,7)		(avg stage 3,4)					
	post									-0.71	0.013	0.50	0.037				
	delta																
	% diff																
T1 : T2	pre	-0.54	0.008	-0.42	0.045			0.55	0.007							0.56	0.0117
	post															(avg stage 1,3,4,5)	
	delta																
	% diff																
T:MHC	pre							0.42	0.044	(avg stage 3,4)						0.43	0.04
	post	-0.48	0.047					0.60	0.009	0.49	0.038			(avg stage 3,4,6)		(stage 4)	
	delta													-0.07	0.019	-0.62	-0.617
	% diff													-0.53	0.0257	(avg stage 2,6,7)	

Table 9a. Correlation matrix for all muscle protein data as it relates to the other markers. For simplicity, only statistically significant correlations are provided Also in the interest of brevity, associations with data from the gas analysis were pooled such that all stages for which a significant correlation existed were averaged together (sometimes could be anywhere from 1-4+ stages). The pertinent stages are given within the grid above/below the r and p values.

		VO_2_1		VO_2_2		VO_2_3		VO_2_4		VO_2_5		VO_2_6		VO_2_7		VL active stiffn		VL passive stiff		VL stff. diff.		VL stiff. %d	
		r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Total Titin	pre																						
	post																						
	delta	0.3+	NS	0.3+	NS	0.62	0.031	0.78	0.003	0.72	0.013	0.58	NS	0.76	0.08								
	% diff					0.44	0.048	0.73	0.007	0.66	0.026			0.79	0.06	0.66	0.02						
T1	pre					-0.47	0.049																
	post																						
	delta					0.68	0.014	0.77	0.004	0.73	0.01					0.63	0.027						
	% diff	0.59	0.046					0.74	0.006	0.71	0.014	0.60	0.158			0.73	0.009						
T2	pre																						
	post																						
	delta							0.62	0.031														
	% diff																						
MHC	pre																						
	post																						
	delta									0.69	0.018												
	% diff																			-0.60	0.038	-0.69	0.013
T1 : T2	pre							-0.51	0.029	-0.51	0.016												
	post																						
	delta																						
	% diff																						
T:MHC	pre					-0.45	0.031																
	post																						
	delta							0.47	0.039					0.67	0.049								
	% diff							0.67	0.017							-0.45	0.045						

Table 9b. Muscle Protein Correlations: RE and Muscle Stiffness (in the VL). N = 12.

Correlations were carried out using muscle values PRE with PRE measures, POST with POST measures, and as the change from PRE to POST (delta) and as this change scaled as a percent difference PRE to POST (% diff) with delta measures. PRE data includes all 25 original participants.

VO_2 on stages		1		2		3		4		5		6		7	
Measure		r	p	r	p	r	p	r	p	r	p	r	p	r	p
RUNNING PERFROMANCE	PRE	-0.17	0.413	0.02	0.912	-0.24	0.244	-0.27	0.196	▼-0.52	0.009	-0.30	0.220	-0.33	0.247
(3Km TT time)	DELTA	0.22	0.329	0.33	0.135	0.11	0.623	0.14	0.528	-0.07	0.765	0.18	0.511	0.17	0.607
Anaerobic Power	PRE	0.19	0.372	0.09	0.686	0.20	0.338	0.22	0.280	0.38	0.068	0.12	0.627	0.23	0.430
(VJ Height)	DELTA	-0.14	0.543	-0.23	0.302	0.00	0.994	0.32	0.142	0.07	0.766	0.13	0.635	0.37	0.261
Flexibility	PRE	0.38	0.059	0.26	0.207	0.23	0.275	▼0.43	0.030	0.39	0.060	< 0.0 1	0.995	0.19	0.516
(Sit-and-Reach distance)	DELTA	-0.07	0.766	-0.12	0.608	0.30	0.182	0.11	0.631	-0.06	0.802	-0.03	0.925	0.54	0.088
Body Composition	PRE	-0.03	0.869	-0.17	0.416	-0.17	0.428	-0.01	0.954	0.09	0.679	0.14	0.572	0.08	0.796
% Body Fat	DELTA														
BMI	PRE	▼-0.48	0.015	-0.29	0.156	▼-0.45	0.024	▼-0.59	0.002	▼-0.60	0.002	-0.25	0.304	-0.26	0.376
	DELTA	-0.31	0.162	-0.11	0.639	-0.35	0.109	-0.08	0.720	0.12	0.614	-0.08	0.783	-0.45	0.166
VO_{2MAX}	PRE	▼0.46	0.022	0.29	0.156	▼0.48	0.016	▼0.57	0.003	▼0.76	<0.001	▼0.72	<0.001	▼0.84	<0.001
	DELTA	0.34	0.126	0.41	0.057	0.34	0.126	▼0.53	0.012	▼0.60	0.004	▼0.51	0.046	0.58	0.064
Speed @ OBLA	PRE	-0.04	0.832	-0.19	0.351	-0.17	0.428	-0.02	0.931	0.09	0.682	0.12	0.629	0.05	0.856
	DELTA	-0.05	0.836	-0.18	0.416	-0.26	0.251	0.05	0.830	-0.03	0.914	0.21	0.445	-0.42	0.197
VO_2 @ OBLA	PRE	▼0.42	0.035	0.23	0.272	▼0.45	0.023	▼0.44	0.028	▼0.64	0.001	▼0.50	0.028	0.46	0.099
	DELTA	0.28	0.20	0.32	0.149	0.25	0.254	▼0.63	0.002	▼0.54	0.011	▼0.76	0.00	0.15	0.66
% VO_2 @ OBLA	PRE	-0.06	0.76	-0.11	0.614	-0.04	0.863	-0.22	0.287	-0.24	0.259	-0.28	0.251	-0.43	0.123
	DELTA	-0.01	0.97	-0.06	0.800	-0.04	0.877	0.15	0.498	0.01	0.975	0.33	0.21	-0.28	0.41
VE (stage matched)	PRE	△0.74	p<0.001	△0.69	p<0.001	△0.74	p<0.001	△0.51	0.009	△0.52	0.010	△0.59	0.007	△0.73	0.003
	DELTA	0.23	0.309	-0.05	0.843	△0.55	0.009	0.26	0.239	0.29	0.202	0.16	0.554	0.07	0.845
VE/VO2 (stage matched)	PRE	△0.42	0.037	0.38	0.059	△0.52	0.008	0.25	0.221	0.11	0.623	0.22	0.376	0.27	0.347
	DELTA	▼-0.54	0.010	▼-0.43	0.044	-0.28	0.213	-0.13	0.572	0.16	0.48	0.10	0.701	0.75	0.11
RER (stage matched)	PRE	0.21	0.322	0.04	0.839	0.08	0.701	-0.06	0.787	-0.34	0.102	-0.19	0.438	-0.14	0.637
	DELTA	▼-0.77	<0.001	-0.34	0.126	-0.12	0.609	-0.20	0.37	-0.12	0.608	-0.42	0.105	△0.782	0.004
VCO_2 (stage matched)	PRE	△0.74	<0.001	△0.66	<0.001	△0.68	<0.001	△0.54	0.006	△0.57	0.003	-0.38	0.197	-0.57	0.140
	DELTA	0.16	0.480	0.12	0.591	△0.61	0.003	0.27	0.228	0.10	0.683	0.14	0.608	-0.20	0.556
% of VO_{2MAX} (stage matched)	PRE	0.24	0.256	0.32	0.119	0.14	0.521	-0.01	0.972	-0.33	0.113	-0.04	0.863	-0.16	0.593
(relative fitness)	DELTA	△0.48	0.025	0.36	0.096	△0.56	0.007	0.39	0.073	0.18	0.446	0.27	0.305	0.16	0.643
[Blood Lactate] (stage matched)	PRE	0.24	0.252	0.25	0.225	-0.10	0.630	-0.38	0.064	-0.02	0.944	0.22	0.407	0.46	0.299
	DELTA	0.30	0.169	-0.13	0.56	0.09	0.703	-0.42	0.061	0.29	0.214	0.50	0.083	0.56	0.330

Table 10a. Correlation matrix for O_2 consumption on all individual to performance and all other metabolic measures. Data was analyzed both cross-sectionally using PRE values and longitudinally for correlations in the changes PRE-POST. Values obtained at each stage are correlated only to the corresponding stages VO_2 (stage matched). All values are included, both significant and non-significant. n-values diminish as you read to the right because of participants inability to complete faster stages. Significant interactions are demarcated as follows:

△Significant correlation defined by an inferior score for this measure associating with a superior value for RE.

▼Significant correlation defined by a superior score for this measure associating with a superior value for RE.

- Active but not passive muscle stiffness and higher [protein] are considered superior for tables 10b and c below with rankings as follows: Titin > MHC & T1 > T2.

VO_2 on stages		1		2		3		4		5		6		7	
Measure		r	p	r	p	r	p	r	p	r	p	r	p	r	p
Vastus Lateralis active stiffness	PRE	-0.29	0.162	-0.23	0.275	-0.32	0.114	-0.22	0.285	-0.30	0.158	-0.06	0.795	-0.09	0.750
	DELTA	-0.15	0.508	0.15	0.515	0.28	0.214	0.39	0.072	△0.45	0.039	0.17	0.530	0.40	0.222
Vastus Lateralis passive stiffness	PRE	-0.19	0.357	-0.20	0.335	-0.18	0.389	-0.10	0.621	-0.03	0.904	-0.13	0.593	-0.15	0.619
	DELTA	0.15	0.495	0.17	0.461	0.08	0.731	-0.15	0.512	-0.30	0.184	-0.13	0.636	0.04	0.906
Vastus Lateralis stiffness diff.	PRE	0.11	0.603	0.05	0.811	0.15	0.478	0.12	0.581	0.25	0.239	-0.04	0.875	-0.04	0.901
	DELTA	0.19	0.406	0.00	0.983	-0.15	0.520	-0.37	0.091	△-0.53	0.013	-0.20	0.465	-0.01	0.978
Vastus Lateralis stiffness % diff.	PRE	0.21	0.322	0.13	0.531	0.24	0.258	0.16	0.431	0.30	0.156	0.02	0.930	0.04	0.893
	DELTA	0.18	0.412	-0.06	0.796	-0.20	0.383	-0.34	0.121	△-0.53	0.013	-0.21	0.442	-0.24	0.480
Gastrocnemius active stiffness	PRE	▼-0.43	0.033	-0.29	0.157	▼-0.52	0.008	▼-0.49	0.013	▼-0.56	0.004	-0.29	0.225	-0.28	0.332
	DELTA	0.22	0.323	△0.51	0.016	0.28	0.215	0.01	0.953	0.22	0.335	0.37	0.153	0.45	0.164
Gastrocnemius passive stiffness	PRE	-0.30	0.148	-0.18	0.384	-0.29	0.166	-0.38	0.062	-0.36	0.087	-0.13	0.594	-0.12	0.687
	DELTA	▼0.55	0.008	0.24	0.278	-0.05	0.836	0.06	0.793	-0.17	0.462	-0.07	0.791	-0.52	0.098
Gastrocnemius stiffness diff.	PRE	0.34	0.095	0.24	0.244	▼0.44	0.026	0.38	0.065	▼0.47	0.020	0.20	0.411	0.20	0.494
	DELTA	0.25	0.255	-0.20	0.370	-0.23	0.309	0.08	0.734	-0.26	0.256	-0.25	0.346	△-0.74	0.010
Gastrocnemius stiffness % diff.	PRE	0.37	0.065	0.27	0.190	▼0.48	0.015	▼0.41	0.043	▼0.50	0.013	0.26	0.287	0.26	0.377
	DELTA	0.01	0.982	-0.30	0.178	-0.13	0.570	0.11	0.635	-0.21	0.366	-0.32	0.230	-0.56	0.073
Soleus active stiffness	PRE	-0.31	0.131	-0.13	0.520	-0.16	0.434	-0.16	0.454	-0.31	0.139	-0.36	0.128	-0.33	0.251
	DELTA	△0.50	0.018	△0.50	0.018	0.38	0.083	0.20	0.365	-0.04	0.869	0.23	0.386	-0.09	0.798
Soleus passive stiffness	PRE	-0.12	0.555	-0.03	0.869	-0.10	0.632	-0.12	0.553	-0.05	0.810	-0.03	0.893	0.00	0.997
	DELTA	-0.02	0.929	0.17	0.451	0.01	0.967	0.03	0.886	-0.07	0.779	-0.28	0.291	-0.57	0.068
Soleus stiffness difference	PRE	0.25	0.219	0.13	0.540	0.10	0.628	0.07	0.733	0.32	0.123	0.38	0.110	0.31	0.285
	DELTA	△-0.43	0.044	-0.19	0.389	-0.23	0.294	-0.03	0.890	0.10	0.662	-0.21	0.434	-0.24	0.475
Soleus stiffness % diff.	PRE	0.29	0.161	0.13	0.542	0.14	0.507	0.13	0.538	0.36	0.088	0.42	0.072	0.38	0.180
	DELTA	△-0.43	0.046	-0.29	0.186	-0.28	0.203	-0.11	0.624	0.05	0.823	-0.26	0.337	-0.25	0.450
Achilles Tendon active stiffness	PRE	-0.15	0.484	-0.10	0.636	0.00	0.987	-0.09	0.682	-0.03	0.901	-0.36	0.126	-0.19	0.514
	DELTA	▼-0.46	0.031	-0.27	0.227	-0.04	0.860	-0.14	0.525	-0.06	0.792	-0.37	0.157	-0.06	0.872
Achilles Tendon passive stiffness	PRE	-0.06	0.793	-0.02	0.911	-0.04	0.851	-0.01	0.968	-0.05	0.805	-0.04	0.858	-0.06	0.847
	DELTA	0.02	0.926	0.26	0.235	0.24	0.276	-0.01	0.950	-0.14	0.534	0.21	0.424	-0.27	0.413
Achilles Tendon stiffness diff.	PRE	0.01	0.954	0.02	0.926	-0.03	0.874	0.03	0.899	-0.03	0.891	0.12	0.628	0.04	0.891
	DELTA	0.16	0.477	0.34	0.127	0.24	0.274	0.03	0.893	-0.12	0.590	0.39	0.138	-0.15	0.654
Achilles Tendon stiffness % diff.	PRE	0.10	0.638	0.06	0.781	-0.02	0.912	0.06	0.765	0.00	0.996	0.29	0.232	0.13	0.655
	DELTA	0.38	0.078	0.26	0.244	0.04	0.858	0.06	0.775	-0.02	0.947	0.40	0.129	-0.02	0.964

Table 10b. RE Correlation Matrix continued…

VO_2 on stages		1		2		3		4		5		6		7	
Measure		r	p	r	p	r	p	r	p	r	p	r	p	r	p
Total Titin	PRE	-0.29	0.176	-0.29	0.181	-0.39	0.064	-0.25	0.244	-0.33	0.132	-0.36	0.159	-0.33	0.264
	DELTA	0.37	0.232	0.28	0.376	0.49	0.105	▼0.73	0.007	▼0.66	0.026	0.58	0.174	0.73	0.098
Total T1	PRE	-0.32	0.132	-0.31	0.157	△-0.41	0.049	-0.31	0.155	-0.40	0.065	-0.45	0.071	-0.42	0.156
	DELTA	0.37	0.232	0.28	0.376	0.49	0.105	▼0.73	0.007	▼0.66	0.026	0.58	0.174	0.73	0.098
Total T2	PRE	-0.12	0.596	-0.17	0.428	-0.23	0.281	0.00	0.986	-0.02	0.940	0.05	0.835	0.01	0.970
	DELTA	0.21	0.513	0.16	0.627	0.04	0.890	0.45	0.147	0.18	0.606	0.27	0.557	0.17	0.741
Total MHC	PRE	-0.24	0.270	-0.23	0.283	-0.32	0.133	-0.26	0.222	-0.31	0.164	-0.28	0.268	-0.19	0.535
	DELTA	0.25	0.425	-0.03	0.923	0.49	0.108	0.46	0.134	▼0.68	0.021	0.28	0.547	0.65	0.158
T1 : T2 ratio	PRE	-0.19	0.385	-0.13	0.551	-0.32	0.141	-0.37	0.082	▼-0.51	0.016	-0.47	0.057	-0.35	0.240
	DELTA	0.05	0.882	-0.15	0.635	-0.21	0.518	-0.17	0.591	0.01	0.967	-0.10	0.839	-0.21	0.696
Titin : MHC ratio	PRE	-0.33	0.128	-0.36	0.096	△-0.45	0.031	-0.30	0.166	-0.38	0.080	-0.42	0.095	-0.44	0.128
	DELTA	0.36	0.249	0.27	0.394	0.34	0.273	▼0.67	0.017	0.53	0.092	0.48	0.271	0.72	0.107

Table 10c. RE Correlation Matrix continued. Only includes participants completing both PRE & POST testing sessions with protein data (n=12)

.

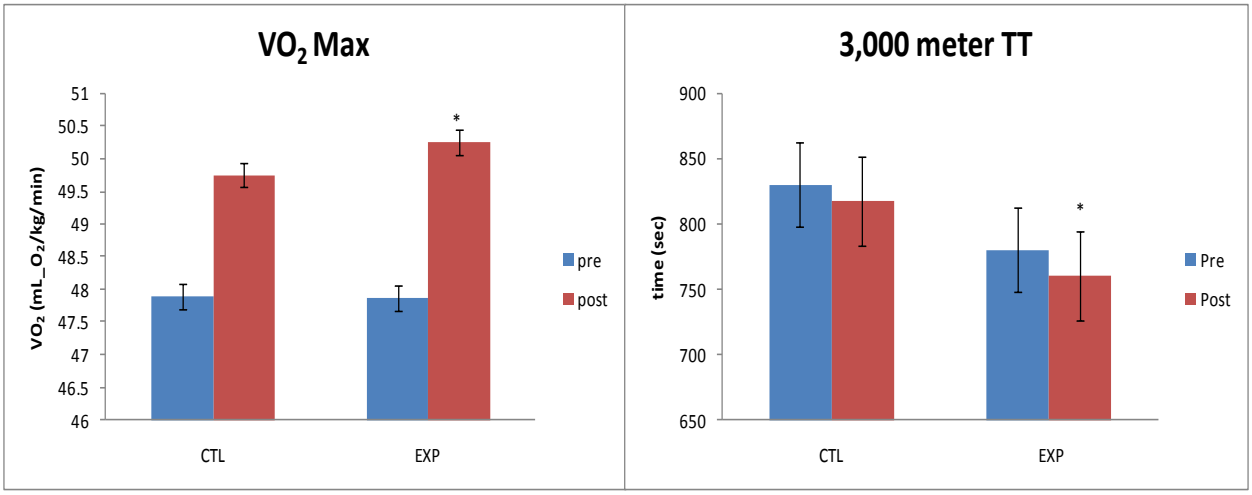


Figure 1. Changes in Fitness (as per VO_{2MAX} and performance in EXP/CTL groups.
*Significant changes marked with asterisk.

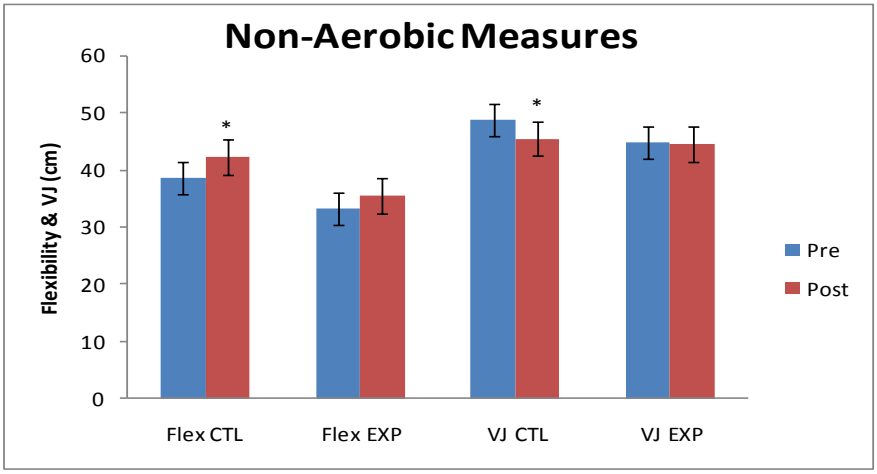


Figure 2. Changes in EXP/CTL groups for non-aerobic fitness data. *Significant data marked with an asterisk.

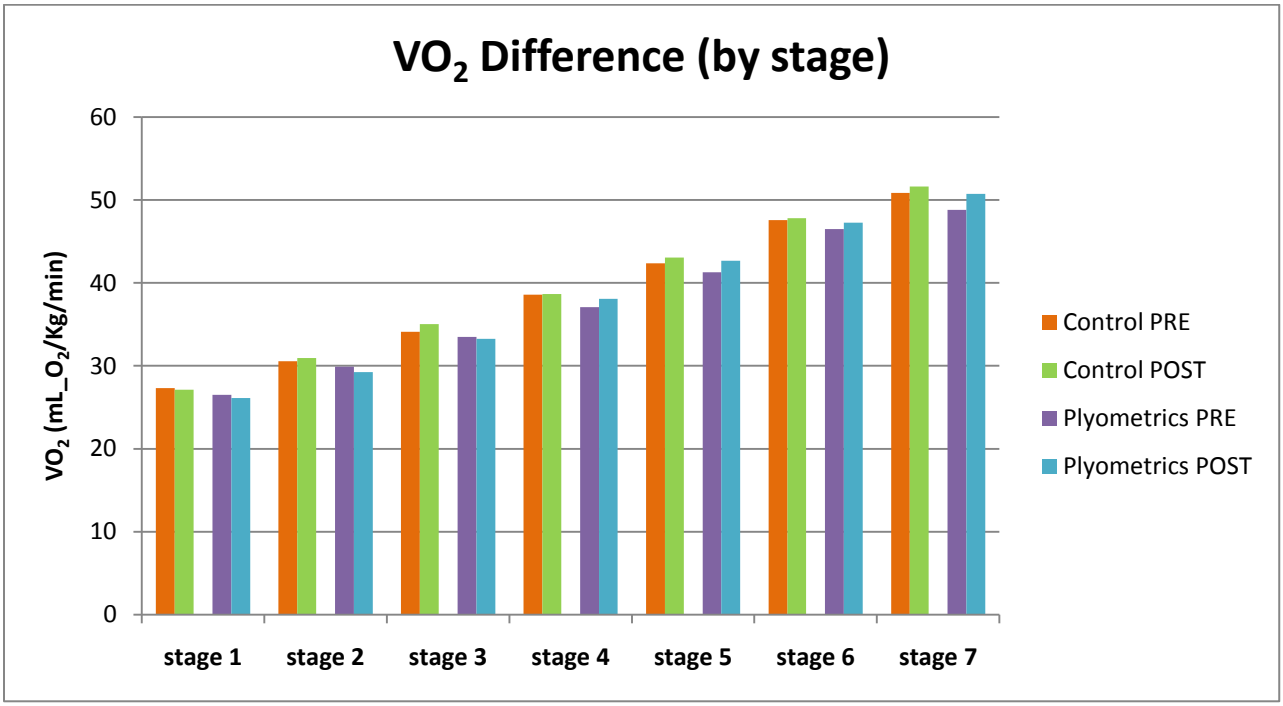


Figure 3. VO_2 consumption for CTL & EXP at PRE & POST. No significant differences were found in either group. Values nearly identical PRE-POST and between groups for all stages.

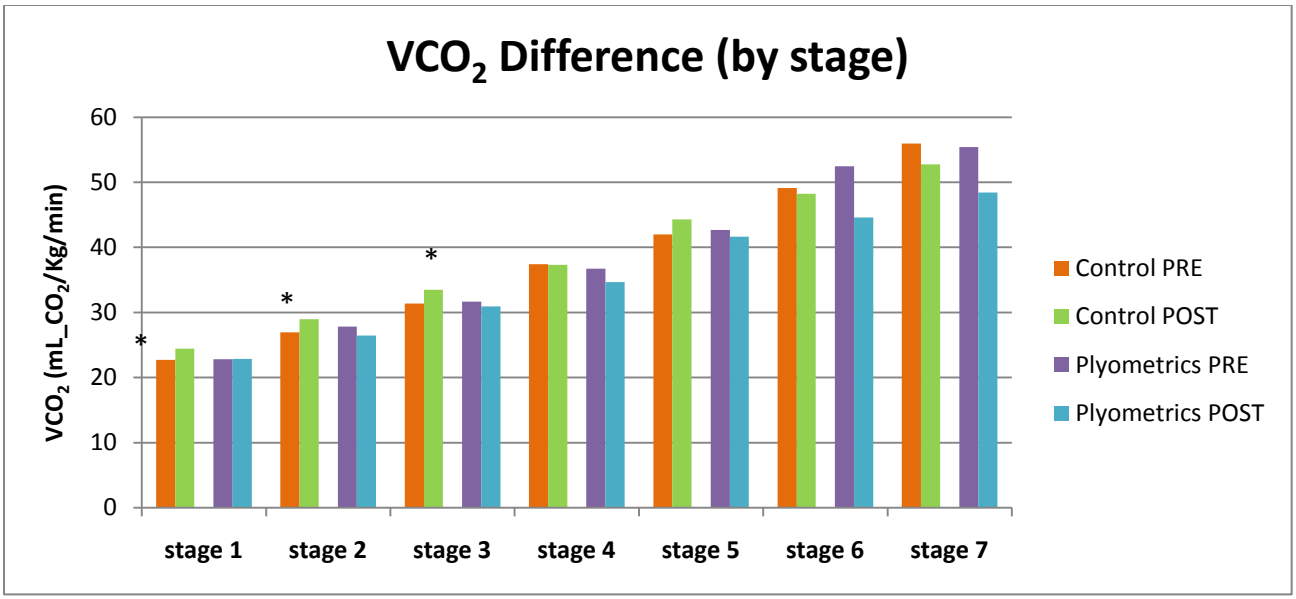


Figure 4. VCO_2 production for CTL & EXP at PRE & POST. Significant differences denoted by an asterisk.* Note that CTL showed variability in VCO_2 production (and in fact significant increases for the slower stages) while EXP continually decreased.

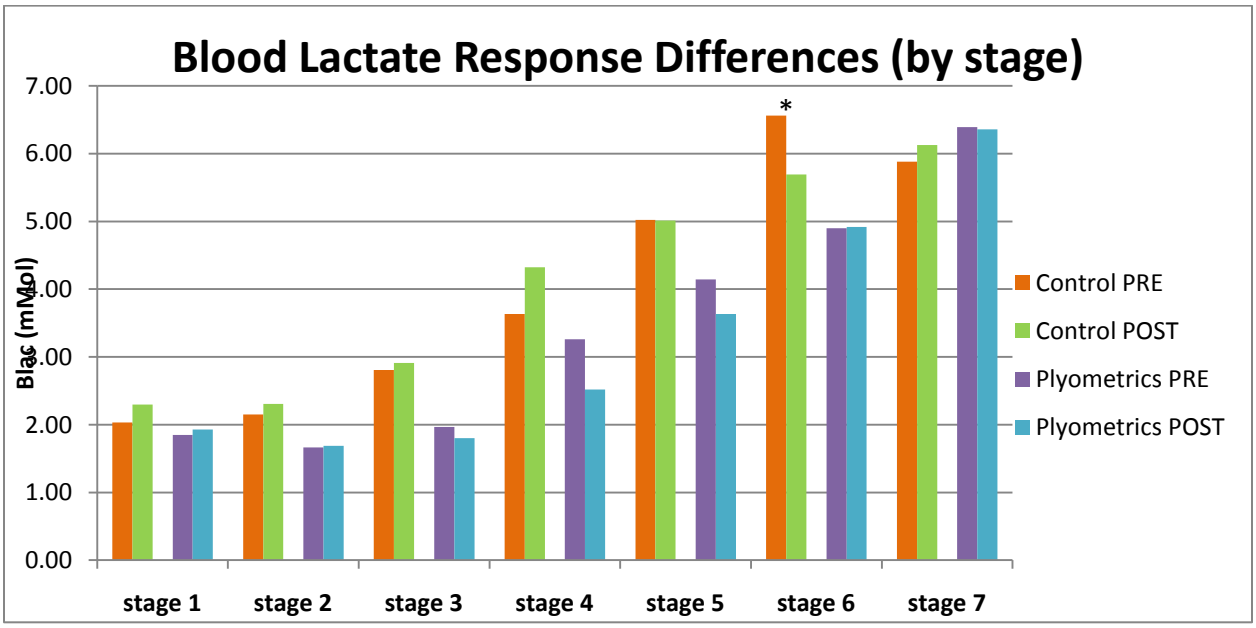
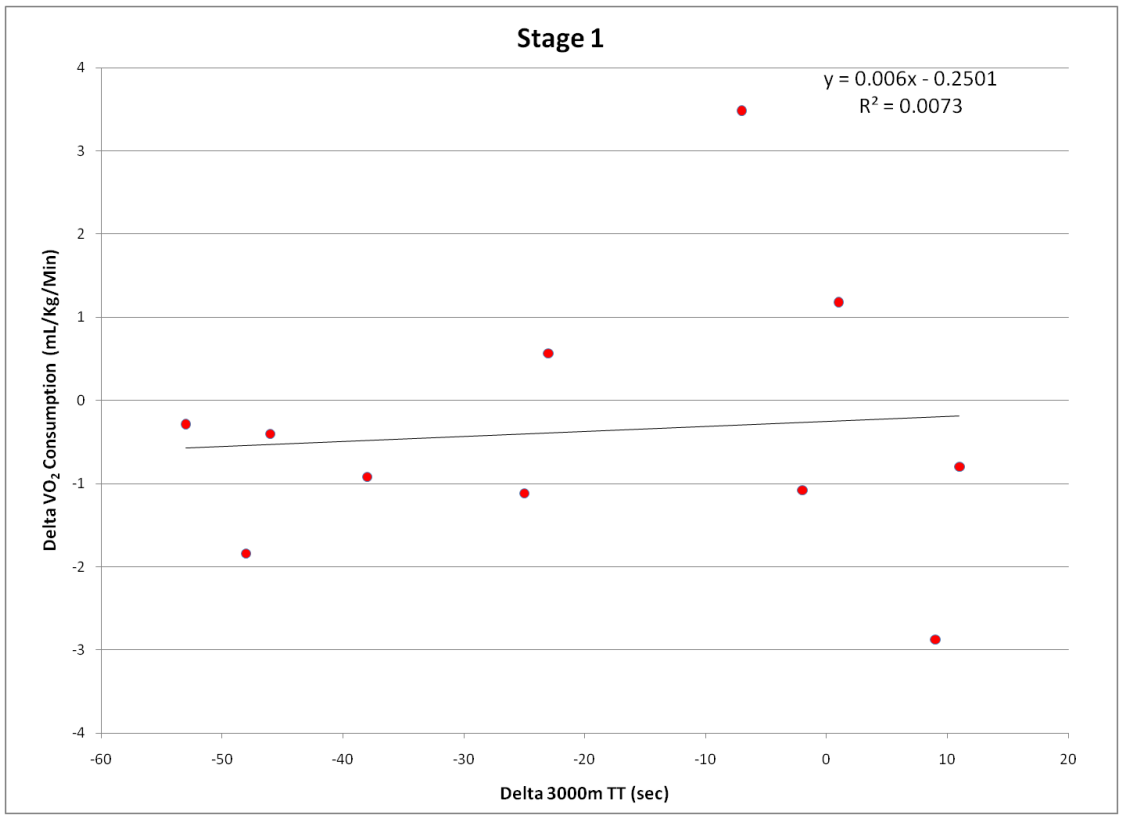
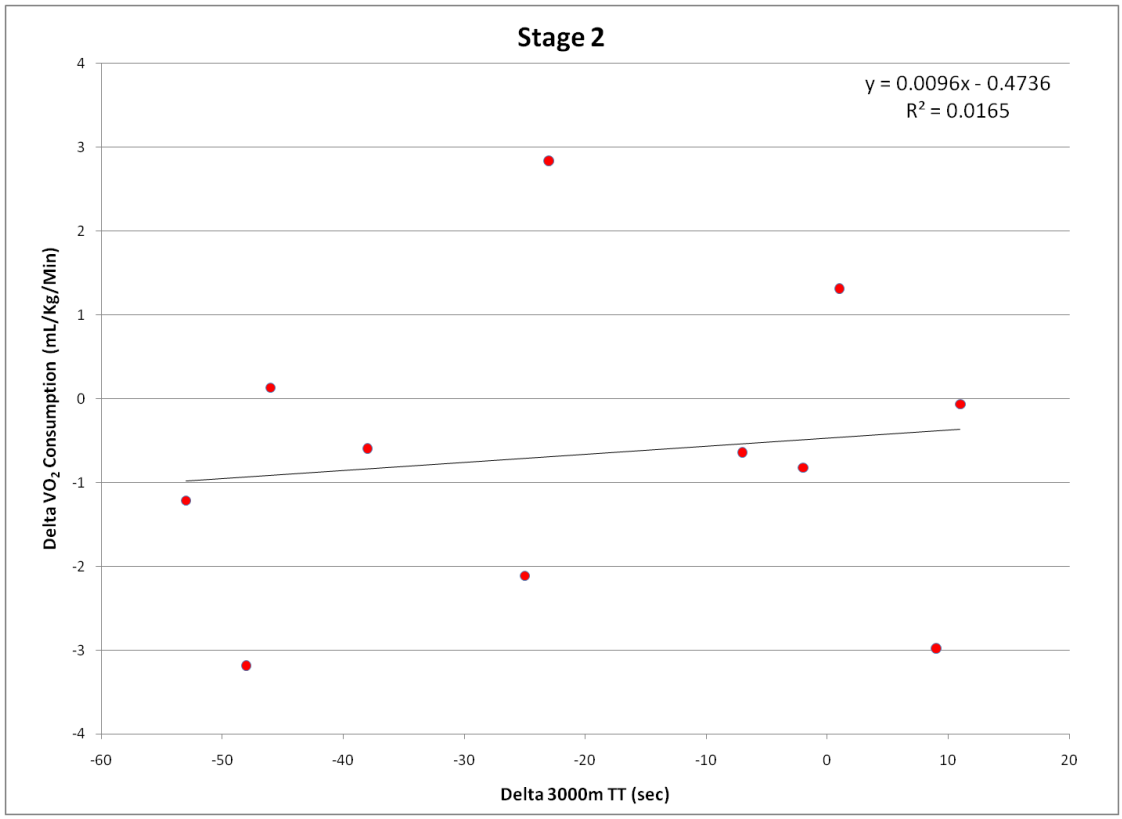


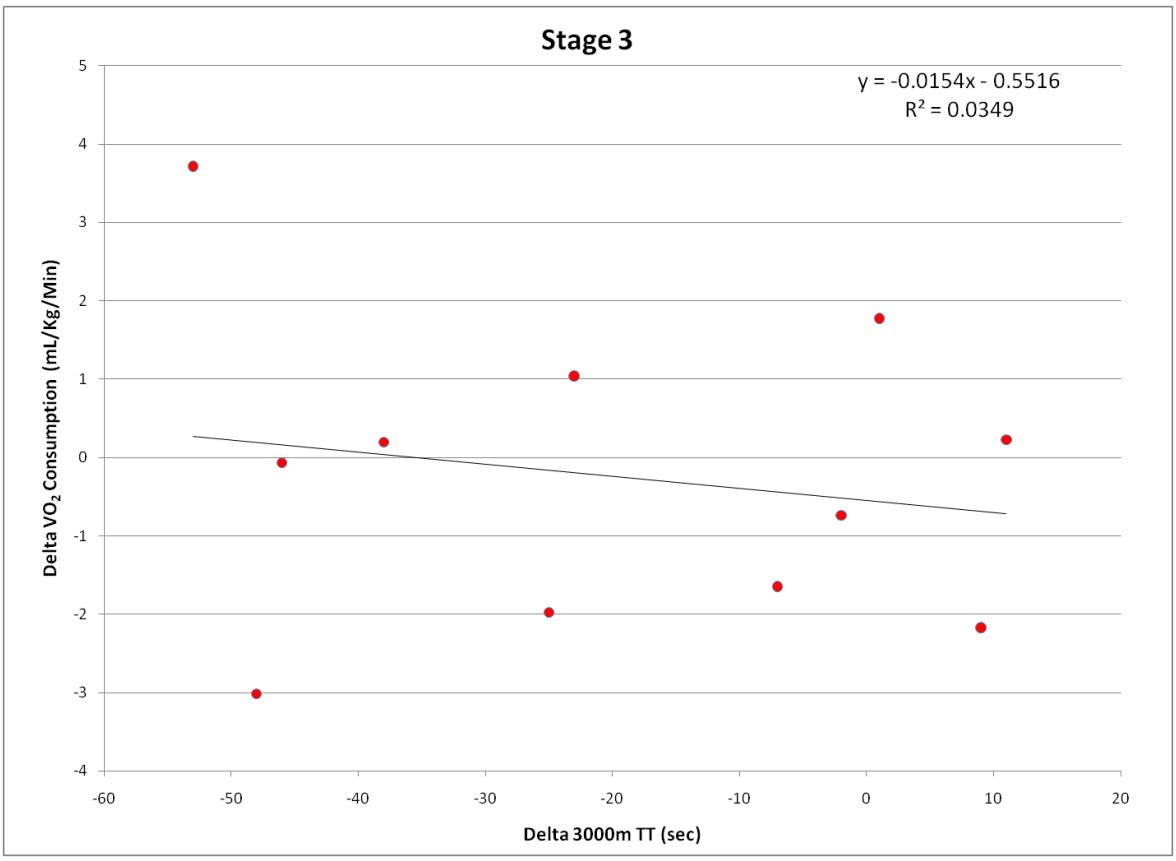
Figure 5. Blac accumulation for CTL & EXP at PRE & POST. Only significance difference identified in CTL for stage 6. However, note the trend for a decrease in EXP, particularly for moderate speeds, with variability in CTL by stage.


Stage 1
y = 0.006x - 0.2501
R² = 0.0073
Delta VO2 Consumption (mL/Kg/Min)
Delta 3000m TT (sec)
4
3
2
1
0
-1
-2
-3
-4
-60
-50
-40
-30
-20
-10
0
10
20

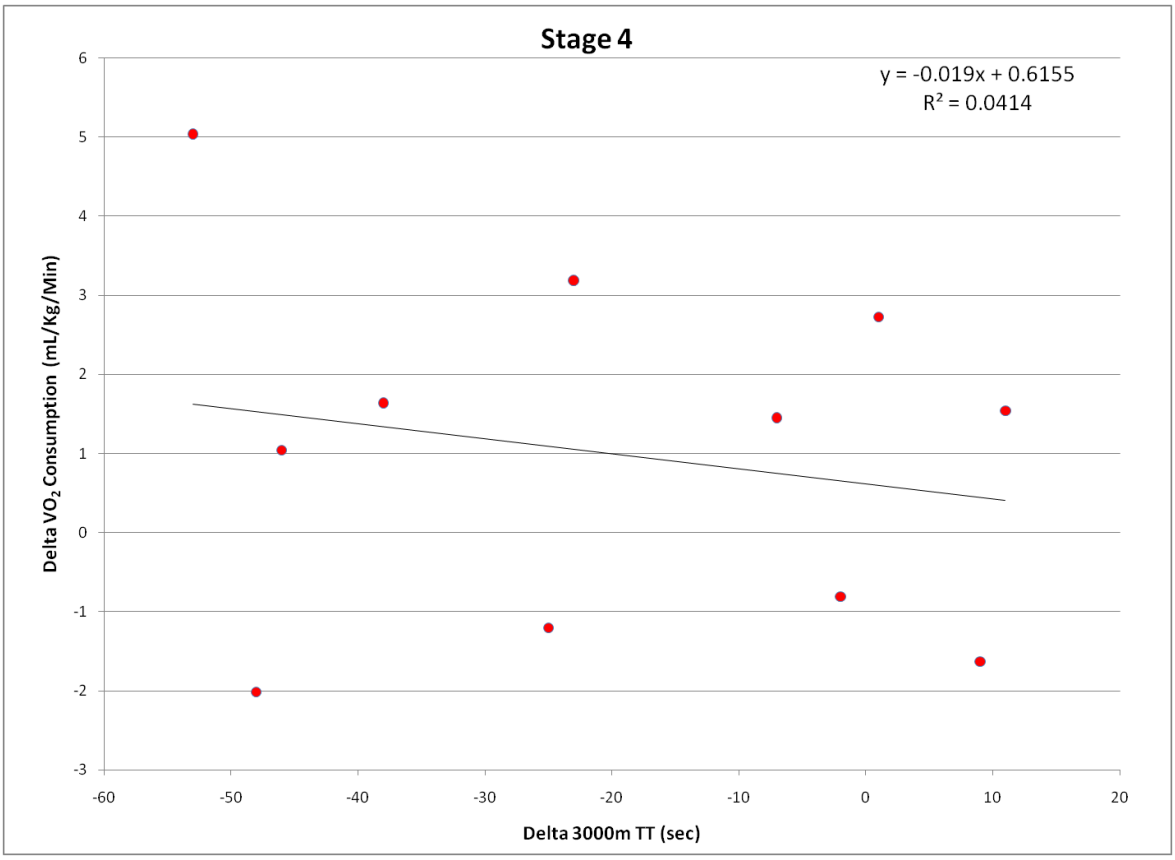
a


Stage 2
y = 0.0096x - 0.4736
R² = 0.0165
Delta VO2 Consumption (mL/Kg/Min)
Delta 3000m TT (sec)
4
3
2
1
0
-1
-2
-3
-4
-60
-50
-40
-30
-20
-10
0
10
20

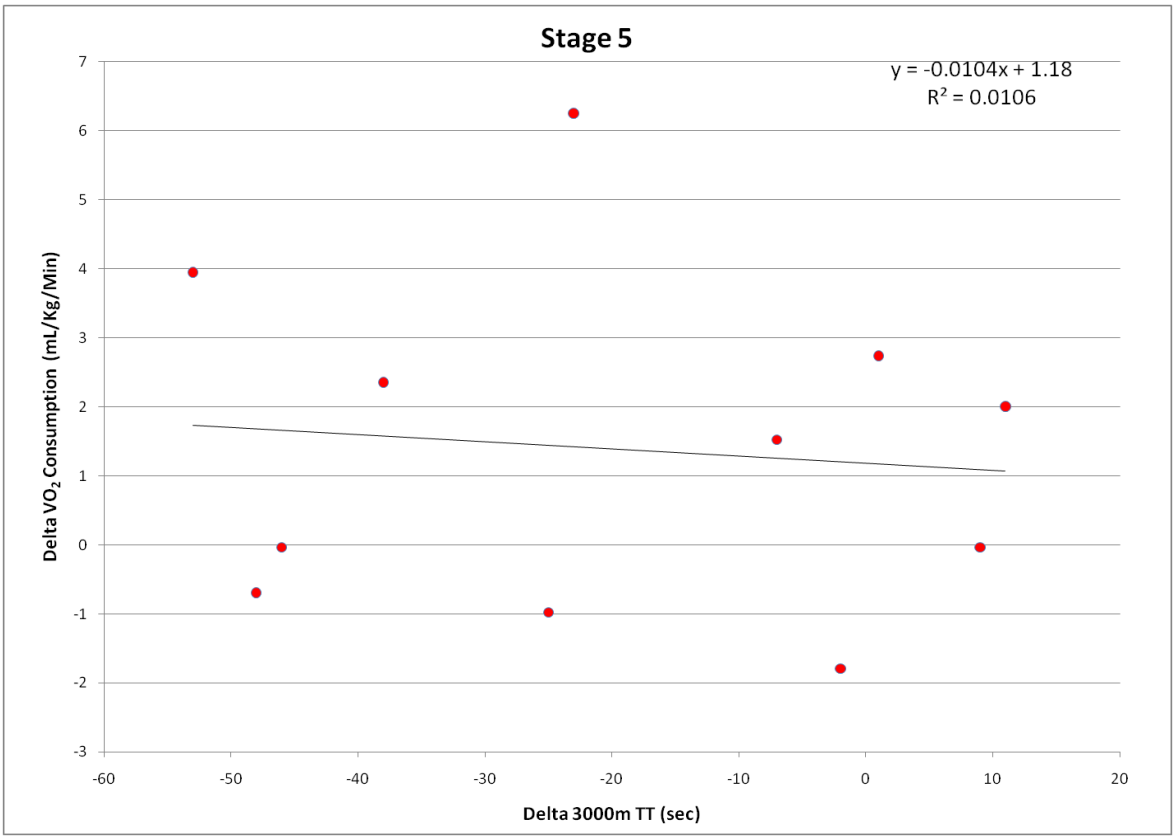
b


Stage 3
y = -0.0154x - 0.5516
R² = 0.0349
Delta VO2 Consumption (mL/Kg/Min)
Delta 3000m TT (sec)
5
4
3
2
1
0
-1
-2
-3
-4
-60
-50
-40
-30
-20
-10
0
10
20

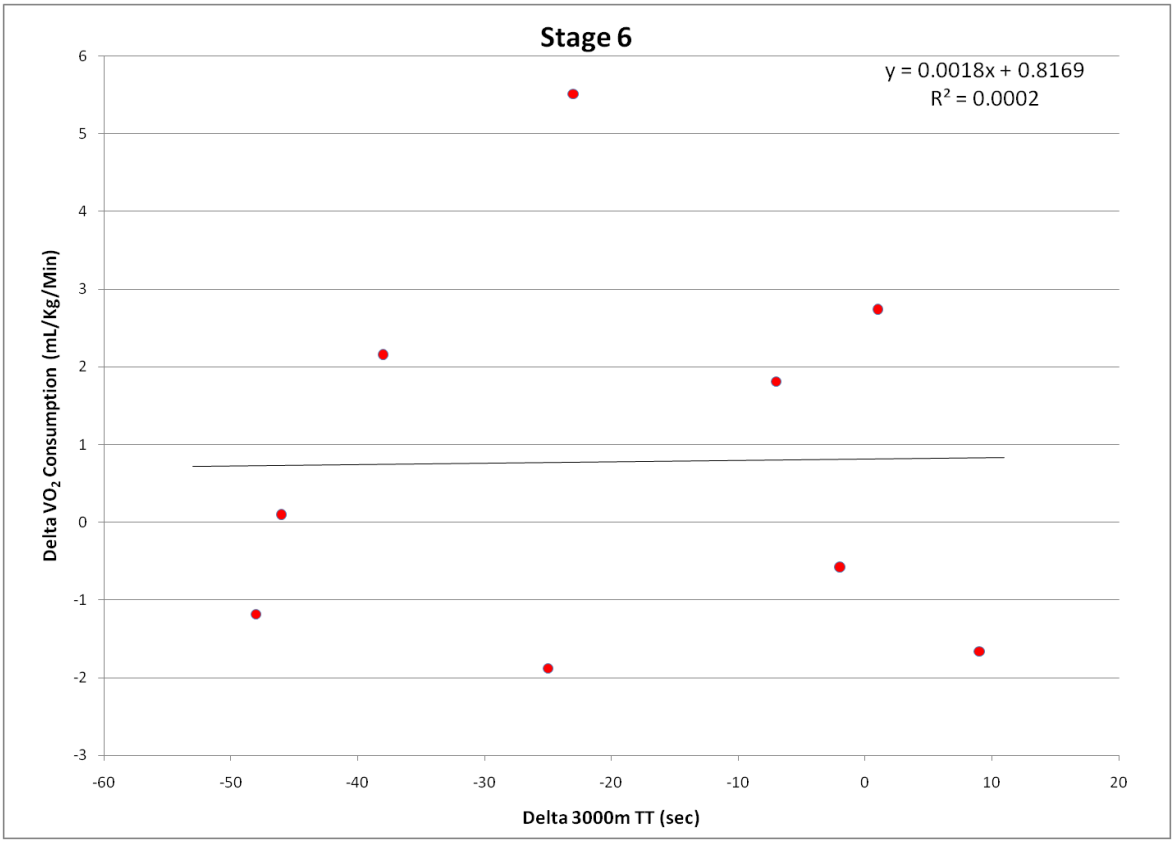
c


Stage 4
y = -0.019x + 0.6155
R² = 0.0414
Delta VO2 Consumption (mL/Kg/Min)
Delta 3000m TT (sec)
6
5
4
3
2
1
0
-1
-2
-3
-60
-50
-40
-30
-20
-10
0
10
20

d

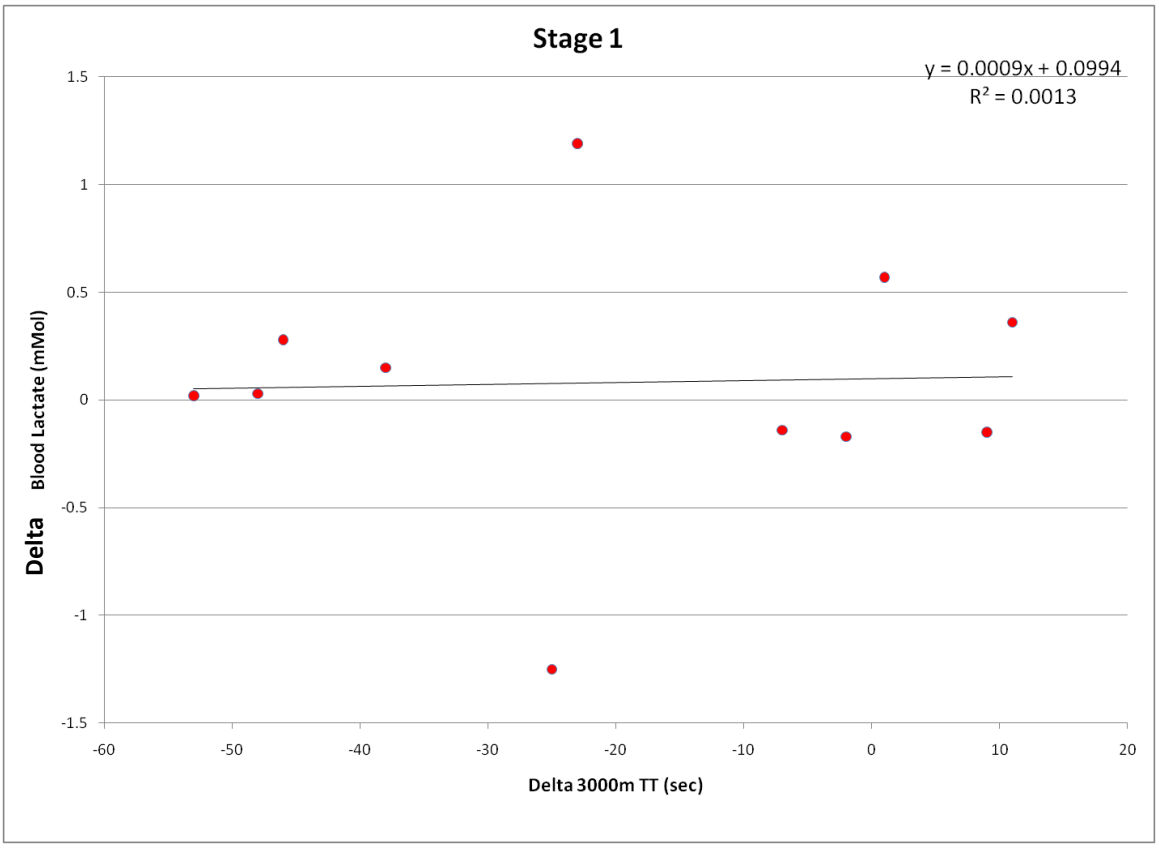


e

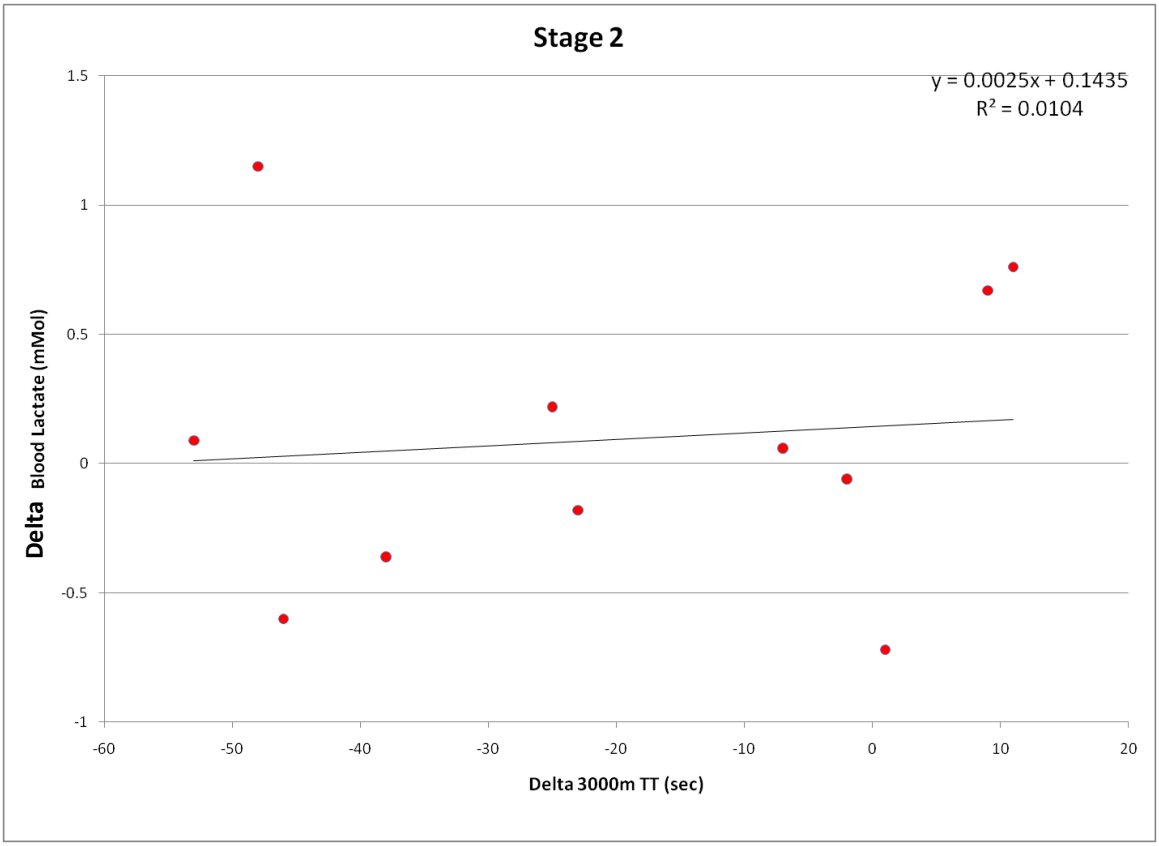


f

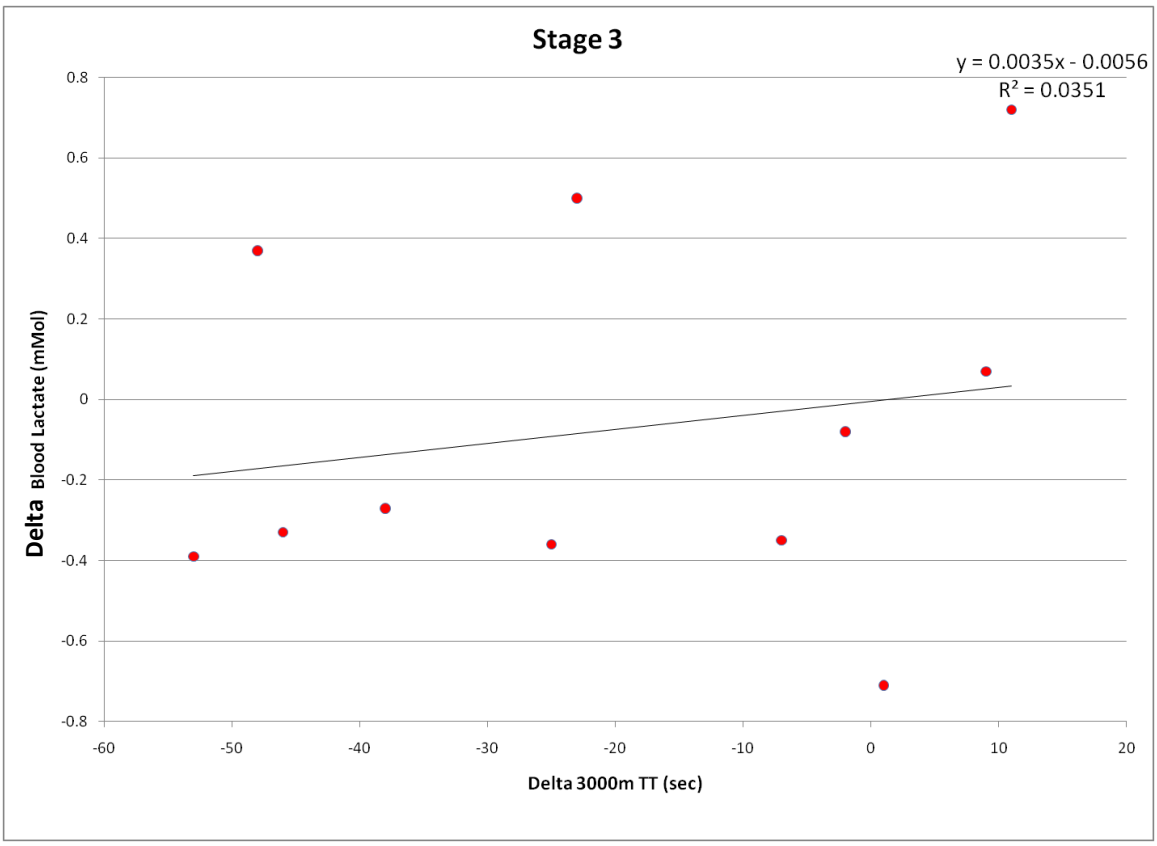
Figures 6. **(a-f)** Correlation plots for TT Performance improvements and VO_2 use changes.


Stage 1
y = 0.0009x + 0.0994
R² = 0.0013
Delta Blood Lactate (mMol)
Delta 3000m TT (sec)
1.5
1
0.5
0
-0.5
-1
-1.5
-60
-50
-40
-30
-20
-10
0
10
20

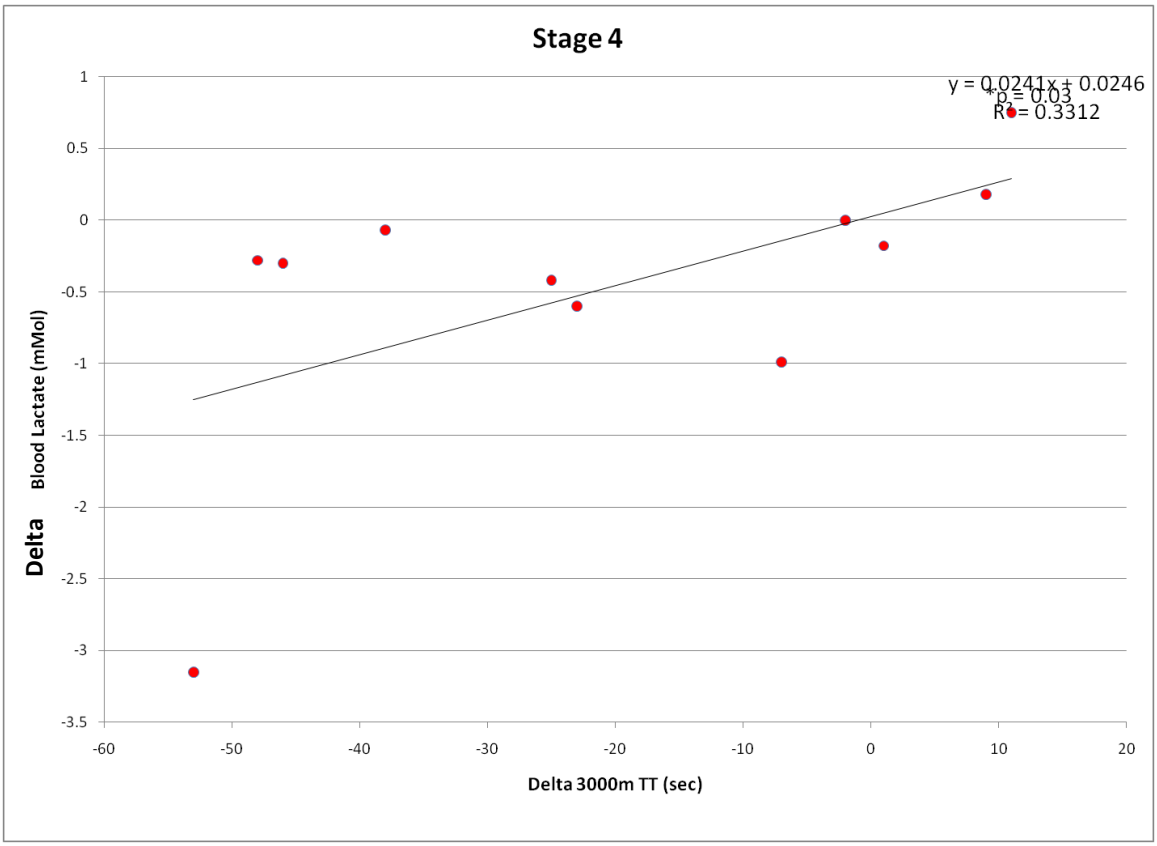
a


Stage 2
y = 0.0025x + 0.1435
R² = 0.0104
Delta Blood Lactate (mMol)
Delta 3000m TT (sec)
1.5
1
0.5
0
-0.5
-1
-60
-50
-40
-30
-20
-10
0
10
20

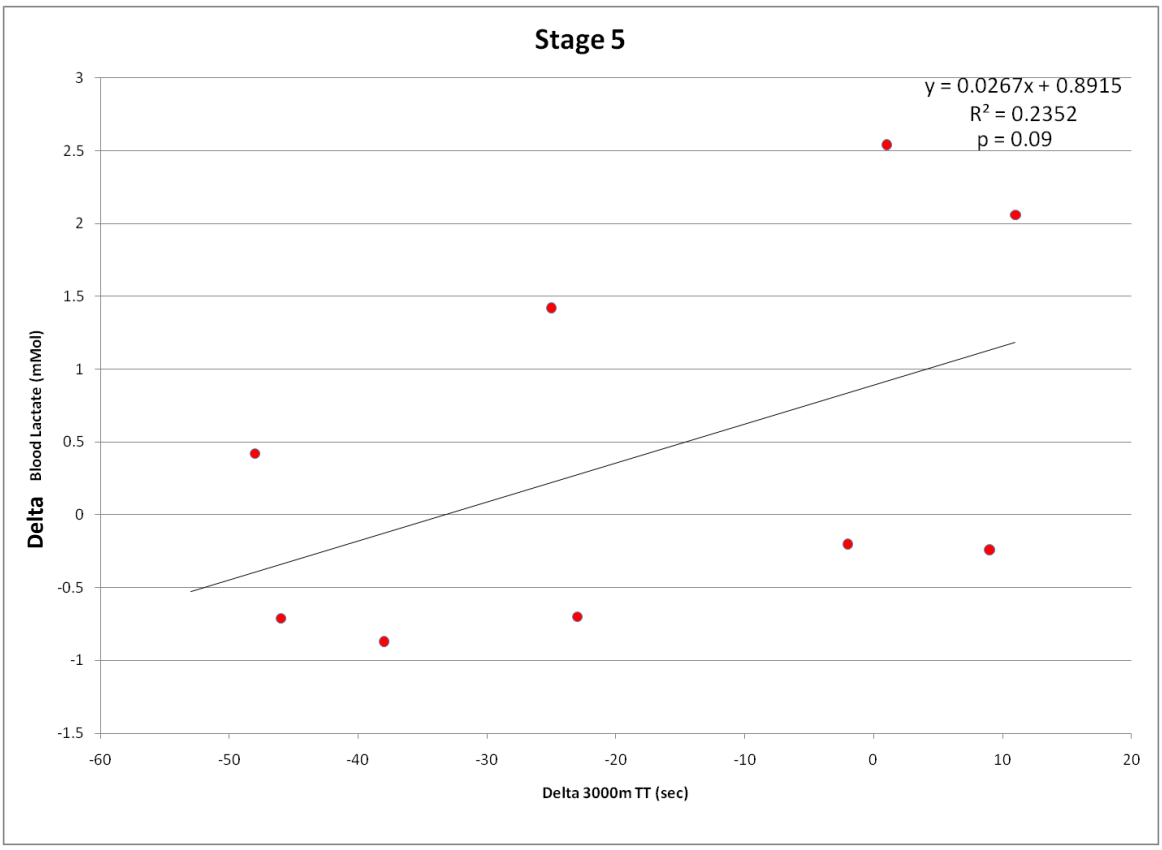
b


Stage 3
y = 0.0035x - 0.0056
R² = 0.0351
Delta Blood Lactate (mMol)
Delta 3000m TT (sec)
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-60
-50
-40
-30
-20
-10
0
10
20

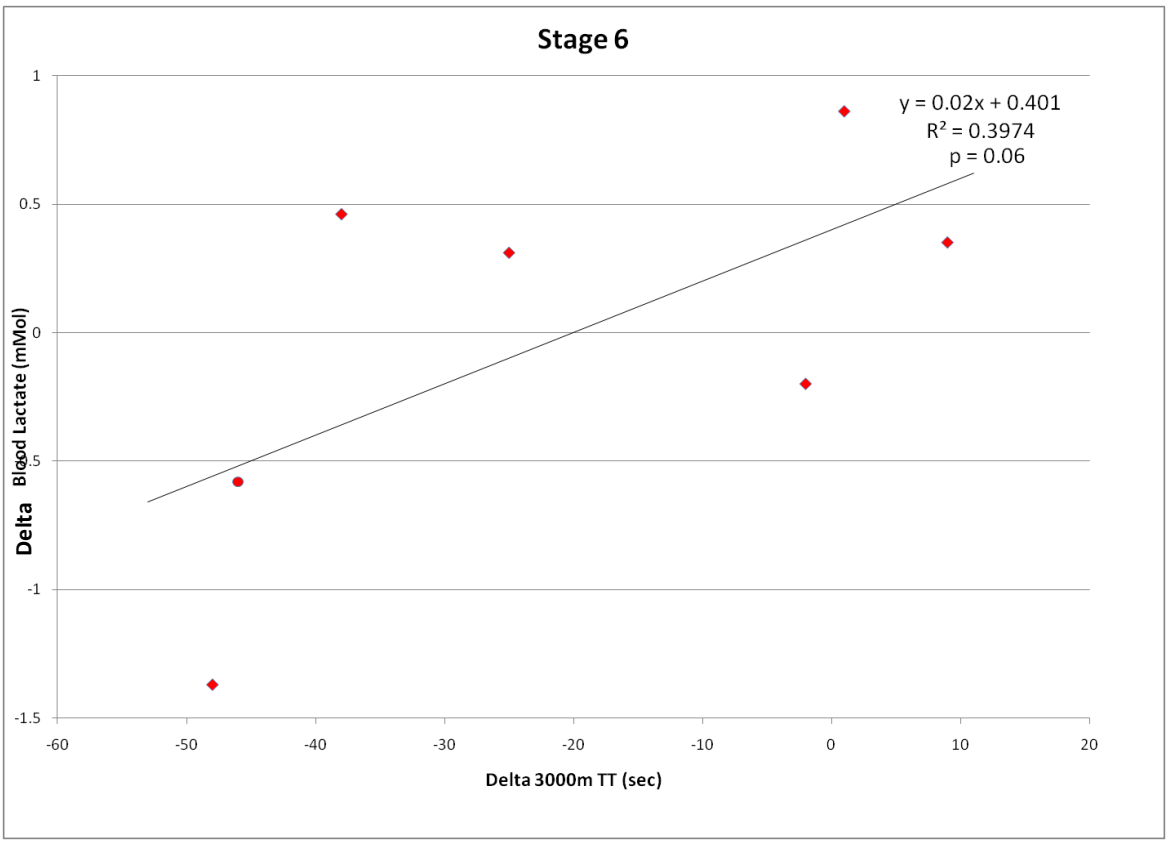
c


Stage 4
y = 0.0241x + 0.0246
*p = 0.03
R² = 0.3312
Delta Blood Lactate (mMol)
Delta 3000m TT (sec)
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-60
-50
-40
-30
-20
-10
0
10
20

d

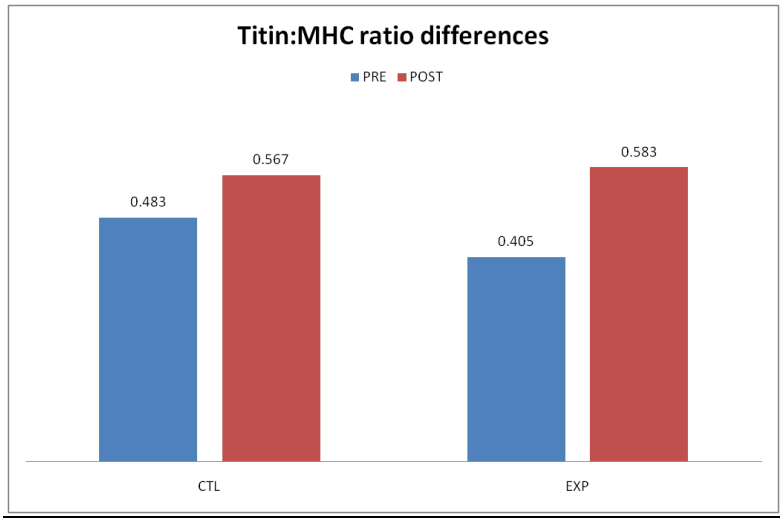


e

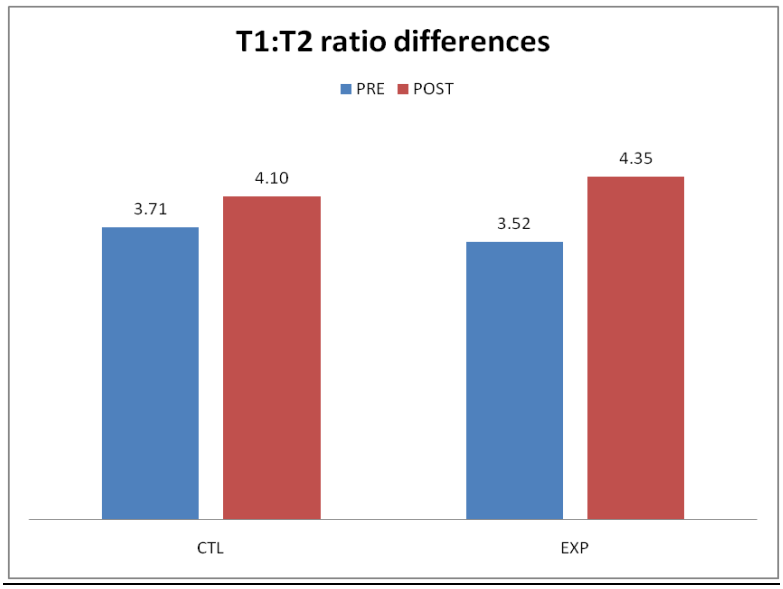


f

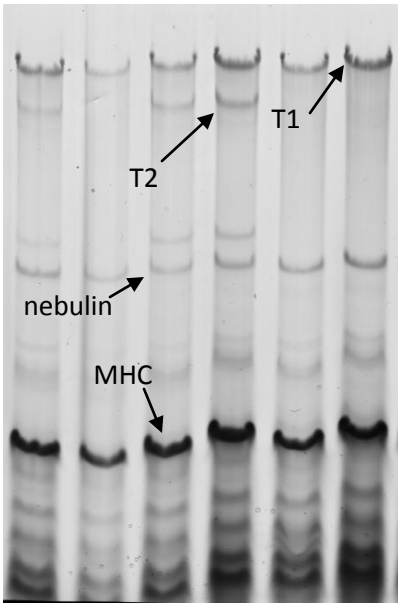
Figures 7. (a-f) Correlation plots for TT Performance improvements and Blac response changes.



a



b



c

Figures 8. (a-c) [a] Ratio amounts of total titin in relation to MHC (total titin : ½ MHC). [b] Titin isoform ratios. [C] 1% VAGE selection with samples showing presence of both isoforms and T1 only.

DISCUSSION

Plyometrics Training. The training routine was developed by adapting a protocol used by Spurrs, et al. (2003) to achieve improved RE in a group of trained male runners. That result was not reproduced here. After speaking with the authors, amendments were made to the training plan to fit the fitness level of this study's participants. Firstly, there was more variability in jumping ability, and so more individualized training was used. Secondly, due to the muscle biopsy data collection at the site of the VL, some exercises that more specifically targeted the triceps *surae* group were substituted with jumps to better activate the muscles of the thigh (see appendix 5 for a complete description). Finally a large incidence of injuries was experienced during the training, about 50% of the participants reported some acute injury or soreness beyond that expected from the novel exercise. Remarkably, only 2 of the 6 incurred injuries below the waist. The majority of complaints involved core musculature of the trunk. It is possible that the lack of sufficient core stability coupled with the violent nature of plyometric training resulted in strained muscles of the back, abdominals and shoulder girdle. Because of this high rate of injury even under supervision, it is not advisable to initiate a plyometric training routine without executing some prior strength training. While RE was not improved through the plyometric training in this study, a more traditional protocol accentuating the distal joints and musculature may be better suited to this end. Muscle stiffness data relating greater muscle stiffness of the *gastrocnemius* to better RE substantiates this belief. Still, the outcome upheld the findings regarding implementation of this type of training: employment of a plyometrics training routine results in improved running performance.

Performance Data. Physiologically CTL and EXP were virtually identical across the board at the onset of the study. With that in mind, any differences incurred by the conclusion of the study can be assumed to have been more probabilistically a result of the training intervention rather than initial differences due to random assignment. While the plyometrics did not carry out their purported function of improving RE, the EXP group did reap performance benefits as indicated by their faster 3Km TT. However, the only change in any of the other performance markers for this group was a decrease in flexibility. Relating this to what was observed in the CTL group, it is possible that the plyometrics were able to stave off the decrease in VJ and reverse the increase in flexibility. A lack of decreasing anaerobic power however is hardly evidence for an effective training modality. It is possible that general activity levels outside of training were low as the study took place during the winter, but certainly not to the levels needed to see detraining. Numbers for all other performance measures were nearly identical PRE-POST in EXP. The most reliable effect seems to be that the plyometric training decreased, while lack thereof allowed for an increase in low-back flexibility over the 6-week intervention period.

Metabolic and Muscle Stiffness Data. Gas analysis and Blac's gathered from the treadmill testing tell much the same story. There were only sporadic disparities between PRE-POST in EXP or when compared to what was seen in CTL as a rule. The propensity for lower VCO_2's in the EXP likely accounts for the lower RER and VE, as CO_2 is the numerator of the calculation of RER, and CO_2 is the major driving force behind respiration. This decrease in turn acted to lower VE/VO_2, as again the numerator in the equation is decreasing. This data does not suggest any change in RE at all. If in fact changes were occurring to improve economy, it may be that they were too subtle for whole body measurements of

gas analysis, though that is problematic because this is precisely how RE is determined in the lab. Despite the lack of evidence for real differences in VO_2 or other respiratory markers, the dependability of the decreases in the aforementioned respiratory markers warrants further consideration. Knowing that the absolute workloads PRE and POST were perfectly matched, and that as such VO_2 would be identical (which is precisely what was observed), without some sort of adjustment in terms of energy derivation, i.e. increased spring-action, all other metabolic markers should have remained equally unaffected. Several hypotheses were explored to see if they could explain these trends.

First, it was possible that fitness increased due to the high intensity nature of the plyometric training. It should be noted that the sole significant evidence of fitness improvements came from the faster TT and increased VO_{2MAX} within EXP. However, there were non-statistically significant reductions in percent of VO_{2MAX} utilized at all speeds, meaning EXP was running at a lower reserve of their maximal aerobic capacity at a matched relative workload, but not significantly so. Moreover, this same trend was seen in CTL, and so should not be considered resultant of the plyometrics intervention. A fitness improvement would likely lead to a shift towards more fat utilization and less carbohydrate, thereby increasing O_2 demand and decreasing CO_2 production at a given workload. While O_2 use was unchanged, CO_2 did repeatedly come out lower POST than PRE. RER data also supports a greater fat utilization POST. In light of these facts, substantiation of this hypothesis necessitates an explanation for the consistent VO_2 response (or lack thereof).

A second rationalization would be that the anaerobic nature of the plyometric training did not do anything to increase aerobic function, but instead increased fitness through a greater anaerobic capacity. This seems unlikely, as there is no anaerobic power improvement in VJ height to back this claim up. Additionally, RER and VCO_2 data refute this change. Most tellingly, Blac values were blunted at several of the running speeds tested. Had the anaerobic contribution been enhanced, one would expect to see just the opposite.

It is also possible that the MTU did in fact increase the spring action during running (as was the objective of this study). While there is no longitudinal muscle stiffness data to support such a change, it is possible that the nature of the measurement was not sensitive to recoil action of the MTU. Myotonometer measures are taken in a motionless, relaxed or isometrically contracted state for passive/active values. Furthermore, as a user-sensitive device it is possible that the novelty of utilizing the device skewed measures, as it seems odd that almost every measure came to be significantly higher (less stiff) POST for all subjects. In fact, only the *Achilles* tendon came out lower POST. Being that two different testers administrated the testing (one PRE, another POST) it was possible inter-tester variability could have accounted for the disparities seen (Aarrestad, Williams, Fehrer, Mikhailenok & Leonard, 2003). Although when three individuals were each tested by both of the testers in sequence, there was no divergence between the two sets of values. Also switched PRE to POST was the actual unit implemented for the measurements. Part-way through post-testing the original myotonometer was replaced by another unit, and no inter-unit testing could be carried out. Taking into consideration these possible sources for error, myotonometer stiffness changes PRE-POST are disregarded for this study, and only cross-sectional correlations are considered.

Because of this, it is entirely possible that stiffness changes were in fact present, but not discernable. This could provide just the explanation required to explain the predilection for lower respiratory values in EXP in conjunction with fitness enhancement. If in fact the plyometrics did induce a stiffness alteration in the muscle it would be expected that this would lower O_2 demand during each of the stages of the treadmill test, or at least at some grouping of speeds for which maximization of the pre-stretch/recoil spring action takes effect. In fact, VO_2 seemed to be the only measure that did not repeatedly, even if not significantly, decrease PRE to POST within EXP. Possible reasons for this are discussed below.

Muscle Protein Data. Muscle data is provided for all values collected, but the most meaningful values are the T1 to T2 titin isoform ratio and the total titin to MHC ratio. No differences were seen in the muscle data between groups or within group by time. Given that no RE changes were observed, this does not substantiate or disprove any of the conjectures regarding the role of different proteins and their role in determination of RE. There is only limited data to support/refute in this arena. The cross-sectional analysis carried out by Kyrolainen, et al. (2003) reported all eleven of their subjects as having the T2 isoform present while only one had both T1 and T2. This group also used the VL as the biopsy site, so similar findings would be expected. The T2 isoform is shorter and found in higher abundance in fibers that tend to generate higher force, i.e. Type II fibers. As a mixed muscle (containing all fiber types) the VL would be predicted to contain a higher count of Type I fibers, and therefore T1. This is precisely what was seen from the VAGE carried out for titin analysis in this study. One possible explanation for this discrepancy is that while participants in this study were recreationally trained, the subjects in the 2003 report were highly trained competitive middle-distance runners, and may have thus displayed a different titin protein makeup. As it is unknown how or even if titin expression changes with training in human skeletal muscle, this incongruity suggests more studies need to be completed to answer these questions.

RE Changes Grouping. The sham grouping by RE improvement was evaluated to offer insight to what factors may link to RE improvements, and identify possible commonalities within this group. Others have looked for muscle quality and “responders” to types of training in the past (Fukashiro, Abe, Shibayama, & Brechue, 2002). As such the improved RE economy group (IRE) saw decreased energy cost of running POST as compared with PRE on stages 1-4, and the degenerated RE group (DRE) experienced higher costs of running POST on stages 4-7. To this extent, the groups are set up to accomplish what was originally intended: to compare a group of individuals with improved RE to those without. Unfortunately, no important differences between the two groups were identified. Intriguingly, this includes the muscle protein data. This fact does not support the speculation that titin isoform makeup or total titin content (when scaled to MHC) has any bearing on RE. Aside from VO_2, the only value from the treadmill testing that significantly changed was RER. RER was consistently higher POST and this difference reached significance on three of the four stages for which RE was seen to improve (stages 1-3, incidentally the three lowest speeds).

Performance Data. Improvements in performance attained through RE are thought to be achieved without concurrent improvements in fitness. Data in this set verifies that notion. VO_{2MAX}, and VO_2 at OBLA were already higher in the IRE group, perhaps near max capacity for these individuals. As

such in order to run faster, they would need to improve economy rather than fitness. This is precisely what occurred. The fitter group saw changes in economy, while the less conditioned group was able to improve their fitness with no change or even a decrease in RE. Of interest is that the group that did see improvements in RE had much faster 3K times both PRE and POST, but the end result (running performance) showed that neither group was able to improve 3K run time -both groups did see large yet non-significant reductions in run time (just missing significance in IRE). So, improvements in fitness (VO_{2MAX}, OBLA, BMI, flexibility) were positively correlated to O_2 use during the treadmill test, and hence a worsening of RE. Faster runners tended to be able to improve RE, but not aerobic fitness. Pairing this data with between groups differences reveals that the IRE group PRE was running essentially the same speed, but consuming significantly more O_2 at OBLA than DRE (so they were not as economical). POST, IRE was able to be running significantly faster that DRE without increasing VO_2 at OBLA (so they become faster by improving economy, not metabolic work capacity). In fact DRE increased VO_2 at OBLA such that there was no longer a difference in metabolic workload at OBLA POST. Despite this, IRE became faster because of the improved economy at their OBLA speed. In summary, viable changes in RE may only come after, but certainly not with enhancement of fitness characteristics.

Muscle Stiffness & Muscle Proteins. Muscle stiffness data was virtually identical to that seen for the experimental grouping. This confirms the notion that PRE/POST measurements were not well matched for comparison. Muscle protein data again did not depict any evidence concerning RE alterations and protein content of the muscle fiber. Conclusions from this data set are limited to associations not cause/effect relationships, and the data supporting any changes is sporadic even within this predetermined data set. The findings were not completely without merit though, as they allowed for tracking physiological dynamics alongside shifting RE. Perhaps the most remarkable upshot was that no convincing evidence could be found supporting the notion of RE's connection to muscle protein alterations.

Correlation Matrix Data. Essentially the IRE/DRE grouping tells much the same story as the correlations with treatment groups, but isolating PRE and delta PRE/POST information enabled a complete picture. Additionally run, but not provided were correlations for all variables with POST data only, muscle and RE-related PRE to delta for all variables as well as delta muscle and RE-related to PRE all variables. Unfortunately this does not allow for cause/effect determination, but speculations based on the other data from this study will be presented concerning mechanistic changes in the conclusions section.

Data within respiratory marker recordings interacted as expected with respect to one another. More consequential were the muscle stiffness PRE correlations and the muscle protein PRE and delta correlations. Considering the former, no potent links to RE could be identified. However, when a Pearson's correlation to performance (using TT) was run on the PRE data, a tie to muscle compliance was exposed as a factor in the distal portion of the leg. Specifically the *gastrocnemius* was seen to be stiffer in the activated state ($r = 0.65$, $p < 0.001$) while the adjoining *Achilles* tendon was found to be significantly more compliant in the relaxed state ($r = -0.52$, $p = 0.008$). This is in line with the findings of Kawakami et al. (2002) that muscle stiffness and tendon compliance in concert would act as the most efficient MTU. It is not surprising that this finding lay within the more distal portion of the leg, as this is

where spring-like function would be most influential. The further removed from the point of contact one gets, the more energy is lost from the impact through lateral forces and shock absorption, and the less remains to be used for recoil. This finding endorses previous research regarding stiffness and performance data (Dumke, et al., 2010; Ettema, 2001; Fletcher, eta al., 2010; Kawakami, et al., 2002; Kubo, et al., 2010; Morgan et al., 1978), however without a cause/effect component or a direct link to RE, it adds little to the body of literature at large.

Correlations pertaining to muscle proteins demonstrated a lower proportion of T1 to T2 isoforms in more fit individuals, as moderate to strong correlations existed in the PRE data for VO_{2MAX}, speed at OBLA, and 3Km TT. As T1 fibers are associated with Type I aerobic fibers in animal models (Prado et al., 2005), and these measures are predominantly aerobic by nature, this was somewhat surprising. It is entirely possible that higher intensity training leading to higher fitness also acts to increase T2 expression of titin. This is feasible in that greater T2 expression, and a lower T1:T2 ratio would be expected for individuals with higher intensity training, which in turn is more likely to be carried out by those with higher fitness levels as defined by these markers. Unanticipated was the link between fitness/performance (TT/VO_{2MAX}) and the titin to MHC ratio. T:MHC was seen to decrease with increasing fitness in the delta correlation, and a higher T:MHC was found with a slower 3Km TT performance from the PRE data correlation. It was hypothesized that a greater relative expression of total titin would have led to and/or been associated with faster running times, but just the opposite was observed. An explanation for this is not offered at this point, however it is worth pointing out that the T:MHC ratios, about 2:5 - 3:5 are slightly higher than those seen in previous research, about 1:5 – 1.5:5 (Granzier & Irving, 1995), though that was in different muscles (psoas and semitendinosus) and was carried out using SDS-PAGE. Overwhelming evidence for a connection to RE existed for neither the T1:T2 ratio nor T:MHC.

Suggestions For Further Research. Initial examinations of the individuals recruited for the study revealed that the participant pool well represented the intended population (recreationally trained runners). Percent body fat was in the healthy range, while BMI was just at the normal/overweight boundary (Aside: This supports the growing evidence against using BMI as a substitution for body composition). As expected given the active lifestyles of the participants, fitness values were in the above average categories for flexibility (37.1cm ranks “very good” for both males and females: Baechle & Earle, 2008), vertical jump (47.4cm, well above the adult averages of 20cm female; 41cm male: Heyward, 2006) and VO_{2MAX} (48.1ml/kg/min as compared to male and female averages of 40 and 45ml/kg/min respectively for active adults: McArdle, Katch, F., & Katch, V., 2001). While not completely unfounded, it was surprising that values for anaerobic power were so far above the mean given the participant’s lack of power training prior to this investigation. Also of note were the RE values in ml/kg/Km that were computed. These numbers were consistently around 84% of those calculated from the standard American College of Sports Medicine metabolic equation.

% below ACSM	12%	15%	17%	17%	17%	16%	17%
Study group	209	198	191	188	186	188	183
ACSM Est.	237	232	229	227	225	223	222

Table 11. RE values in $mL\_O_2/Kg/Km$. Study participants used consistenly 16% less O2 than predicted by the ACSM estimation equation.

Intentions of this study were to induce changes in running economy in a group of runners and examine the possible courses of action through which this change occurred. In this regard, the experiment was not successful. One possible explanation for this may be the superior RE that existed within the sample studied. Other than the first stage, which was set at a speed of 7.5Km/hr (just fast enough to prompt a very slow run in most of our subjects) there was only an economy change of 15ml (7.5%) whereas speed increased by 7.5 Km/hr or by 8:00/Km (93%). Typically these values are not given in research, as the energy cost of running is traditionally reported simply as the VO_2 consumption at the given speed or speeds tested. However, given the stark contrast between "average" efficiency and that measured, it seems reasonable to presume this played a role in the failure to induce improvements in RE. Owing to this, it is proposed that values be calculated and reported in the literature concerning future investigations of RE. This not only would help identify RE changes between speeds within a given individual, but also help those in the practical field identify paces where a runner is particularly economical (or uneconomical). Furthermore, it is postulated that the improved economy seen as speed increases may link to marked changes in running gait, physiological thresholds, or other factors. By scaling O_2 use by distance rather than time, different speeds can be compared.

Muscle data has yet to undergo final analysis (anticipated summer 2011), and may yet reveal significant divergence between groups or correlations with RE, muscle stiffness or the changes seen therein. Despite the consequences of these pending results, the current data, along with the lack of uniformity in the literature supports the need for further investigations, particularly of longitudinally designed studies to disinter the role of the influential factors concerning RE. Lastly, the role of the protein titin is still ambiguous in skeletal muscle function. While this study was able to contribute a limited window into the mechanical function of this protein, ancillary investigations will need to be carried out as to what role it plays in force production and free energy during running. Further research should be performed with particular attention to structural proteins that are positioned in the sarcomere to allow for a possible spring-like function (costameric proteins, titin, nebulin, twitchin, tropomyosin, etc.) and proteins of the tendon, i.e. collagen. Additional studies are also needed to determine the role of phosphorylation on the titin protein within the context of skeletal muscle force production, as this was not completed in this investigation, but is another possible source of differential regulation of muscle stiffness.

CONCLUSIONS

No evidence from this study exists to support the use of plyometrics to increase RE, or that RE is related to the muscle protein titin. It is possible that the intervention was simply too short to induce the changes in RE expected, however, as it has been shown in the past that 6-week programs were able to produce significant changes in RE, this is not likely (Spurrs, et al., 2003; Turner et al., 2003). Cross-sectional analysis revealed strong correlations for stiffer muscles and more compliant tendons within the *triceps surae* group to better performance. This is in agreement with previous findings Arampatzis, De Monte, & Karamanidis, et al., 2006; Dumke, et al., 2010; Fletcher, et al., 2010; Kubo, Kanehisa, & Fukunaga, 2001) but not others (Ettema et al., 2001; Morgan et al., 1978). Unfortunately, PRE/POST changes in stiffness could not be analyzed without reservation. Furthermore, the site of RE stiffness dependency and of the biopsy was not the same (distal versus proximal leg). In light of these complications, no conclusions could be drawn regarding muscle protein and muscle stiffness. Finally, there were recurring trends in the metabolic records hinting at diminished CO_2 production following the 6-week exposure to plyometric training. This prompted an auxiliary investigation to be discussed below.

As stated above, TT performance was significantly improved for EXP and not CTL. It was assumed that the increase in VO_{2MAX} was the reason for this, since it was significantly increased in EXP and not CTL. However correlations run for performance change to physiological measure differences showed no correlation between any of the fitness data and TT performance (see table 12). As a result of this, a correlation was run between changes in TT performance and Blac by stage. A second correlation was carried out using the actual speed run during the TT. For this, only data points from those stages for which the participant was within 1Km/hr of their 3Km TT average pace were taken into account. Because of the limitations of reducing the n, only stages 4 and 6 were considered. These results are tabulated in table 13. Blac deltas did in fact correlate strongly in the positive direction with TT performance changes, particularly when speed at TT was used as a limiting criterion. No such relationships existed within the CTL group. Moreover, VO_2 deltas did not associate with performance improvements at all. Meaning runners who became faster were the same that saw attenuated Blac (anaerobic output) at the same given workload and vice versa. This is not a novel concept itself, but the proposed mechanisms leading to this physiological phenomenon are. These data add support to the suggestion that the plyometric training supplements performance through assuaging anaerobic, and not aerobic work output.

vo2 max	obla speed	obla vo2	obla %vo2
-0.069	-0.136	-0.007	0.028

Table 12. TT r values. None significant.

3KTT/Blac	stage 4	stage 6	stage 6
r value	0.576	0.630	0.855
p value	0.058	0.059	0.032
EXP group	all	all	speed matched

Table 13. Correlation for delta-TT performance to delta-Blac in EXP group for stages near TT pace

Both T1:T2 and titin:MHC showed significant positive correlations with faster TT PRE (and POST for T:MHC), it was speculated that this may be the means through which the plyometric training rook their effect within this study's EXP group. When statistics were run on just EXP for delta-TT and protein, similar strength correlations were calculated for T1:T2 and titin:MHC, but due to an n of 6, it was difficult to reach significance ($r = -062$, $p = 0.09$ and $-.54$, $p = 0.13$ respectively, $p > 0.05$). This shift in direction of the relationship is peculiar, and is unexplained. Similarly correlations within EXP only were run for changes in Blac during each stage of the treadmill protocol with both titin measures to test the hypothesis that the spring model would parallel changes seen in this anaerobic marker. This yielded inconsistent results from stage to stage for total titin: MHC, but did give link shifts to a higher T1 percentage to a lower Blac response (stage 1: $r = -0.34$, $p = 0.256$; stage 2: $r = -0.63$, $p = .092$; stage 3*: $r = -0.81$, $p = .024$; stage 4: $r = -0.43$, $p = .197$; stage 5: $r = -0.29$, $p = .317$; stage 6: $r = -0.66$, $p = .467$ $n = 6$ satges 1-4, $n = 5$ stage 5, $n = 4$ stage 6.). From this preliminary data it seems that titin may play a role in spring action of the muscle, and that the T1 isoform is the more economical of the two.

Recalling the Kyrolainen et al. (2003) study, the data here does not agree with previous findings. The most blatant difference is that they identified T2 in all subjects, and T1 in only one, where this study identified T1 in all participants, and T2 in nearly all (only 1 lacked T2 completely). With respect to the animal models, the findings in this study seem much more likely (as T1 should be the more prolific of the 2 isoforms). Additionally, while this study implemented VAGE for titin analysis, the previous investigation used SDS-PAGE to isolate the protein, the former being much more reliable. However, final analyses are pending, and so results are not finalized. It is feasible that the different sample compositions (Kyrolainen et al. used highly trained middle-distance runners, whereas less trained more diverse runners were examined here. It would make intuitive sense that higher T2 isoform content would be found in the former, and lower in the latter, but the dearth of research allows only for speculation. Moreover, without established length-tension relationships for sarcomere stretch and titin force production within human skeletal muscle (Prado et al. (2005) delineated these relationships in rabbit skeletal muscle), scientists are left to guess which isoform (if either) is able to act as a more efficient spring.

The spring model propounds that a less compliant MTU provides usable energy for mechanical work. This energy contribution to work output allays the metabolic demand at a given workload. Traditionally, this is purported to enhance RE by engendering a reduction in aerobic demand. In short, the energy supplied by the spring action substitutes for what would otherwise need to be accounted for by the aerobic system. Upon examining the reason for this it becomes apparent that it is simply because of the bulk of the energy cost at the speeds tested for RE being aerobic by nature. In addition to this, it is very difficult to quantify anaerobic supply. However it is entirely possible that if the spring model is correct, the reduced metabolic load may come not from attenuation of the aerobic system, but rather from curtailing the anaerobic involvement (or both).

A suggestion such as this is not founded in the literature, but rather in basic physiology. The body as a whole and the individual cells function under the basic principle that maintenance of a homeostatic balance trumps all else. On both a cellular as well as a holistic level, anaerobiosis wreaks havoc on the system. And so, it would intuitively follow that when energy demands are relieved, the

anaerobic component would be the primary target for such reductions. Again, this is normally not considered, mainly due to the fact that the speeds examined when determining RE are low enough to discount (and subsequently ignore) the anaerobic component. When workload is diminished (by slowing down during a run for example), the physiological response is not to maintain anaerobic kinetics and alleviate aerobic demand, but rather the reverse. Reducing workload by supplementing energy production with elastic recoil of the MTU is akin to just such a situation.

The best measure to provide evidence for such a phenomenon would be a blunted Blac response. In fact, a rightward shift in the lactate curve for an individual is canonical evidence of fitness gains. However, this same phenomenon would be possible through a stronger spring-action of the MTU if in fact the energy savings were to be reaped within the anaerobic system. Data reported here does not unequivocally support that this took place with statistically significant figures, but at the higher running speeds (where anaerobic contribution is considerable and lactate accumulation relevant) there is a inclination for mitigated Blac levels in the plyometric training group (non-statistically significant reductions of 8.4, 23, 12, -0.39(increase) & 0.52% in stages 3,4,5,6(increase) & 7 respectively). Still this fails to answer the question of where energy savings from supplementary spring-action may be taking effect.

To get a clearer picture of the aerobic:anaerobic ratio, data collected was used to establish this mathematically. VO_2 values were divided by Blac to provide a quantifiable measure of this proportion (ml_O_2*L_blood/kg/min/mol_Lac). Given that total work output was equivalent PRE and POST, any work supplied by a stiffer MTU would enact to lessen either the aerobic or anaerobic work of the system, hence either lowering or raising this ratio respectively. Ignoring stages 1 and 2, as Blac concentrations were not yet significantly increased from resting, and stage 7, as only a few participants had Blac values recorded for this stage, the ratio was lower (meaning energy shifts were towards proportionately less anaerobic workload) across all stages. When these values were converted to a percent (to eliminate the cumbersome units and to scale relative changes in Blac) and averages computed, it was found that for stages 3-6, the average for participants in the plyometrics group decreased by 8%. For comparison, values for CTL averaged an increase of 2.5%. These values are analogous to the inverse of the slope on a standard lactate threshold graph with VO_2 as the unit measure of intensity. While this effect supports the concept posited, it does not relate this presented outcome to performance.

It is the contention of this author that plyometric training, which failed to augment RE as measured by O_2 consumption and neglected to increase VJ, but still improved running performance was able to do so through an increase in muscle stiffness and tendon compliance in the distal portion of the leg. Based on correlations carried out, this change is thought to be related to total titin concentration, as expressed by T:MHC as well as the T1:T2 ratio. It is postulated that significant changes in RE evaded detection in part because of the superior RE displayed by the sample population at the outset of this study, and in part because of the mixed ability levels. Given an increased spring component to energy production, it is thought that at lower intensities RE will improve as there is a negligible anaerobic contribution. At higher intensities O_2 consumption will not be affected, and rather a mollified anaerobic component will be observed. For the participants in this study, statistically significant changes in either of these measures eluded detection as a result of the heterogeneity of fitness levels. Through the use of

RE calculations give in O_2 use per unit distance rather than time (as suggested above), it may be possible for future studies to avoid this conundrum and compare runners of various abilities to one another for RE values at relative rather than absolute speeds. In conclusion figure 9 displays the traditional and contemporary propositions through which greater muscle stiffness may enact. The contemporary model put forth here is thought to better relate to common physiologic events associated with exercise, training and performance than the traditional explanation.

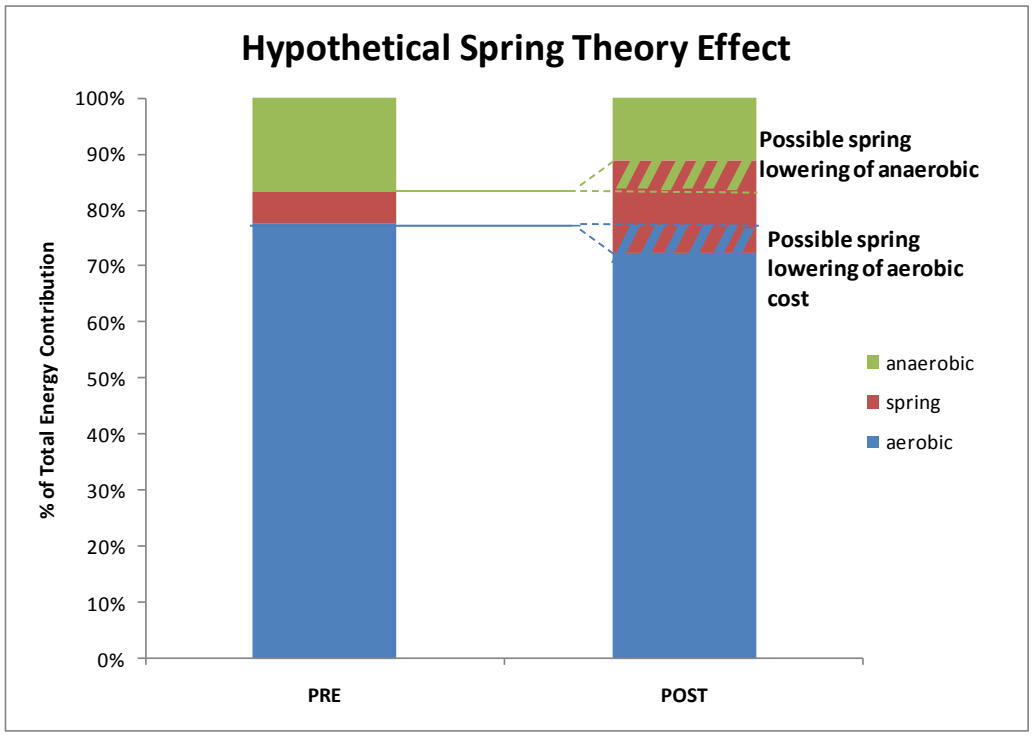


Figure 9. At the same workload, energy demand can be apportioned more or less toward any given input. Specifically, it is typically assumed that energy derived from spring action will lower aerobic cost. Depending on relative intensity, it is just as likely that energy potentiated through a spring action of the MTU would act to supplement anaerobic contribution, or possibly both.

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Physical Activity Readiness Questionnaire (PAR-Q) and You

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly:

YES	NO		
☐	☐	1.	Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?
☐	☐	2.	Do you feel pain in your chest when you do physical activity?
☐	☐	3.	In the past month, have you had chest pain when you were not doing physical activity?
☐	☐	4.	Do you lose your balance because of dizziness or do you ever lose consciousness?
☐	☐	5.	Do you have a bone or joint problem that could be made worse by a change in your physical activity?
☐	☐	6.	Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
☐	☐	7.	Do you know of any other reason why you should not do physical activity?

If you answered:

YES to one or more questions

Talk to your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want – as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- Start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- Take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively.

Delay becoming much more active:

- If you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- If you are or may be pregnant – talk to your doctor before you start becoming more active.

Please note: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Appendix 2

Date: _____ Subject #: _____

RUNNING QUESTIONNAIRE

How Long have you Been Running? _____

How many times do you run per week on average? Yearly _____ Last 6 wks _____

On Average how many miles/week do you run? Yearly _____ Last 6 wks _____

What experience do you have with power/strength Training? _____

_____

What race distances do you currently compete in/train for? _____

_____

What are your PR's at these distances (w/date)

Distance	Time	Date
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

What injuries have you incurred in the last 6 months? _____

_____

What injuries have you incurred in the last 12 months? _____

_____

Are there any chronic injuries you have had over the years? _____

_____

When was the last time you were not running for an extended period of time? _____

Why? _____

Do you wear orthotics or use any other devices to alter your running gait? _____

RE/VO_2max/LT PROTOCOL

Stage	TIME	SPEED	SPEED
1	(min:sec)	(m/min)	(Km/hr)
2	0:00-3:00	129	7.5
3	4:00-7:00	153	9.0
4	8:00-11:00	177	10.5
5	12:00-15:00	201	12.0
6	16:00-19:00	225	13.5
7	20:00-23:00	249	15.0
8	24:00-27:00	274	13.5
9	28:00-31:00	288	15.0
10	32:00-35:00	322	17.5
11	36:00-39:00	322_3%	17.5
12	40:00-43:00	322_5%	17.5

Myotonometer Sites & Positions

All passive measurements were taken followed by all active in the following sequence: VL, *gastrocnemius, soleus,* Achilles (tendon).

All measures were taken in triplicate and averaged prior to analysis.

Vastus Lateralis

Passive: Individual was instructed to sit on a bench with heels on the floor and legs straight but completely relaxed. The muscle was located visually and through palpation. If necessary the tester would ask the individual to isometrically contract their leg I order to better visualize the VL. Limb laxity was checked by gently rocking the leg manually and feeling for any resistance just before the measure was taken. The site was marked so as to repeat the exact position in the active state. The distance from the proximal lateral patella was recorded for replication of the same site during post-testing.

Active: All active positions were demonstrated prior to instruction/measurement. Individuals were asked to stand under a lever arm type affixed squat machine with feet shoulder width apart. They were then asked to lower by swatting down, keeping knees at or slightly off from their toes until apporimately 85-90^{O} flexion of the knee joint was achieved.

Soleus

Passive: Following passive VL individuals were asked to simply remain seated and place the feet flat on the floor in a neutral position. Again a light manual rocking of the leg was employed to check for a completely relaxed state. The medial *soleus* was located through palpation, and when possible visualization was employed as an aid. The site was marked at the center of the muscle and distance measured to the center of the medial *malleolus*.

Active: The individual was positioned in the squat machine as with the VL but was instructed to mimic a running pose. Slight flexion of the hip and knee in conjunction with unloading of the heels was the target position. They were instructed to shift all of their weight forward to their forefoot without actually lifting their heel from the platform.

Gastrocnemius

Passive: The same position as for the *soleus* was used for the *gastrocnemius* with a check to full relaxation of the leg. The center of the medial head of the *gastrocnemius* was located in the same manner as the VL, distance marked and recorded from the medial *malleolus*.

Active: The active reading was taken on the squat machine with feet in a neutral stance and ankles in maximal plantar flexion and all weight on the toes and forefoot –as in doing a calf raise exercise. Individuals were instructed to get as high up “on their toes” as possible.

***Achilles* Tendon**

Passive: The individuals remained seated on the bench and were instructed to cross their legs (literally as in sitting with your legs crossed), as this was found to allow the least stiffness in the tendon. The site was measured at the center of the *Achilles* just above the back of their shoe's upper. Relaxation check was carried out by rotating the foot at the ankle clockwise/counterclockwise and verbally encouraging relaxation.

Active: The active reading was taken in the same position as for the *gastrocnemius*, only with ankles in a neutral position.

Exercise _____ Week	Squat jump	Split scissor jump	2-legged forward jump for distance	Alternate leg bound	Single leg forward hop	Stepping-drop jump (12-18")	Lateral line jumps	180-turn line jumps	Split scissor jump w/step	High-box double jump (14-42")	**Total Contacts**
1	2·10	2·10	2·10								**60**
1	2·10	2·10	2·10								**60**
2	2·10	2·10	2·10	4·12							**108**
2	2·10	2·10	2·10	4·12							**108**
3		2·12	2·12	4·12	2·10						**136**
3		2·12	2·12	4·12	2·10						**136**
4			3·10	4·12	3·10	2·6					**150**
4			3·10	4·12	3·12	2·8					**154**
4			3·10	4·12	3·12	3·8					**158**
5					2·10*	3·10	3·10	3·10	3·10	2·6	**172**
5					2·10	3·10	3·10	3·10	3·10	3·8	**184**
5					2·10	3·10	3·10	3·10	3·10	3·10	**190**
6					2·12	3·12	3·12	3·12	3·12	3·12	**228**
6					2·12	3·12	3·12	3·12	3·12	3·12	**228**
6					2·12	3·12	3·12	3·12	3·12	3·12	**228**
					With each leg	Height adjusted for ability				Height adjusted for ability	

* advanced movement for those capable

DESCRIPTIONS

Warm-up consisted of jogging, leg swings, skipping, light bounding, bouncing and submaximal jumps as well as stretching for 20:00 each session.

Descriptions

All jumps were encouraged to be as high and as fast as possible… Maximizing flight time while minimizing contact time. This direction was repeated throughout all the training sessions.

SQUAT JUMP: 2-legged maximal vertical jumps done in succession as quickly as possible.

SPLIT SCISSOR JUMP: 2-legged staggered stance maximal vertical jumps done in succession, switching the forward and rearward foot with each jump. Done as quickly as possible

2-LEGGED FORWARD JUMP FOR DISTANCE: 2-legged jumps in succession (like a frog hopping of a kangaroo) across the gymnasium. Done for maximal distance rapid-fire.

ALTERNATE LEG BOUND: Jumping for maximal distance from leg-to-leg as far and as fast as possible. Similar to running strides, only more explosive in nature.

SINGLE LEG FORWARD HOP: Performed with each leg: successive maximal jumps for distance on one leg. Emphasis again on rapid jumping with maximal force application.

STEPPING-DROP JUMP: Participants walked across a mat and stepped as if the mat continued… upon "falling" off the end, they brought both feet underneath them and rebounded from contact with the floor into a maximal vertical jump (slight forward movement).

LATERAL LINE JUMPS: Jumping from side to side over a line on the gymnasium floor. They were encouraged to jump as if over an object (which for safety was not actually there).

180-TURN LINE JUMPS: As per Lateral line jumps except that a 180-degree turn was completed with each jump. The direction of the turn reversed with each single contact (forward/backward/forward…).

SPLIT SCISSOR JUMP WITH STEP: This was done as per the Split scissor jump, only the forward foot was elevated -on a step (12-16" based on height and ability) during both the take off and the landing. Jumps were still completed in succession as quickly as possible, and were still done with maximal jump height.

HIGH-BOX DOUBLE JUMP: Participants jumped onto a secure box and then immediately jumped again (maximally) slightly backwards and for height. A mat was placed on the floor to soften the final landing, as total jump height for the 2 jumps was as high as 60-70 inches). They were instructed to jump onto the box landing with bent legs, and to attempt to complete the second jump as soon after landing as possible with a maximal effort. The boxes were of varying heights based on vertical jump ability... sufficient to encourage significant knee flexion upon landing. A few seconds in between each pair of jumps was permitted, but not required.

Several of these jumps were designed intentionally to activate the quadriceps muscle and specifically the VL in order to maximize gains in that muscle. While this is not typical of plyometrics, it is a fair variation, and still includes the plantar flexion at the ankle that is associated with traditional plyometric training.

All rest times between sets of jumps were between 1:30 and 3:00 to allow for near complete rest and reasonable duration of the session (45-60min).

Appendix 6

MHC SDS-PAGE Protocol

After collection of muscle sample:

Cold Homogenize muscle samples in liquid N_2.Dissolve muscle sample in 40x (40 μL/1mg) of 2x Sample Buffer (1.4ML MQ water), 2.0ml glycerol, 4.0ml 10% SDS, 2.5ml Tris buffer (0.5M pH 6.8), 0.1 ml Bromophenol Blue, 0.5ml β-mercaptoehtanol). Mix and pour Bis:acrylamide gels (4% stacking, 7% resolving). Boil samples at 100^{O}C approximately 2 minutes before loading on gel. Using different Running; Upper; Lower Buffers (6.057g Tris-Base, 28.826g glycine, 10ml 10% SDS, H_2O to volume (1L); 50ml 5xRunning Buffer, 447.5ml H_2O, 10% SDS, 400μL β-mercaptoethanol (add immediately before use); 400ml 5x Running Buffer, 3580ml H_2O, 20ml 10% SDS) run under conditions: 14.5-54^{O}C, 275V, for 24 hours on 16x18cm Hoeffer gels. Stain using Sigma Aldrich silver staining kit.

Common steps (weighing of sample, homogenization procedure) have been abbreviated for brevity, as they are given in full for the titin analysis description (appendix 7). Protocol is an adaptation of methods used for individual fiber samples.

Titin VAGE Protocol

After collection of muscle sample:

SOLUBILIZATION: Weigh muscle samples on ice. Individual cold homogenize muscle samples in liquid N_2 by grinding with glass on glass homogenizing pestle for approximately 4 minutes. Keep samples at -20^{O}C when not in liquid N_2. Add 40 volumes of Urea Buffer (8M urea, 2M thiourea, 3% SDS w/v, 75mM dithiothreitol, 0.03% bromophenol blue, & 0.05M Tris-Cl, pH 6.8). Mix gently approximately 10x. Add cold glycerol buffer (10% Glycerol, 1% β-mercaptoethanol, 4.3mM Tris-HCl, pH, 8.8). Mix gently approximately 10x. Place in water bath at 60^{O}C and mix vigorously without spilling approximately 10x. Switch to larger pestle, and continue with gentle pumping for 4 minutes. Allow sample to sit in water bath for 10 additional minutes. Remove and pipet sample into microcentrifuge tubes. Spin for 5 minutes at 1300rpm at 4^{O}C. aliquot samples to desired storage volumes and keep in -80^{O}C freezer or in liquid N_2 until use.

GEL POURING: Clean 16x19cm glass gel plates with 70% ethanol. Grease edges of each plate (1 per pair). Use 1.5mm spacers to assemble sandwich and clamp. Set gel sandwich in cassette holder. Pour 12% acrylamide plug (10ml: 4ml 30% acrylamide, 5x Running buffer 0.250M Tris-Base, 1.92M glycine, 0.5% w/v SDS, diluted to 1L with H_2O), 1.89ml dH_2O, 0.1ml 10% Ammonium persulfate, 8.75μL TEMED) to about 1-2cm. Layer plug with isobutanol or 70% alcohol and allow to polymerize at RT 20-30 minutes. Dump isobutanol and wash with 1X Running Buffer. Place gel cassette and 60cc syringe in 60^{O}C incubator oven for at least 30 minutes. Prepare 100ml 1% agarose and pour immediately: Mix 30ml 30% w/v , 20ml 5X Running Buffer (filtered), 1.0g Agarose (1% w/v) and 48ml nanopure water to a final volume of 100ml with constant mixing. Heat covered until boiling, 9-10 minutes and check final volume for evaporation. Remove cassette and syringe from incubator and pour gel immediately. Add comb and allow to settle for 4-5minutes adding excess agarose to eliminate air bubbles. After allowing to cool to RT approximately 15-20 minutes, seal with plastic wrap and store overnight at 4^{O}C.

GEL LOADING AND RUNNING PARAMETERS: Prepare all Running Buffers (Upper: 100ml 5x Running Buffer, 0.4ml β-mercaptoethanol –add immediately prior to use; Lower: 1x Running buffer fill unit –concentration and volume varies based on size of chamber). Remove gel from fridge and clean out individual wells. Rinse wells with 1x Running Buffer +BME. Fill wells with fresh buffer after cleaning. Pour cold Lower Buffer into gel rig, and set temperature. Thaw samples in hot water bath (approximately 30 seconds at 60^{O}C) and aliquot appropriate amounts into each well (intial gels use 4.5 and 9μL; analysis gels use 6 volumes 2-12μL). Assemble Upper Buffer Chamber unit and add a small amount of cold buffer to check for leaks. Add remaining buffer. Run gel at 4^{O}C for 3 hours, 20 minutes at 15mA (220-400V) with constant stirring. Stop the run when blue dye is 2-3cm from bottom of gel.

Stain with Coomassie Brilliant Blue and read using densitometric scanning. First round should be run as initials to give an estimate of loading amount and ensure adequate preparation procedures were

carried out. Second round will consist of increasing loading amounts and a linearity check of the densitomety will serve to quantify titin based on the slope of this line.

Appendix 8

Additional Measures Recorded But Not Discussed

Electromyography. To substantiate the VL's contribution during running, an electromyography recording (EMG) was placed on this muscle during the treadmill testing procedures using a Delsys surface EMG and a Nexus 1.5 VICON data acquisition package (Boston, MA). This was done in part to add support for the VL biopsy, and possibly identify any significant changes in motor recruitment patterns during the running stride (brought about presumably from the training intervention). If motor recruitment patterns were unaltered significantly enough for the sensitivity of EMG recording to pick up, this measure would at the least add merit to the use of the VL biopsy for running data.

Biomechanics. Biomechanics data was collected during the treadmill portion of the test during all stages using an 8 camera VICON motion capture system (F40 cameras) with appropriate markers on the limbs and trunk (Los Angeles, CA). The results of this testing are beyond the scope of this paper and will not be discussed below, but procedures were repeated exactly pre to post, so as not to effect any of the other measures.

Data from these measurements are being analyzed as of the writing of this document.