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UNIVERSITY OF CALGARY

Changes in tendon compliance and muscle energetics of in vivo human skeletal muscle

by

Jared R Fletcher

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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Abstract

Recently published reports suggest the role of the muscles and tendons of the lower limbs are an important factor in determining the energy cost of running (E_{run}). Specifically, there exists a link between the mechanical properties of the Achilles tendon (AT) and E_{run} but the impact of the muscle's energy cost is not considered. To date, very little is known regarding the interaction between AT stiffness, muscle energetics and E_{run} . Further, little is known about the AT stiffnessenergetics relationship in female runners. Therefore, the overall goal of this thesis was to explore the relationship between AT stiffness and muscle energetics in male and female distance runners.

The first study revealed AT stiffness of female runners was lower than in males, but E_{run} was similar to males. Further, the relationship between E_{run} and Achilles tendon stiffness was not different between the sexes. Results from the second study demonstrated that when reductions in AT stiffness were simulated, the rate of muscle energy use was elevated and the magnitude of muscle activation needed to reach a target force was increased.

A novel method of assessing AT moment arm was assessed in study four. A key finding was that moment arm did not change through ankle range of motion. These results were used in the fifth study which demonstrated using estimates of muscle energetics, along with kinematics and kinetics during running that strain energy release from the AT during running was significantly lower than the muscle energy cost required for strain energy storage to occur. Lastly, using a prolonged run as an acute method of reducing AT stiffness, the impact of changes in AT stiffness during running on muscle energetics and E_{run} was evaluated. Results from this final study suggest that prolonged running reduces AT stiffness, the impact of which is an elevated muscle energy cost and increased whole-body E_{run} without a significant increase in estimated AT strain energy release. Together these findings support the notion that the role of the AT in running is to accommodate muscle-tendon unit length change, thereby reducing the amount of muscle fascicle shortening and therefore muscle energy cost.

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Preface

Chapters two through seven, respectively, are based on the following manuscripts:

Fletcher, J.R., and B.R. MacIntosh. Energetic aspects of running economy. In preparation.

- Fletcher, J.R., Pfister, T.R. and B.R. MacIntosh. Energy cost of running and Achilles tendon stiffness in male and female trained runners. Physiol Rep, 1(7), e00178, doi:10.1002/phys2.178, 2013. Used under the terms of the Creative Commons Attribution License.
- Fletcher, J.R., Groves, E.M., Pfister, T.R. and B.R. MacIntosh. Does muscle shortening alone explain the energy cost of muscle contraction in vivo? European Journal of Applied Physiology, 113: 2312-2322. Used with kind permission of Springer Science+Business Media.
- Fletcher, J.R., and B.R. MacIntosh. Estimates of Achilles tendon moment arm length at different ankle joint angles: effect of passive force. Re-submitted to Experimental Physiology, November 5, 2014. EXPPHYSIOL/2014/084004
- Fletcher, J.R. and B.R. MacIntosh. Achilles tendon strain energy in distance running: consider the muscle energy cost. Journal of Applied Physiology, in press. DOI: 10.1152/japplphysiol.00732.2014. Used with kind permission of the American Physiological Society.
- Fletcher, J.R. and B.R. MacIntosh. Changes in Achilles tendon stiffness and energy cost following a prolonged run in trained distance runners. Submitted to Applied Physiology, Nutrition, and Metabolism, October 21, 2014, apnm-2014-0446.

This dissertation is based on a collection of stand-alone manuscripts, and there may be some redundancy in the introduction, methods and discussion of chapters two through seven.

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List of symbols, abbreviations and nomenclature

Symbol	Definition
AT	Achilles tendon
ATP	Adenosine triphosphate
[BLa ⁻]	Blood lactate concentration
BM	Body mass
Ca ²⁺ ATPase	Calcium adenosine triphosphate
CSA	Cross-sectional area
$d_{\rm L}$	Elongation
EC	Energy cost
EC _M	Rate of muscle energy use
E _M	Rate of muscle use per unit volume
EM	Elite male
EMG	Electromyography
E _{run}	Energy cost of running
F _M	Muscle force
Fz	Vertical ground reaction forces
HbO ₂	Oxyhaemoglobin
HHbO ₂	Deoxyhaemoglobin
ISO	Isometric
K	Achilles tendon stiffness
KIN	Isokinetic
L_{f}	Fascicle length
LG	Lateral gastrocnemius
MA	Moment arm
M _C	Corrected moment
MG	Medial gastrocnemius
M_{M}	Measured moment
MVC	Maximal voluntary contraction
NIRS	Near-infrared spectroscopy
RER	Respiratory Exchange Ratio
RMS	Root Mean Square
RUN	90 minute prolonged run
S	Speed
SERCA	Sarco-endoplasmic reticulum Ca ²⁺ ATPase
	-

symbol	Definition
SL	Stride length
sLT	Speed of lactate threshold
SOL	Soleus
SR	Sarcoplasmic reticulum
SWC	Smallest worthwhile change
TE	Tendon Excursion (method)
TF	Trained female
ТМ	Trained male
TS	Triceps Surae
V/V _{max}	Velocity of shortening relative to maximal unloaded shortening velocity
VL	Vastus lateralis
ΫO _{2max}	Maximal rate of oxygen uptake
<i>V</i> 0₂	Rate of oxygen uptake

"Great ideas originate in the muscles."

-Thomas A. Edison

Chapter One: Introduction

1.1 General introduction

This thesis presents an investigation into the changes in tendon stiffness and muscle energetics of in vivo human skeletal muscle and is compiled as a series of independent manuscripts. Therefore, each chapter contains an introduction specific to that investigation. A general introduction to the thesis is presented here.

The energy cost of running (E_{run}) has been a unique trait to human evolution, as it allowed our *Homo sapiens* ancestors to cover great distances on a finite energy supply. This allowed these individuals the unique advantage of hunting by foot over great distances while generating very little metabolic heat. The reduced metabolic heat generation, as well as the ability to dissipate that heat which is generated offered a unique advantage to *Homo sapiens* over the rest of the animal kingdom, who did not have this advantage of economy of locomotion and thermoregulatory capacity (Bramble and Lieberman, 2004). Since quadrupeds cannot simultaneously pant and gallop (Bramble and Jenkins, 1993), the technique of forcing animals to gallop over long distances particularly during the hottest times of day, allowed early *Homo sapiens* to literally follow the hunted animal until the animal was driven to hyperthermia and died.

Today, the importance of E_{run} is highlighted in that it is an important physiological predictor of distance running performance among elite runners and may be the key performance variable in breaking the two-hour marathon. As it relates to health, understanding the factors which dictate a good exercise economy may be beneficial in that it allows one to perform exercise or activities of daily living at a low metabolic cost. This becomes important when individuals are faced with a rapidly-declining maximal oxygen uptake in the face of aging or

disease; a good exercise economy, defined as a low metabolic cost, may be the sole way of accomplishing these routine tasks.

The energy cost of exercise is primarily determined by the energy cost of muscle contraction yet much of the work studying the energy cost of running does not consider what the muscles are doing. More specifically, few studies have investigated the interaction between muscle and tendon in minimizing the energy cost of muscular contraction and how this may translate to improving the whole-body E_{run} . Furthermore, virtually nothing is known about muscle- tendon interactions in female runners. Most research on this topic has been done on male runners.

It has become apparent in recent years that the role of the muscle-tendon unit in the lower limbs may be an important determinant of E_{run} . During running, muscular contractions are repeated. The energy cost of these contractions is thought to be dependent on the required force and the amount of fibre shortening during the contractions. The tendon, if sufficiently compliant, may allow the fibres to remain isometric during a stretch-shorten cycle of the whole muscle-tendon unit. As a result, the tendon alone can accommodate the joint range of motion, keeping the energy cost of the contraction low (Roberts *et al*, 1997). Also during these actions, elastic energy is stored in the tendon (Voigt *et al*, 1995) during the tendon stretch, and a portion of this elastic energy is returned during the shortening phase (Cavagna *et al*, 1968, Voigt *et al*, 1995). The tendons of the lower limbs act as a spring. There is growing evidence to suggest that the elastic recoil provided by the tendon contributes a significant portion of the energy for propulsion (Arampatzis *et al*, 2006, Hof *et al*, 2002, Lichtwark and Wilson, 2008, Scholz *et al*, 2008). The elastic properties of tendons can enhance muscle performance, as well as reduce the energetic cost of contraction, during stretch-shortening cycle activities because tendon stretch and recoil reduces the muscular work (Gabaldon *et al*, 2008, Lichtwark *et al*, 2007), and muscle work is thought to require additional energy beyond that required for isometric force maintenance (Ryschon *et al*, 1997, Chasiotis *et al*, 1987, Bergstrom and Hultman, 1988, Russ *et al*, 2002).

It has previously been shown that in a group of trained distance runners, the most economical runners displayed a higher stiffness of the Achilles tendon (AT) compared to the less economical runners (Arampatzis *et al*, 2006, Fletcher *et al*, 2010). To date, it is unclear how and why a stiff AT may be associated with a lower E_{run} .

There are apparent advantages of stiff tendons in some cases, and compliant tendons in other cases. The lengthening of a tendon for energy storage is relevant in stretch-shortening cycles where a substantial pre-stretch of the tendon occurs early in a contraction. A compliant tendon allows more energy conversion of either kinetic or gravitational energy to potential energy. This energy can subsequently be released upon shortening. A compliant tendon may also help by allowing the tendon to lengthen during the stretch phase of the stretch-shortening cycle thereby keeping fascicle shortening velocity low. This permits high active force to be generated. In contrast, a stiff Achilles tendon is associated with lower E_{run} , in spite of lower capacity for elastic energy storage and return.

Differences in the mechanical properties of the tendon will result in differences in the storage and release of tendon strain energy as well as the muscle energy cost to allow that tendon strain energy to occur. To date, the interaction between tendon strain energy and the associated muscle energy cost has not been examined, nor has this interaction been studied where AT mechanical properties are altered acutely.

1.2 Purpose of the research

Therefore, the purpose of this thesis was to examine changes in tendon compliance and skeletal muscle energetics in vivo. Examining the difference in muscle and whole-body energy cost between stiff and compliant Achilles tendons was accomplished in several ways:

- By examining differences in E_{run} between males and females, where it was anticipated the AT mechanical properties would differ.
- 2. By acutely altering AT stiffness by allowing additional shortening of the muscle tendon unit during contraction and examining the change in the rate of muscle energy use.
- 3. By examining differences in E_{run} and AT mechanical properties between elite and trained runners, where it was anticipated the AT mechanical properties would differ.
- 4. By estimating the amount of AT strain energy released per stride, and the associated muscle energy cost for this tendon strain energy storage/release to occur in elite and trained male and female runners.
- 5. By estimating the change in AT mechanical properties and the associated energy cost of muscle contraction before and after a prolonged run, where it was predicted the AT mechanical properties and whole-body E_{run} would be altered as a result of the run.

1.3 Hypotheses

It was hypothesized that:

 E_{run} and AT mechanical properties would differ between similarly-trained male and female runners.

- Additional muscle fascicle shortening, as measured by ultrasonography would result in an elevated muscle energy cost measured using near-infrared spectroscopy.
- E_{run} measured at a common relative speed would differ between elite and trained runners. These differences would be partially attributable to the differences in AT mechanical properties between elite and trained runners.
- 4. The differences in AT mechanical properties would result in differences in both the estimated amount of tendon strain energy and muscle energy cost for storage of tendon strain energy to occur between elite and trained male and female runners.
- 5. A prolonged submaximal run would result in:
 - a. A reduction in AT stiffness
 - b. An increase in the estimated tendon strain energy storage/release
 - c. An elevated muscle energy cost in order for tendon strain energy to occur, a result of elevated muscle fascicle shortening following the run.
 - d. An elevated E_{run} following the prolonged run, some of the elevated energy cost being attributable to the elevated triceps surae muscle energy cost post-run.

1.4 Overview of separate chapters

Chapter two is dedicated to understanding the major factors that influence E_{run} from a muscle energetics perspective. Many reviews have been dedicated to understanding the specific factors dictating E_{run} and how they may be improved with various forms of training (Saunders *et al*, 2004, Anderson, 1996, Barnes and Kilding, 2014). However, most studies do not consider the fact that our skeletal muscles require energy to contract and this is where the energy is going. Here, we review the general factors that influence the energy cost of running, and try to put them

into the context of understanding the role that muscle contraction and muscle energetics plays in contributing to the variability in the E_{run} and how, from a muscle energetics point of view, the energetic factors which likely can and cannot be changed with training.

In Chapter 3, we address the question of whether or not there are differences in E_{run} between similarly-trained male and female distance runners. While this question has been addressed previously by several authors (Ingham *et al*, 2008, Daniels and Daniels, 1992, Helgerud *et al*, 2010, Pate *et al*, 1992), there is still contention as to whether differences between male and female runners exist. We believe much of this controversy arises from the inappropriate measurement of E_{run} itself. Furthermore, very little is known regarding the specific AT mechanical properties in female runners and how this relates to E_{run} . If E_{run} does not differ between the sexes, yet the AT mechanical properties do, why might this be the case? Is AT stiffness 'tuned' to a higher compliance in the slower runners in order to minimize muscle shortening, and therefore run with a lower muscle energy cost?

In Chapter 4 we address whether additional muscle shortening and/or work contributes to an elevated muscle energy cost, and therefore likely an elevated E_{run} . To accomplish this, we measured muscle fascicle shortening, the level of muscle activation and the rate of muscle energy use in vivo during isometric and isokinetic contractions. These data have allowed us to quantify *in vivo* the differences in the rate of muscle energy use between isometric and isokinetic contractions, where it was anticipated the amount and rate of muscle shortening would differ.

In order to appropriately estimate triceps surae muscle and Achilles tendon forces from joint moments during running, it is important to accurately estimate the AT moment arm at different ankle angles. In Chapter 5 we propose a novel correction for passive force in estimating the AT moment arm length. These data allowed us to accurately estimate tendon

forces during running in order to estimate AT tendon strain and muscle energy cost during running.

Chapter 6 presents the relationship between AT strain energy storage and release and the estimated corresponding muscle energy cost for this strain energy storage to occur, allowing us to reconsider the role of the AT during distance running. Given that elite runners have considerably stiffer ATs, they should have reduced capacity of the AT to store tendon strain energy. Here, we further evaluate the idea that the role of the AT in running is to reduce muscle shortening, thereby reducing muscle energy cost.

In Chapter 7 we continue with the idea that the role of the AT during distance running is not to store and release strain energy but to reduce muscle shortening; we have evaluated the change in AT stiffness and E_{run} following a prolonged run in trained distance runners. It was anticipated that the prolonged run would result in dynamic creep and thus reduced AT stiffness. Therefore the amount of strain energy stored and released during each stride should increase. However, we also anticipated that this reduced AT stiffness would result in an elevated muscle energy cost, thus reducing the effectiveness of the additional storage and release of tendon strain energy.

Lastly, we offer some general conclusions regarding the role of the AT during distance running and how this relates to muscle energetics *in vivo*. Furthermore, commentary regarding how this work can be applied to future research, with particular attention to the interaction between *in vivo* tendon mechanical properties and muscle energetics during exercise.

Chapter Two: Energetic Aspects of Running Economy

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2.1 Abstract

The economy of running has traditionally been studied as the oxygen cost of running, and there is a considerable body of literature that presents economy this way, without consideration of the energy equivalent. Fundamentally, the understanding of the major factors that influence the energy cost of running can be obtained with this approach. However, a recent return to presenting economy as an energy equivalent (Fletcher et al., 2009, Shaw et al., 2014) has allowed a refocusing of the topic on the origin of the need for energy and this approach will provide a more basic understanding of the factors affecting the energy cost of running (E_{run}).

Without muscle contraction, running would be impossible. Yet most of what we understand about E_{run} ignores the fact that our skeletal muscles require energy to contract and this is where the energy is going. Here, we review the general factors that influence E_{run} , and try to put them into the context of understanding the role that muscle contraction and muscle energetics plays in contributing to the variability in the E_{run} . In order to achieve this approach successfully, it is important to understand the determinants of muscle energy cost that are not affected by training. These include, environmental factors, surface characteristics and certain anthropometric features. We contrast this with a presentation of the factors affecting E_{run} that are affected by training, including certain anthropometric features, muscle and tendon properties and running mechanics. Summarizing the key features that dictate muscle energy cost during distance running has allowed us to consider the influence of biomechanics (limb weight and length, AT and vertical ground reaction force etc) and physiology (force-length-velocity properties of muscle) in dictating the muscle energy cost, and therefore E_{run} .

2.2 Introduction

2.2.1 Importance of E_{run} to distance running performance

Endurance running performance is determined by a combination of physiological factors. These include a high maximal oxygen uptake $(\dot{V}O_{2max})$, the ability to minimize disturbances to homeostasis at a higher fraction of $\dot{V}O_{2max}$ and the energy cost required to run (E_{run}) at that high fraction of $\dot{V}O_{2max}$. The ability to tolerate disturbance to homeostasis may also be important. With few exceptions, world-class marathon running performances are achieved in runners who possess $\dot{V}O_{2max}$ values above 75 ml·kg⁻¹·min⁻¹ and the portion of $\dot{V}O_{2max}$ that can be sustained for the marathon distance is at least 80% of $\dot{V}O_{2max}$ (Foster and Lucia, 2007). Using the ACSM metabolic equations for the energy cost of running over level ground, a mean $\dot{V}O_2$ of 71.9 ml·kg⁻ ¹•min⁻¹ is required to achieve the current marathon world-best (2:03:23, IAAF). Given this assumed $\dot{V}O_2$ to run at this speed (342 m•min⁻¹) and assuming the runner could maintain 80% of $\dot{V}O_{2max}$ for the marathon, this effort would require a $\dot{V}O_{2max}$ of nearly 90 ml·kg⁻¹·min⁻¹. Values this high have rarely, if ever, been reported in distance runners. If an elite marathoner could sustain 90% of $\dot{V}O_{2max}$ for over two hours, this would require a $\dot{V}O_{2max}$ of 80 ml·kg⁻¹·min⁻¹ to surpass the current world marathon best time. $\dot{V}O_{2max}$ values this high have been reported in elite runners but it seems unlikely that elite runners could sustain relative intensities of 90% $\dot{V}O_{2max}$ for this time frame (Billat et al., 2001).

So how are such phenomenal running performances possible? It is likely the energy demanded by an elite marathoner to cover the distance in world-record time is significantly lower than that estimated by the ACSM metabolic equation. Assuming the marathon distance is sustained at a relative intensity of 80% of $\dot{V}O_{2max}$, and E_{run} was reduced by 10%, then the

required $\dot{V}O_{2max}$ would *only* be 81 ml•kg⁻¹•min⁻¹. Values this high have been frequently reported in elite distance runners (Pollock, 1977; Zhou et al., 2001). Thus, E_{run} can greatly influence the speed at which the marathon can be sustained, and will likely be a key determinant in breaking the 2-hour mark for the marathon (Joyner et al., 2011). These estimates, however, do not consider that E_{run} may increase over the course of the run (Brueckner et al., 1991; Petersen et al., 2007). This phenomenon, which has been largely unexplored in elite distance runners, may be one of the main contributors preventing a sub 2-hour marathon (Fletcher et al., 2011) and further highlights the important impact of E_{run} on distance running performance.

It is known that E_{run} is likely influenced by a number of physiological and biomechanical factors and several excellent reviews have been written on the topic in the last 25 years (Anderson, 1996; McCann and Higginson, 2008; Morgan et al., 1989; Morgan and Craib, 1992; Saunders et al., 2004). Recently, we have estimated that the active muscle energy cost represents a substantial portion of the total metabolic cost of running (Fletcher and MacIntosh, 2014). Specifically, we have estimated that the energy cost of triceps surae muscles contraction during the running stride of highly-trained runners represents nearly 25% of the total metabolic cost of running. This proportion increases to nearly 40% in lesser-trained male and female runners (Figure 2.1). The energy cost of other active muscles would also contribute to the total metabolic cost of running. Consequently, understanding the specific factors that dictate the muscle energy cost during running offers a unique understanding of the underlying factors which might also dictate E_{run} . However, to date the factors which dictate the muscle energy cost during running have not been given appropriate consideration.

2.2.2 Quantifying the energy cost of running

It has long been established that there exists a linear relationship between running speed and oxygen uptake in humans (Henry, 1951; McMiken and Daniels, 1976). E_{run} has predominantly been expressed as the steady-state $\dot{V}O_2$ (ml·kg⁻¹·min⁻¹) at a particular running speed (Daniels, 1985); a low $\dot{V}O_2$ at a given speed implying a 'good running economy'. A good running economy (low E_{run}) further suggests success in or previous training for athletic endurance performance (Conley and Krahenbuhl, 1980; Daniels and Daniels, 1992; Daniels, 1985); therefore, considerable effort was made in assessing distance running performance potential by measuring $\dot{V}O_2$ at a given submaximal speed. For example, Dill (1965) found that the $\dot{V}O_2$ of a champion marathoner was 17% lower than a champion miler running at the same speed. Similar findings that marathoners may be more economical than runners specializing in shorter distances have frequently been reported (Costill et al., 1973; Pollock, 1977). This observation is probably a consequence of the measured speed being better suited to the marathoners' typical training paces. Furthermore, $\dot{V}O_2$ is frequently found to vary in similarly-trained runners of equal abilities (Daniels, 1974) and has been identified as an important factor in the success of East African distance runners (Lucia et al., 2006; Wilber and Pitsiladis, 2012).

ATP is resynthesized from ADP and P_i from the energy released during oxidative phosphorylation. O₂ is consumed when it accepts electrons at the end of the electron transport chain to form ATP via ATP synthase. Thus, $\dot{V}O_2$ reflects the quantity of ATP used when aerobic metabolism can provide all of the energy at a given running speed. This is only true: 1) when sufficient time is given to achieve a physiological steady-state and 2) when the speed is less than that which results in accumulation of blood lactate. This latter point is important because at speeds greater than this, steady-state conditions are unlikely as a result of the $\dot{V}O_2$ slow component and non-aerobic metabolism contributes to the energy cost.

Berg (2003) conceded that measurement of $\dot{V}O_2$ was reliable but suggested that using the $\dot{V}O_2$ to assess E_{run} may not be entirely sound. He recognized that $\dot{V}O_2$ does not account for the type of substrate utilized, a factor that modifies the energy equivalent per volume of oxygen metabolized. This fact has been given previously to mistakenly justify the expression of E_{run} as simply a $\dot{V}O_2$. However, substrate selection may be important in long duration events where sparing of muscle and liver glycogen is important. As a consequence, Berg (2003) suggested it was important to consider the respiratory exchange ratio and complete the calculation of E_{run} . Based on this notion, we suggested that the measurement of the energy cost to run a given distance (kcal or kJ per km) was a more sensitive and more appropriate measure of E_{run} than $\dot{V}O_2$ alone (Fletcher et al., 2009). Recently, these suggestions were confirmed, showing that this method of expressing E_{run} in terms of energy cost is a more valid and reliable measure of E_{run} compared to $\dot{V}O_2$ or O_2 cost alone (Shaw et al., 2013; Shaw et al., 2014).

Despite the vast array of research on the aerobic demand of running at a common speed, comparing athletes at the same absolute running speed does not account for differences in the speed associated with the lactate threshold (sLT), which is an estimate of the anaerobic threshold. The anaerobic threshold is defined as the highest sustained intensity of exercise for which measurement of oxygen uptake can account for the entire energy requirement (Svedahl and MacIntosh, 2003). Consequently, by not accounting for differences in sLT between runners, the same absolute running speed represents a different relative speed for each runner. Expressing E_{run} at a common relative speed below the lactate threshold is important to ensure that a $\dot{V}O_2$ steady-state can be achieved and to minimize differences in substrate use. We (Fletcher et al., 2009) and others (Costill et al., 1979) have demonstrated that the respiratory exchange ratio (RER) increases as a function of running speed and with it, the energy made available per liter of oxygen increases (Lusk, 1928). Lastly, it seems logical to measure E_{run} at a speed that relates to some competition distance, like the marathon (ie. at a similar intensity relative to the lactate threshold) as long as that speed is below the speed at which lactate accumulates.

Despite the expression of E_{run} in terms of energy having been established in the 1960s (Margaria et al., 1963), it is only recently that studies have taken advantage of the additional information given by the RER (Albracht and Arampatzis, 2013; Di Prampero et al., 1986; Di Prampero et al., 1993; Fletcher et al., 2013a; Franz et al., 2012; Mooses et al., 2013; Pialoux et al., 2008; Shaw et al., 2013). Therefore, we suggest appropriate expression of E_{run} should be done in terms of units of energy (kcal or kJ) and per unit distance (per km) and per body mass (kg) rather than simply a $\dot{V}O_2$ to best reflect the actual energy cost and to permit comparisons when the absolute speed of running is different. In calculating E_{run} in this way, we were able to address such issues as the apparently paradoxical negative relationship between $\dot{V}O_{2max}$ and E_{run} (Morgan and Daniels, 1994; Pate et al., 1992) and apparent differences in E_{run} in similarly-trained male and female runners (Daniels and Daniels, 1992; Fletcher et al., 2013a; Helgerud, 1994; Helgerud et al., 2010).

2.3 Skeletal Muscle energetics

Without muscle contraction, running would be impossible. Yet most of what we understand about E_{run} ignores the fact that our skeletal muscles require energy to contract and

this is where the energy is going. Here, we review the general factors that influence the energy cost of running, and try to put them into the context of understanding the role that muscle contraction and muscle energetics plays in contributing to variability in the E_{run} . The energy cost of muscle contraction is primarily dictated by the rate at which ATP is hydrolyzed. The primary use of ATP within the muscle can be divided into two portions: the energy cost of cross-bridge cycling and of ion pumping.

2.3.1 Skeletal muscle energetics

2.3.1.1 Cross-bridge turnover and activation (ion pumping)

Muscle energy cost in vivo arises from cross-bridge turnover as well as non-cross bridge processes. The latter being the energy cost of ion pumping, primarily from the Na⁺-K⁺ ATPase and the sarco-endoplasmic reticulum Ca²⁺ ATPase (SERCA) pumps. The energy cost associated with Ca²⁺ re-uptake represents the majority of the energy cost associated with ion pumping (Homsher and Kean, 1978).

There are two main methods to estimate the non-cross-bridge energy cost of contraction. Typically, this energy cost is calculated during isometric contractions when muscle length is changed above optimal length and estimated for a long length where filament overlap would be prevented (Barclay et al., 1993) or by preventing cross-bridge interactions pharmacologically. In doing so, cross-bridge cycling, and thus ATP use at the cross-bridge site, is prevented (Young et al., 2003). Eliminating energy for cross-bridge interactions yields the energy use for non-crossbridge function. This is assumed to be primarily for ion pumping.

Excitation-contraction coupling (E-CC) is the sequence of events in a muscle including the following: an action potential is propagated along the surface membrane and into the

transverse tubules; Ca^{2+} is released from the sarcoplasmic reticulum (SR); Ca^{2+} is then bound to troponin, causing a re-configuration of tropomyosin and the ultimate binding of the myosin head to actin; ATP is broken down by actomyosin ATPase, releasing energy and causing the myosin head to swing resulting in force development and/or translation of the actin filament. This E-CC includes passive activation as well as the energy requiring cross-bridge interactions. Energy is not required for action potential propagation or Ca^{2+} release. Chemical energy is required in order to regenerate ATP that is used for crossbridge turnover (section 2.3.1.2).

Relaxation occurs when the Ca^{2+} is actively transported out of the cytoplasm and back into the SR. Energy is required by the SERCA pump, which shuttles 2 Ca^{2+} ions per ATP hydrolyzed (Rüegg, 1986). When contractions are of short duration, the energy cost is greater because of the fibre shortening against the series elastic structures during force development. . Combining the energy cost of SERCA and Na⁺-K⁺ ATPase pumps accounts for 30-40% of the energy used during an isometric contraction, where the energy associated with cross-bridge cycling as a result of shortening is not considered (Barclay et al., 1993; Barclay, 1996; Homsher et al., 1972). When shortening is considered, the proportion of the energy cost attributed to noncrossbridge ATPases is much less because shortening considerably increases the cross-bridge turnover (Smith et al., 2005). Considering this cost of shortening, an isometric contraction is more costly in the initial part of the contraction when force is rising because the fibres shorten against the series elastic components of the muscle. During locomotion, most muscle contractions are of short duration, so this is a relevant aspect of the energy cost of locomotion.

2.3.1.2 Turnover of cross-bridges

2.3.1.2.1 Isometric contractions

During an isometric contraction, energy cost is elevated compared to the resting state. Since, by definition no external work is performed during an isometric contraction, the energy cost must arise primarily from time-dependent cross-bridge cycling. Barclay et al. (2010a) have previously estimated this rate to be 1.5 ATP split•s⁻¹ in frog sartorius muscle at 0°C. In human muscle, assuming the ATP turnover rate is 1 mmol•kg⁻¹ wet wt•s⁻¹ (Katz et al., 1986) and a crossbridge concentration of 0.18 mM (Barclay et al., 2010b), each cross-bridge splits 5.6 ATP per second. That is, the ATP splitting cycle requires 180 ms. A cross-bridge duty cycle of 0.3 (Barclay et al., 2010a) would require the cross-bridge be attached for 60 ms; the remaining 120 ms presumably being required for the cross-bridge to return to a state from which it can attach again. This duty cycle would be fibre-type specific; a shorter duty cycle associated with fasttwitch fibres. Thus, the energy required during an isometric contraction is dependent solely on the required force (which dictates the number of cross-bridges required to support that force) and the contraction duration. However, recognizing that as force develops in an isometric contraction, the tendon is stretched thereby requiring shortening of the fibres, additional crossbridge cycles will be required.

2.3.1.2.2 Shortening contractions

Shortening at a velocity greater than a critical velocity can increase the rate of turnover of individual cross-bridges. This critical velocity depends on the rate of shortening being fast enough that the isometric crossbridge turnover is exceeded and is different for myosin isoforms: faster for fast-twitch myosin isoforms. This critical velocity is the equivalent of a cross-bridge sweep (per half sarcomere) per isometric cross-bridge cycle time for each half sarcomere fibre length. At the optimal velocity, that for which efficiency is maximal, the energy cost of a

shortening contraction is 2-3 fold greater than that expected during an isometric contraction. This increase in energy demand is referred to as shortening-induced increase in ATP turnover (Woledge et al., 1988). The amount of ATP split, and therefore energy use by the muscle is proportional to the amount of shortening within each half sarcomere and is dependent on the working stroke (or cross-bridge sweep) of each cross-bridge. This working stroke distance increases as a function of shortening velocity (Barclay et al., 2010a). At velocities greater than that associated with optimal efficiency, the cross-bridge remains attached beyond the filament displacement at which their force reaches zero, opposing filament movement generated by cross-bridges at earlier stages of their attachment cycle. These cross-bridges thus contribute to the decreasing average force per cross-bridge as shortening velocity increases.

Therefore the shortening energy cost is also proportional to shortening velocity (Hill, 1938; Homsher and Kean, 1978).

2.3.2 Energy cost of muscle contraction

Running can be considered a series of voluntary muscle contractions; the force of contraction being dictated by the running speed and the controlled motion of the lower leg. The required level of voluntary muscle activation is primarily determined by the force-length-velocity relationships of the muscle and the need for force or movement through a specific angular displacement. The level of muscle activation a combination of motor unit recruitment and rate coding as measured by surface EMG, dictates the energy cost since the rate of energy consumption depends on the number of fibres activated and the rate and number of cross-bridge cycles required. The volume-specific rate of energy consumption is greater in fast-twitch
muscles during isometric contractions and slow shortening because faster muscles have higher rates of time-dependent cross-bridge cycling (Katz et al., 1986; Rall, 1985).

The energetic cost of generating force is also dependent on the average length of the activated muscle fibre. For muscles having similar fibre type compositions and operating under similar levels of activation and shortening velocities (relative to length), muscles with shorter fascicles can be expected to consume proportionally less ATP per unit force generated compared to muscles with longer fascicles (Roberts et al., 1998b). The volume of active muscle recruited to generate the required force is the product of fascicle length and active cross-sectional area. Consequently, a muscle with longer fascicles will require a greater active volume of muscle and therefore, a greater amount of ATP will be consumed. Thus, it seems likely that muscle architecture is adapted to economize the metabolic cost of generating the required force. The link between muscle fascicle length, tendon stiffness and muscle contractile energetics has been modeled previously (Lichtwark and Wilson, 2008). These authors have demonstrated that maximum *efficiency* is achieved over a wide range of muscle fascicle lengths and tendon stiffness values; however, most importantly, these authors demonstrated that different muscle fascicle lengths and tendon compliance combinations are required to maximize contractile efficiency depending on the gait conditions (walking vs running) and speed. Their results suggest different fascicle length-tendon compliance combinations are selected to keep muscle shortening velocity (and therefore the amount of muscle work) low. This reduces the required volume of activated muscle according to the force-velocity relationship. This idea is wellmatched with the energy cost of running considering that during running, the triceps surae muscles perform little work (Ishikawa et al., 2007; Roberts et al., 1997) and economy of muscle force generation, rather than *efficiency*, may be the more relevant feature.

2.3.2.1 Force-length relationship

The force of contraction during running is dictated by the running speed and the controlled motion of the lower leg. It has been known for some time that the force a muscle can produce depends on its average sarcomere length whereby an optimal muscle length exists. Muscle contraction at longer or shorter lengths than this optimal length results in less muscle force (Gordon et al., 1966; Ramsey and Street, 1940). As it relates to E_{run} , for a given amount of muscle force required to run a particular speed, the required level of activation can be minimized if the muscle is operating near optimal length. In keeping the level of activation low, muscle energy cost and therefore E_{run} can be reduced (Stainsby and Lambert, 1979).

The classic experiments of Gordon et al. (1966) were the first to relate this force-length curve to the amount of myofilament overlap and the corresponding number of cross-bridges available to develop force. At sarcomere lengths above and below optimum, the measured tension decreases as a function of the number of active cross-bridges available to develop the tension. The force-length relationship has been expanded to in vivo human sarcomere length estimation (Walker and Schrodt, 1974) and the quantification of the force-length relationship of whole muscle using ultrasound (Austin et al., 2010; Finni et al., 2001; Ichinose et al., 1997; Maganaris, 2003). An important consideration, however, is that the classic force-length curve of Gordon et al. (1966) applies only to maximally active muscle fibers. As it relates to whole-body energy cost, very few exercises are performed under conditions of maximal activation.

During submaximal stimulation, the length at which peak force occurs is longer than the optimal length associated with maximal stimulation. This is apparent both in situ (Rack and Westbury, 1969) and in vivo (Ichinose et al., 1997) and appears to be associated with enhanced

contractile response at long muscle lengths (Lambert et al., 1979; Rassier et al., 1999). This probably relates to a decrease in intermyofilament spacing and subsequent changes in calcium sensitivity at long sarcomere lengths (Fuchs and Wang, 1991; Stephenson and Wendt, 1984; Wang and Fuchs, 1994).

At short muscle-tendon unit lengths, the tendon is slack (Huijing et al., 1989). Thus, muscle shortening is required simply to take up the tendon slack prior to the development of any appreciable joint moment. This can be seen in vivo as a large toe region of the force-tendon elongation curve where considerable stretch the tendon occurs with little force development (Figure 2.2). For a given muscle-tendon unit length, a higher force is associated with more tendon elongation and a corresponding additional muscle shortening. This additional shortening could result in the muscle sarcomere length being shorter than the optimal and would necessarily increase the level of muscle activation needed to reach a given force (Ichinose et al., 1997).

2.3.2.2 Force-velocity relationship

The relationships between mechanical work, efficiency and speed of shortening were first proposed by AV Hill almost 100 years ago (Hill, 1922). Since the rate of mechanical work (or power output) is the product of force and velocity, the maximum power output that can be generated by a muscle, or group of muscles, is defined and limited by their force-velocity relationships. The now commonly used hyperbolic force-velocity equation of Hill (1938) dictates the conditions for power output.

Since power is the product of force and velocity, the power-velocity relationship is also bound by this equation. However, under conditions of submaximal activation, the velocity at which peak power is achieved is slower and peak power achieved is lower. This has been

extensively described previously (Chow and Darling, 1999; MacIntosh et al., 2000; Sargeant, 2007). For a given force requirement, the level of activation can be minimized if the muscle can operate at a lower shortening velocity (Figure 2.3). In running, where a set force must be generated, the level of activation can be minimized if the velocity of shortening is low (Fletcher et al., 2013b).

2.3.2.3 Motor unit recruitment

The muscle's in vivo force-length and force-velocity relationships dictate the magnitude of activation required to achieve a given force and velocity of shortening (Praagman et al., 2006). The force-velocity relationship dictates that force production for a given level of activation is maximal when that force can be developed isometrically (Roberts et al., 1997, Gabaldon et al., 2008, Biewener, 1998, Fenn and Marsh, 1935) and decreases as shortening velocity increases. Stainsby and Lambert (1979) suggest that the major determinant of metabolic cost of contraction in voluntary movement should be motor unit recruitment. This notion is consistent with the observed increase in EMG during cycling, which has a minimum at a unique cadence associated with a given power (MacIntosh et al., 2000), and this cadence is closely related to the optimal cadence for best efficiency (Coast and Welch, 1985). Load, shortening and velocity of shortening have less impact on the magnitude of energy requirement (Stainsby and Lambert, 1979). For submaximal contractions like those imposed during running, the level of activation needed to generate a given force can be minimized when the fascicles are allowed to develop force isometrically.

Keeping the underlying factors dictating energy use in muscle in mind, attention will turn to the energy cost of running: those factors that are not affected by training will be considered. This will be followed by an examination of those factors which can be affected by training.

2.4 Factors not affected by training

 E_{run} can acutely change by factors other than those factors related to training. These factors include environmental factors (wind, temperature, altitude), surface features and footwear.

2.4.1 Environment

2.4.1.1 Wind

The energy required to overcome wind-resistance is a function of the runner's frontal surface area (section 2.4.3.3), air density (altitude, humidity and pressure) and the wind velocity. Pugh (1971) found that work required to overcome wind resistance was a linear function of running speed and wind velocity-squared. As such, when running at speeds approaching the 2-hour marathon barrier (5.8 m•s⁻¹), the extra energy required to overcome air resistance is approximately 8% higher compared to running with no air resistance (Pugh, 1970). This extra energy can be nearly completely abolished by drafting behind other runners, which saves 80% of the extra energy required to overcome wind resistance (Pugh, 1971). However, wind also serves a thermoregulatory function in that cooler air crosses the skin during running, allowing for greater heat loss by convection. This may result in lower heat storage, and a longer exercise duration as a consequence. Presumably the extra energy is required due to the need to generate

greater propelling force. This would relate to the need for increased motor unit recruitment in muscles contributing to the forward propulsion.

2.4.1.2 Temperature

The environmental temperature can certainly limit running performance since high environmental temperatures lowers a runner's ability to dissipate heat. Heat exchange between the body and the environment is defined by the relative impact of the metabolic rate (ie. E_{run}), which is balanced by heat loss by convection, radiation and evaporation (Cheuvront and Haymes, 2001). Thus, a lower E_{run} for a given environmental temperature and humidity will result in less heat storage, and a longer exercise duration is permitted. Similarly, a runner with a comparatively low E_{run} can perform at a higher metabolic rate, corresponding to a faster speed, for the same level of heat storage. Where less heat is generated, less energy is required for peripheral circulation as warmed blood from the core is transferred to the skin (MacDougall et al., 1974; Rowell et al., 1969). Pulmonary ventilation is also elevated in hyperthermic conditions (Chu et al., 2007), which may also explain the elevated E_{run} , owing to an elevated work of breathing (COAST et al., 1993).

2.4.1.3 Altitude

Measured at a common absolute speed (255 m•min⁻¹), sea-level oxygen cost of running is approximately 4.5% greater than that measured at an altitude of 2300 m (Daniels et al., 1977). The difference in oxygen cost measured on the treadmill is 4%, so most of the altitude dependent difference is not related to overcoming air resistance. The only mechanism suggested for the lower oxygen cost at altitude was the possibility of differences in the anaerobic energy

contribution at altitude (Daniels et al. 1977). The possibility for anaerobic contribution at altitude when there was not at sea level relates to the compromised maximal oxygen uptake and the lower intensity associated with the anaerobic threshold. Any contribution by anaerobic metabolism would decrease the oxygen demand, even if the total energy cost was not different.

There is another possible contributing factor. When converting the oxygen cost to the energy cost, the energy equivalent of the oxygen uptake increases at altitude, so for the same energy yield, oxygen uptake would be lower. When running the same absolute speed at altitude as at sea level, this speed represents a greater speed, relative to the compromised lactate threshold. At a greater relative speed, the RER will be closer to 1 and the energy yield per volume of oxygen will be higher. At an altitude of 2300m, the measurements of oxygen cost were likely made at a speed at altitude which represents a relative speed which is approximately 10-15% faster as a result of the compromised lactate threshold at altitude (Daniels, 1970; Faulkner et al., 1968; Friedmann et al., 2004). This elevated relative speed is accompanied by a higher RER and a corresponding increase in the caloric equivalent of oxygen uptake. An increase in the speed relative to the lactate threshold (sLT) of 10-15% represents an increase of more than 1.5% in the caloric equivalent of oxygen. For example, at 90% sLT, the caloric equivalent of oxygen is approximately 4.97 kcal•L⁻¹ (Fletcher et al., 2009). A 10% increase in the running speed (to 99% sLT) raises the caloric equivalent to nearly 5.04 kcal·L⁻¹; an increase of 1.2% in the energy equivalent of oxygen uptake. This would impact the measurement of E_{run} between sea-level and altitude conditions.

If the sea-level measurements were made at a relative intensity of 90% sLT, the RER at this speed should be approximately 0.93 (Fletcher et al., 2009). Using the $\dot{V}O_2$ data presented by Daniels et al. (1977) at 255 m•min⁻¹, this represents a caloric cost of 0.99 kcal•kg⁻¹•km⁻¹ for his

58 kg runners. That same running speed at altitude would represent a speed approaching (or exceeding) the sLT. At this speed, the RER would be close to 1.00 and the equivalent caloric cost becomes 0.96 kcal•kg⁻¹•km⁻¹. Taking into account the energy yield per litre of oxygen accounts for more than half of the 4% difference in oxygen cost between altitude and sea-level treadmill running and yields a difference in sea-level vs altitude E_{run} of 0.03 kcal•kg⁻¹•km⁻¹.

It was also hypothesized that the thinner air at altitude presents less resistance to ventilation, and therefore a lower work of breathing at altitude. However, these authors showed that pulmonary ventilation at altitude was 15-20% greater compared to at sea-level (110 L•min⁻¹ vs. 96 L•min⁻¹) and thus could not explain the lower oxygen cost at altitude. Estimating the energy cost of ventilation at altitude (96 L•min⁻¹) and at sea-level (110 L•min⁻¹) according to Mazess (1968), the energy cost of ventilation would have been 0.11 and 0.08 kcal•kg⁻¹•km⁻¹ at sea-level and at altitude, respectively. Thus, the lower resistance to ventilation at altitude (0.03 kcal•kg⁻¹•km⁻¹) likely explains the lower E_{run} at altitude when E_{run} is presented as an energy equivalent.

Taken together, the differences between sea-level and altitude oxygen cost may likely be explained by the lower work of ventilation, increased energy per litre of oxygen uptake and possible anaerobic contribution that was not accounted for.

2.4.2 Surface Features

2.4.2.1 Friction

Running straight ahead at a constant speed on a dry, smooth, flat surface requires friction between shoe (or foot) and surface (Frederick, 1986). When on a slippery or wet surface or when changing speed or direction, subjects tend to modify their kinematics (and therefore use a less-than-optimal movement pattern) to compensate for surface characteristics (Frederick, 1983). Presumably, this also elevates E_{run} , although further research is required to determine the magnitude of this increase as a result of the less than optimal kinematics.

2.4.2.2 Surface stiffness

Runners are capable of adjusting their leg stiffness, allowing them to run with similar kinematics over a variety of different surfaces with varied stiffness (Ferris et al., 1998). If a runner was not able to quickly adjust leg stiffness based on the surface, the vertical displacement of the centre of mass would be greater as surface stiffness decreased. There exists an optimal surface stiffness over which a runner's best performance can be achieved (McMahon and Greene, 1979). These authors have demonstrated that a 'tuned' track surface, could be built for which ground contact time is decreased, and there is an increase in stride length and ultimately this could result in 2-3% improved performance times. These improvements in performance were most pronounced over long-distance races, for which ground contract time and stride length is related to E_{run}. (Kram and Taylor, 1990). Later, Kerdok et al. (2002) examined the energetic implications of running surfaces of variable stiffness, the lowest of which was within the range of stiffness tested by McMahon and Greene (1979). They showed that while the running mechanics during the support phase were essentially unchanged, E_{run} increased as a function of surface stiffness. The E_{run} was reduced with lower surface stiffness. These authors postulated that an increase in energy rebound from the compliant surface in the latter portion of ground contact contributed to the lower E_{run} .

2.4.2.3 Other surface features

 E_{run} is also elevated on soft and uneven surfaces, as evidence by E_{run} being significantly elevated while running on sand compared to grass or concrete (Lejeune et al., 1998; Pinnington and Dawson, 2001; Zamparo et al., 1992). The elevated E_{run} on sand has been attributed to a reduction in the re-utilization of elastic energy and/or the energy lost due to backwards translation of the foot during push-off. It has also been hypothesized that an elevated muscletendon work while running on sand contributes to the elevated E_{run} (Lejeune et al., 1998). In terms of the muscle energetics presented earlier, these mechanisms (foot slip, increased work and decreased tendon strain energy release) translate to an increased shortening and probably increased motor unit recruitment. Both of these factors would increase the energy cost of muscle contraction.

2.4.2.4 Footwear

It has been suggested that runners attempt to maintain a specific (optimal?) movement pattern during running (Nigg and Wakeling, 2001), which therefore explains the oftendemonstrated lack of significant change in kinematics and/or kinetics between footwear designs (Cole et al., 1995; Dufek et al., 1991; Nigg et al., 1987). Adapting to a less than optimal movement pattern would result in changes in muscle activation and which would manifest as differences in metabolic cost.

It has been suggested that a potential mechanism by which footwear might reduce E_{run} is because footwear serves to reduce some of the impact shock. A reduction in E_{run} of 3% with well-cushioned shoes compared to poorly-cushioned ones support this notion (Frederick et al., 1986). These authors developed a 'cost of cushioning' hypothesis whereby a portion of the

measured E_{run} in well-cushioned shoes is reduced because less muscle activation is required to brace the force of impact with the ground. To support this hypothesis, E_{run} was compared between well-cushioned shod and unshod conditions. The former condition would incur an estimated increase in E_{run} as a result of the mass of the shoes. Despite the added mass of the shoes, E_{run} was not different between shod and unshod conditions. These results contrast with those of Perl et al. (2012) who demonstrate a 2-3% reduction in E_{run} while barefoot running on a treadmill compared to shod, despite accounting for differences in shoe mass, footstrike type (forefoot vs rearfoot) and stride frequency. The authors attribute the reduction in E_{run} in the barefoot condition to more elastic energy storage and release in the longitudinal arch. At the speed at which their subjects ran $(3 \text{ m} \cdot \text{s}^{-1})$, the additional energy release required to account for the 2-3% difference in E_{run} between conditions would be approximately 17-24 J•stride⁻¹. This additional energy release was estimated from the absolute energy cost differences between conditions and the estimated stride length at that running speed (Cavanagh and Kram, 1989). This seems feasible given that approximately 34 J•stride⁻¹ is stored in the arch of the foot during running (Ker et al., 1987). Presumably cushioned shoes reduce this energy storage and release.

To eliminate any confounding factors such as shoe construction, Tung et al. (2014) isolated the effect of cushioning on E_{run} by attaching the same cushioning foam to the belt of a treadmill. In so doing, E_{run} was reduced by 1.6% when runners ran unshod on the cushioned belt in comparison to running unshod without the cushioning. Interestingly, E_{run} was not different between shod and unshod conditions on a normal treadmill belt, likely because the beneficial effects of cushioning were balanced by the detrimental effects of added shoe mass. These results suggest 1) shoe mass can have a meaningful influence on the measured E_{run} and 2) there exists a

trade-off between running in very light running shoes at the expense of extra cushioning in order to minimize E_{run} .

Runners are also able to assess shoe comfort reliably (Hennig et al., 1996) and it has been hypothesized that comfort could relate to performance (Nigg, 2001). In fact, oxygen cost was 0.7% lower in shoes deemed 'most comfortable' compared to those deemed 'least comfortable' (Luo et al., 2009). Further insight into the specific mechanism for a lower E_{run} between footwear (kinematics, kinetics, muscle activity etc) should be investigated.

2.4.3 Anthropometry

2.4.3.1 Ankle & Foot Morphology

 E_{run} is determined primarily by the energy needed for muscle contraction of sufficient force to support body weight during the stride duration (Kram and Taylor, 1990). Therefore, average muscle force and thus muscle energy cost is related to the average F_z during stance, as dictated by body mass and the F_z moment arm and the moment arm of the Achilles tendon (Carrier et al., 1994; Ker et al., 1987). These moment arms are shown in Figure 2.4. The ratio of F_z moment arm to that of the Achilles tendon is referred to as the gear ratio. Often, the F_z moment arm length is interpolated from known forefoot length. In this case, the ratio of forefoot length to AT moment arm is referred to as the foot lever ratio (Kunimasa et al., 2013). Both F_z and AT moment arms can be altered by changing ankle joint kinematics during the stance phase. This has important implications to E_{run} since changes in joint angle configuration at touch-down result in changes in both the F_z and AT moment arms. The relative change in the gear ratio for a given F_z will determine the magnitude of the required muscle force. Reductions in the gear ratio result in a reduction in muscle forces. It has previously been suggested that the Achilles tendon moment arm length changes with ankle angle, the maximum of which occurs at large dorsiflexion angles (Fath et al., 2013; Maganaris et al., 1998; Maganaris et al., 2000). However, we (Fletcher and MacIntosh, submitted) and others (Hashizume et al., 2012) have recently demonstrated that this may not be the case; Achilles tendon moment arm length remains constant throughout the range of motion. Thus, additional ankle excursion during stance may not result in a greater muscle force required to generate a given ankle moment during the stance as a result of a decreasing AT moment arm length.

During running, the ankle angle at touchdown is nearly 90 degrees, with elite runners exhibiting less ankle excursion during stance compared to good runners (Cavanagh et al., 1977; Williams and Cavanagh, 1987). A small excursion translates to lower angular velocity and a corresponding slower velocity of contraction. A slower velocity of contraction results in lower level of activation needed to generate a given force and consequently lower energy cost of muscle contraction.

A shorter AT moment arm, measured at rest, is associated with a lower E_{run} (Mooses et al., 2014; Scholz et al., 2008). The advantage of a short AT moment arm in reducing E_{run} has been attributed to increases in the elastic energy storage/release from the AT during running since larger AT forces for a given joint moment are required with a short AT moment arm; more elastic strain energy is stored and released in a tendon stretched to the same magnitude if AT forces are higher. It has been estimated that a reduction in the AT moment arm of 10% would result in a reduction in running $\dot{V}O_2$ of approximately 4.2 ml•kg⁻¹•min⁻¹ (Scholz et al., 2008). The estimated energy savings of a shorter moment arm are based solely on the extra elastic energy storage from the shorter AT moment arm and ignore the additional muscle energy cost associated

with elevated AT force. This muscle energy cost would be considerably higher (Fletcher and MacIntosh, 2014),

Shorter moment arms also require smaller muscle shortening velocity to achieve a given joint angular velocity (Nagano and Komura, 2003). This effect may be substantial, given the relatively large angular velocities at the ankle joint during submaximal running (KyroLAINEN et al., 2001). As previously suggested however, the elevated AT force associated with a shorter moment arm may also incur a substantial muscle energy cost (Fletcher and MacIntosh, 2014) and as such, a longer AT moment arm may help reduce E_{run} by reducing the required muscle force and level of muscle activation to sustain a given joint moment. To support this hypothesis, elite Kenvan long-distance runners, a population known for their exceptionally-low Erun (Larsen, 2003; Wilber and Pitsiladis, 2012), have longer AT moment arm lengths and shorter forefoot lengths compared to similarly-trained Japanese distance runners (Kunimasa et al., 2013). Furthermore, both long AT moment arm and short forefoot lengths are associated with better endurance performance. Considering a lower gear ratio reduces the energy cost of muscle contraction (Biewener et al., 2004; Carrier et al., 1994). Reducing the gear ratio from 2 to 1.5 reduces the estimated triceps surae muscle energy cost by nearly 40% (Fletcher and MacIntosh, 2014), assuming the same amount of shortening. However, a long AT moment arm also necessitates a greater amount of shortening for a given angular displacement.

The length of the F_z moment arm is also dictated by the length of the forefoot. Forefoot length is another anatomical feature (along with presumably short or long moment arm lengths) for which humans have evolved, presumably to favor economical walking and running. In relation to body mass, humans possess extremely short forefoot lengths (Rolian et al., 2009). This evolutionary adaptation has long been assumed to benefit bipedal locomotion since short

toes require smaller plantarflexor forces to balance the large dorsiflexion moments as a result of F_z (MANN and HAGY, 1979; Weidenreich, 1923). Using kinematic, force and plantar pressure measurements, this hypothesis was tested in a sample of human subjects. It was demonstrated that relatively long forefoot lengths had to generate more than four times the peak flexor force compared to a short-toed individual over a single stance phase (Rolian et al., 2009). The authors suspected that such an increase in force output would lead to at least a small increase in the metabolic cost of running. This seems very likely given that the elevated muscle force would result in a greater active muscle volume and a concomitant increase in energy cost. Thus, it seems logical to suggest that it is the ratio of F_z to AT moment arm lengths, rather than the absolute AT moment arm length itself which dictates the muscle energy cost.

2.4.3.2 Body mass, body composition and mass distribution

Not only is absolute body mass an important energy cost parameter in the energy cost of running, but body composition and distribution of that mass may be equally important. Active skeletal muscle is primarily responsible for the energy use, so a body mass consisting of a high proportion of skeletal muscle mass and low fat mass should be advantageous in reducing the energy cost of running over a fixed distance, since transporting metabolically-inactive tissue like fat would come at a metabolic cost. In fact, Kenyan boys show a lower leg circumference than boys of similar age from other continents (Larsen, 2003). This suggests that even lower muscle mass may be advantageous.

It is estimated that the energy cost of running (measured as $\dot{V}O_2$) was elevated by 4.5% for every additional kg of load carried distally whereas the energy cost was only elevated by 1% when that same weight was carried on the trunk (Jones et al., 1986). Therefore, minimizing the

weight of the swinging limbs, by minimizing fat and muscle weight in these areas should reduce the energy cost of running, as long as the muscle mass necessary to generate the forces and movements is maintained.

Since running involves rotation of the limbs, a substantial portion of the body mass should be located at a close proximity to the joint centre of rotation. This serves to minimize the limb moment of inertia, which comprises a substantial portion of the total metabolic cost of running, since joint moment need to impart an angular acceleration is proportional to the moment of inertia (Cavagna et al., 1964; Fenn, 1930). Swinging the limbs during running may come at a substantial energy cost. Using measurements of blood flow as a proxy for energy use by the active skeletal muscles in running guinea fowl, Marsh et al. (2004) were able to conclude that 26% of the total lower limb blood flow (and thus an equal proportion of the metabolic rate) was responsible for swinging the limb. This proportion of the metabolic rate was independent of running speed. Using a device that pulled the leg anteriorly during the swing phase, reducing the need of the muscles to swing the leg directly, Modica and Kram (2005) showed a reduction in metabolic cost by 20%. This estimate was later refined to ~7% of the metabolic cost of running (Warddrip, 2007), the difference likely a result of the device used by Modica and Kram also aiding in forward propulsion (Arellano and Kram, 2014a).

The metabolic cost of arm swing has also been addressed: is swinging the arms metabolically beneficial or costly (Arellano and Kram, 2014b)? By having subjects hold their arms in different positions, these authors demonstrated that running with a normal arm swing incurred the lowest metabolic cost (Figure 2.5). While swinging the arms might incur a metabolic cost, these data suggest the arm swing serves to reduce the amplitude of shoulder and

torso rotation. Without arm swing, shoulder and torso rotation must increase to counterbalance the rotational angular momentum of the swinging legs.

A reduction in the moment of inertia of the swinging limbs can be accomplished either by decreasing the distally-located mass, such as reducing fat, muscle or shoe weight, or reducing the radius over which that mass is rotated. The latter can be accomplished acutely by increasing the knee flexion angle during the swing phase. Assuming similar knee flexion angles, runners possessing short femur and long tibia lengths would also possess a smaller lower limb moment of inertia compared to runners with relatively long femurs and short tibias. Limb moment of inertia may also be minimized during the swing phase by reducing the angle formed between femur and tibia. Elite runners tend to exhibit a more acute knee angle during the swing phase compared to good runners (Cavanagh et al., 1977). However, these authors did not assess E_{run} and as such it cannot be said with certainty how these kinematic differences are related to E_{run} .

While the assumption that a higher joint moment is required where limb moment of inertia is high remains clear in theory, the influence of limb moment of inertia on the energy cost has only scarcely been examined. Cavanagh et al. (1977) showed small differences in knee joint angle between elite and good distance runners; however these differences were small and not significant. Later, Williams and Cavanagh (1987) showed no difference in limb lengths in runners whose energy costs were different. To date though detecting differences in the energy cost of running as a result of various anthropometric measures and/or masses from the various segments of the body have been difficult, with many studies showing no differences in economy as a result (Cavanagh et al., 1977; Williams, 1985; Williams and Cavanagh, 1987). This can either result from the large sample size requirements and/or the technical error of measurement to detect these small differences in the energy cost. Conversely, the influence of individual

differences in lower limb mass distribution and/or moments of inertia on E_{run} is not as great as theoretically suggested. As suggested by Williams and Cavanagh (1987), there does not appear to be easily identifiable and universally applicable patterns of economical movement that will apply to all runners.

2.4.3.3 Frontal surface area

It is apparent that much of what we now know about E_{run} has been derived from metabolic measurements performed on a treadmill in the laboratory. These measurements are sometimes difficult to extrapolate to overground running given the lack of air and wind resistance in the laboratory. Therefore, it is also difficult to determine with any degree of certainty to what extent the $\dot{V}O_2$ is elevated when running overground. The magnitude of difference in $\dot{V}O_2$ between overground and treadmill running has been the subject of much investigation (Bassett et al., 1985; Jones and Doust, 1996; Maksud et al., 1971; McMiken and Daniels, 1976). The difference between overground and treadmill running would be related to the energy in overcoming aerodynamic drag. A smaller frontal area reduces the drag (resisting force) opposing the runner's forward motion (Pugh, 1971). Many researchers have attempted to correct for the additional energy required to overcome wind resistance by imposing some gradient to the laboratory treadmill when measuring E_{run} .

From measurements made of a model runner in a wind tunnel, A.V. Hill (1928) suggested there might be an equivalent work against vertical forces (as in running up a grade of some slope) and the horizontal work required to overcome air resistance; as such, the equivalent treadmill slope could be calculated in order to best simulate the effect of air resistance on overground running. Later however, Pugh (1970) found, as would be predicted from Hill's

(1928) original equation, that the energy cost of running was proportional to wind resistance and thus running speed, but a precise relationship between grade and overground running (where wind resistance could be considered) was not shown. Using a portable Douglas bag system, Maksud et al. (1971) measured $\dot{V}O_2$ during track and treadmill running and concluded that at speeds faster than 187 m•min⁻¹, track running resulted in a 'generally higher $\dot{V}O_2$ ' compared to treadmill running. These authors attributed this difference primarily to the need to overcome air resistance during track running. This would account for an additional 8% energy cost at 358 m•min⁻¹(Pugh, 1970), equivalent to 14:00 over 5,000m. Later, McMiken and Daniels (1976), using a similar gas collection system (Daniels, 1971), could not demonstrate a difference between overground and treadmill running in elite distance runners at speeds up to 260 m•min⁻¹ and concluded that level-grade treadmill running was a valid instrument for the estimation of E_{run} in distance runners. The following year however, it was reported that track running resulted in a higher E_{run} compared to running on the treadmill, at least at speeds greater than 255 m•min⁻¹ (Daniels et al., 1977).

It would seem from early theoretical observations (Hill, 1928; Pugh, 1971) that overground E_{run} would be higher than level grade treadmill E_{run} , and some measurements made using portable gas-collection/measurement systems would confirm these theoretical findings (Daniels, 1971; Maksud et al., 1971). Thus, attempts have been made to correct for these differences by imparting some gradient to the laboratory treadmill in order to best reflect the metabolic cost of overground running. For example, studies have employed grades of 1-2% (Heck et al., 1985; Helgerud et al., 2010; Jones and Doust, 1996; Tegtbur et al., 1991), but only the study by Jones and Doust (1996) has justified the rationale for choosing such a gradient. These authors suggest a 1% grade best reflects the $\dot{V}O_2$ during overground running; however

only at speeds greater than 225 m•min⁻¹ was overground running significantly different than level-grade treadmill running.

There are three reasons which confound the use of some gradient when measuring E_{run} on the treadmill to compensate for wind resistance First, differences in the imposed treadmill slope make it difficult to compare values for E_{run} between studies. Secondly, the fundamental factors dictating E_{run} on level ground are different than those factors while running up a slope. When running up a slope at progressively faster speeds, E_{run} increases in proportion to body mass since the increased energy expenditure when running up a slope is related to the gain in potential energy (van Ingen Schenau, 1979). This may not be the case in uphill treadmill running because of the potential differences during the support phase of running since the supporting leg is moving down the belt during ground contact; foot contact occurs at a similar vertical position on each step (van Ingen Schenau, 1979). Additionally, if the centre of mass was displaced horizontally along the belt, energy would be required to propel the body back up the belt with each step. Furthermore, over level ground, running at progressively faster speeds, the E_{run} increases as a cubic function of the running speed (Léger and Mercier, 1984), in proportion to body surface area and aerodynamic drag (Pugh, 1971). Lastly, the biomechanics of running up a slope may be different compared to running on the level, as a result of differences in kinematics and/or kinetics of running between the two modes of exercise (Anderson, 1996; Nelson et al., 1972). These differences have been shown to increase the muscle energy cost as a result of greater muscle work during uphill running (Roberts et al., 1997).

Above, we have attempted to outline those factors not affected by training which likely affect the energy cost of muscle, and therefore, whole-body E_{run} . There exist specific anthropometric (eg. limb length) and morphological (eg. ankle and foot anatomy) characteristics

that influence the measured E_{run} . However, it is well-known that E_{run} is lower in trained distance runners compared to lesser-trained runners (Fletcher et al., 2009; Pollock, 1977) thus it is clear that E_{run} is likely altered by both short and long-term training protocols. These training strategies have recently been reviewed (Barnes and Kilding, 2014). Below we outline the various factors of E_{run} that are altered by training and consider the influence of muscle energy cost on those factors.

2.5 Factors affecting E_{run} that are altered by Training

2.5.1 Anthropometry

2.5.1.1 Body mass

Long-distance runners are smaller and lighter than middle-distance runners (Cavanagh and Kram, 1989). Also, elite African runners appear to be of lower body mass (Coetzer et al., 1993) and BMI (Saltin et al., 1995) compared to their Caucasian counterparts. These anthropometric differences appear to have persisted since childhood (Larsen et al., 2004). While little research has examined why body mass confers an athletic advantage, several factors specifically related to the energy cost of muscle contraction may explain this. For example, it is well established that F_z expressed relative to body mass is increased as a function of running speed (Keller et al., 1996). Thus, at a given running speed, the absolute F_z is lower in lighter runners compared to heavier runners. As such, there should be lower energy cost required by the active muscles. Over a wide range of body masses, Taylor et al. (1980) showed that the energy cost of running at a particular speed is proportional to the force exerted by the muscles active during stance. By manipulating the required muscle force by the addition of extra mass it was shown that the increased energy cost was proportional to the mass of the carried load.

2.5.1.2 Allometric-scaling for body mass

Because body mass must be supported during running, the expression of E_{run} is typically done as the energy cost relative to the subject's body mass (in kg). Because oxygen consumption during running does not increase to the same extent as body mass (Bergh et al., 1991; Rogers et al., 1995), allometric-scaling for body mass has been used. The allometric scaling relationship is:

$$\dot{V}O_2 = aBM^b$$
 2-1

where BM is body mass, *a* is a constant and *b* is the scaling exponent. Where the relationship between BM and $\dot{V}O_2$ is linear, the value of *b* should be 1 and E_{run} should be scaled to BM⁻¹. For mammals, ranging in mass from less than 100 g to greater than 1000 kg, the allometric-scaling exponent for basal oxygen uptake is generally taken to be ³/₄ (Kleiber, 1932; Schmidt-Nielsen, 1984).

However, it has been argued that the body mass (BM) scaling factor of $\frac{3}{4}$ for $\dot{V}O_2$ is by no means universal (Glazier, 2005; Heusner, 1987; Welsman et al., 1996) and a wide range of body masses is necessary to accurately assess the relationship. Therefore, appropriate verification of the classic allometric scaling relationship (Equation 2-1) should be used (West et al., 1997; West et al., 2002). This may be particularly important in situations where ranges of BM are relatively small. Such would be the case when scaling within the range of body masses seen in adult human studies (Fletcher et al., 2013a). Furthermore, since the metabolic cost of running is dictated by the muscle energy use, there is no reason to believe the E_{run} follows the same scaling as basal metabolic rate. Since body mass contributes to the metabolic cost of transport in a linear fashion (Roberts et al., 1998a), it is not obvious that a scaling factor other

than b = 1 is justified. The linear relationship between E_{run} and body mass in trained male and female runners is shown in Figure 2.6.

2.5.2 Muscle Properties

 E_{run} at a given relative speed, eg. relative to the speed associated with the lactate threshold, is determined by the total volume of muscle that must be active to support body weight during the stance phase as well as the rate at which that unit volume of muscle transforms energy (Kram and Taylor, 1990; Roberts et al., 1998b). The volume of active muscle is equal to the cross-sectional area (CSA) and the muscle fascicle length. The rate at which the muscle transforms energy is dependent on muscle fibre type. Each of these factors dictating muscle energy cost has been elaborated upon previously (Kram and Taylor, 1990; Roberts et al., 1998b; Taylor, 1985). The rate of muscle energy use (EC_M) is given by:

$$EC_M = (LF/\sigma)E_M$$

Where *L* and *F* are fibre length and muscle force, respectively and σ is the force per unit crosssectional area. *E_M* is the rate at which each unit volume uses energy which for isometric contraction is related to the muscle fibre type; fast-twitch muscles have higher rates of energy use related to the elevated cost of cross-bridge cycling and activation costs (Barclay et al., 2010a; Rall, 1985). E_{run} increases as a function of running speed since force is developed more rapidly, implying activation of additional motor units. Faster running speed also requires a faster velocity of shortening. At some critical velocity of shortening, the time-dependent turnover of cross-bridges becomes inconsequential and the turnover is related to the velocity of shortening. This critical velocity will be faster with slow-twitch muscle. It is well-established that muscle cross-sectional area increases after a period of resistance training which may (Blazevich et al., 2003; Kawakami et al., 1995) or may not (Blazevich et al., 2007; Seynnes et al., 2007) be accompanied by a concomitant decrease in muscle fascicle length, at least in pennate muscle; changes in fascicle length appearing prior to an increase in muscle CSA. However, to run at a given submaximal speed, an increase in absolute strength as a result of increased muscle CSA would result in a lower relative intensity. This lower relative intensity would not require the need to recruit higher threshold motor units, where the muscle energy cost is higher. This may be one of the explanations by which E_{run} is improved following a period of strength training. The effect of strength training on E_{run} has been recently summarized quite well (Barnes and Kilding, 2014). Chronic endurance training may also result in a shift to a higher proportion of slow Type I fibres (Rusko, 1992) further ameliorating the reduction in muscle energy cost at a given speed.

2.5.3 Tendon stiffness

Strength training has also been shown to increase tendon stiffness (Kubo et al., 2001a; Kubo et al., 2001b; Kubo et al., 2002b) and increased tendon stiffness has been proposed to be one of the main mechanisms behind an improved E_{run} following plyometric training (Saunders et al., 2006) despite the apparent reduction in energy storage and return associated with a stiff tendon.

It is not well understood how specific mechanical alterations of tendon can affect the energy cost of muscle contractions. It is suggested that the energy cost of contraction is related to the level of motor unit activation and both the amount of shortening and the shortening velocity (Stainsby and Lambert, 1979). Further, the amount and velocity of shortening are

dictated by the muscle's in vivo force-length and force-velocity relationships (Praagman et al., 2006). In fact, many seminal papers make inferences regarding the energy cost of contraction assuming muscles operate over a similar range of the force-velocity relationship, regardless of speed of locomotion or body size (Kram and Taylor, 1990). As such, it would be of interest to know where the muscles operate and how altering the mechanical properties of the tendon affect the operating range of the muscle on their respective force-length and force-velocity curves.

Recent classic papers from a variety of species and muscle functional tasks highlight the fact that muscle shortening patterns during natural movement are well matched to their contractile properties (Askew and Marsh, 1998; Lutz and Rome, 1994; Roberts et al., 1997). The fact that muscles operate at the most appropriate loads and favorable velocities based on these contractile demands suggest that contractile properties of muscle and the tendon are well matched. For example, Lutz and Rome (1994) found that the semimembranosus muscle of the frog operated at appropriate lengths and shortening velocities to maximize power output during maximal jumping (Figure 2.7). This effect would not be possible unless the tendon was perfectly tuned (with respect to stiffness and proportion of muscle-tendon length occupied) to allow the muscle to operate at the appropriate length and velocity. This effect is also shown during human cycling whereby vastus lateralis muscle fascicle lengths operate on the plateau of the forcefascicle length relationship during maximal cycling (Austin et al., 2010). At submaximal power outputs, the fascicle lengths operate at longer lengths due to less strain of the tendon. This shift to a longer length can presumably take advantage of the shift in the submaximal optimum of the force-length relationship (Ichinose et al., 1997). Thus, a "functional coupling" (Austin et al., 2010) exists between the mechanical properties of the tendon and the muscle fascicle length and velocity.

The tendon can also act in such a way as to minimize the amount of work that is required by the muscle in order to minimize metabolic cost. By minimizing the length change during active muscle contraction, the tendon allows the muscle's force-length-velocity relationship to be optimized. In theory, if the length change of the whole muscle-tendon unit can be accomodated by the tendon alone, the muscle can operate isometrically, thus minimizing the level of muscle activation required to produce the necessary force. By outfitting wild turkeys with surgically implanted strain gauges on the tendon and sonomicrometry crystals on muscle fascicles of the lateral gastrocnemius, Roberts et al. (1997) were able to measure the force and fascicle length changes of the muscle-tendon unit as the turkeys ran on level ground. What they demonstrated was that the fibers of the lateral gastrocnemius developed force but underwent very little length change during the stance phase of running. Thus, the Achilles tendon was able to take up much of the muscle-tendon length change so the fibre shortening could be reduced. The Achilles tendon also accomodates much of the muscle-tendon unit length change during human running. (Ishikawa et al., 2007; Lichtwark et al., 2007) and thus greatly reducing the shortening-induced muscle energy cost (Fletcher and MacIntosh, 2014). Presumably, the mechanical properties of the Achilles tendon was 'tuned' to accommodate the majority of muscle-tendon unit length change. Any change in these mechanical properties would affect the magnitude of length change of the muscle fascicles, and energy cost would necessarily be higher.

The relative shortening velocities in running turkeys has recently been measured directly in which the above hypothetical scenario has been shown to occur (Gabaldon et al., 2008). During level running, the shortening velocity of the lateral gastrocnemius were quite low (~0.05 v/v_{max}), supporting the notion that force can be maximized and activation minimized (as reflected by electromyography) at low shortening velocities. Having to run up an incline required slightly greater V/V_{max} ratios (~0.12 V/V_{max}) and the volume of active muscle that had to be recruited increased in accordance with the muscle's force-velocity properties.

Regardless of the mechanical energy fluctuations of the body that occur during the running stride, running animals (including humans) moving at a constant speed must generate enough muscle force to intermittently support their body weight. As running speed increases, force must be developed more quickly which requires the recruitment of additional motor units, likely related to the faster, less economical muscle fibers. This results in a higher metabolic cost for the same impulse: greater force is developed in a shorter period of time. This is why the cost of generating these muscle forces determines to a large extent the metabolic cost of running, from rodents to horses (Kram and Taylor, 1990).

Of course, this argument is only valid if one considers that during running, the tendon compliance is tuned in such a way that it allows the muscle fascicles to generate force at low shortening velocities. If the tendon is too stiff, then lengthening and shortening is required by the fascicles and the volume of active muscle recruitment increases. If the tendon is too compliant, much of the energy for force generation will be consumed shortening the fascicles even with negligible joint rotation. In the case where high forces need to be generated quickly, as Kram and Taylor (1990) would suggest in fast running, too compliant a tendon would require greater fascicle shortening than that necessary for joint rotation, resulting in higher velocity. This suggests that there may be an "optimal tendon compliance" with respect to minimizing muscle shortening.

2.5.3.1 Does an "optimal stiffness" exist to reduce the EC of running?

It has previously been shown that in a group of trained distance runners, the most economical runners displayed a higher Achilles tendon stiffness compared to the less economical runners (Arampatzis et al., 2006; Fletcher et al., 2010). The former study demonstrated the opposite to be true in the patellar tendon – that the most economical runners had a lower patellar tendon stiffness compared to the less economical runners (Arampatzis et al., 2006). This opposite result suggests that the roles of these two muscles in minimizing the energy cost during running are different. The reason for these apparently contrary observations with respect to the impact of tendon stiffness on the muscle energy cost is not obvious, however. We contend that the role of the tendon in running is to minimize the energy cost of muscle contraction. Is it possible that energy cost is minimized in the quadriceps muscles by a more compliant tendon, while a stiffer tendon reduces energy cost in the triceps surae?

The two muscle groups are known to behave in different ways during running. The quadriceps muscles undergo a stretch –shortening cycle (Gillis and Biewener, 2001), but the triceps surae has little if any stretch and performs predominantly a shortening contraction (Ishikawa et al., 2007). This suggests the role of these two muscles and their tendons during running are different.

There are apparent advantages of stiff tendons in some cases, and compliant tendons in other cases. The lengthening of a tendon for energy storage is relevant in stretch-shortening cycles where a substantial pre-stretch of the tendon occurs early in a contraction. A compliant tendon allows more energy conversion of either kinetic or gravitational energy to potential strain energy. This energy can subsequently be released upon shortening. A compliant tendon may also help by allowing the tendon to lengthen during the stretch phase of the SSC and shorten

during the shortening phase, thereby keeping fascicle shortening velocity low and reducing the necessary level of activation of motor units required to generate the force. If tendon compliance is optimal, the power-velocity relationship can be optimized because the fascicles are shortening at the appropriate velocity (Askew and Marsh, 1998; Gabaldon et al., 2008). This may be the case in the patellar tendon, which would lend support to previous evidence suggesting a more compliant patellar tendon might decrease E_{run} (Albracht and Arampatzis, 2006; Arampatzis et al., 2006).

Conversely, a more compliant AT requires greater muscle fibre shortening and/or velocity of fibre shortening for a given joint movement. In the AT, force transmission to the joint may be favoured over elastic energy storage and release. This is the case because for a given amount and rate of muscle tendon unit shortening, less muscle fibre shortening is needed with a stiff tendon compared to a compliant one where additional fibre shortening is needed to accommodate tendon stretch. We have recently estimated the tendon strain energy release from the AT and compared that to the estimated muscle energy cost in order for this strain energy storage to occur (Fletcher and MacIntosh, 2014). These results demonstrate that the storage and release of tendon strain energy comes at a considerable muscle energy cost. Therefore, reducing shortening-induced energy cost contributes to a reduced E_{run} . Thus, it appears that a compliant tendon may be favoured in one case whereas a stiff tendon can be favoured in another.

2.5.4 Running Mechanics

2.5.4.1 Stride Length and stride frequency

At speeds below the lactate/ventilatory threshold, where E_{run} is most appropriately measured, the lowest E_{run} in humans is generally thought to occur at stride frequencies of 83 to

91 strides per minute (Hunter and Smith, 2007). This freely-chosen stride frequency closely resembles the stride frequency associated with the lowest energy cost (Högberg, 1952; Hunter and Smith, 2007), particularly in trained runners, although the self-selected stride frequency is generally 3-8% lower than the optimal frequency when measured at 80% of sLT (de Ruiter et al., 2013). This is shown in Figure 2.8. The difference between self-selected and optimal stride frequency in terms of oxygen uptake is generally small (< 3 ml•kg⁻¹•min⁻¹); however, a larger increase in oxygen uptake is seen when stride frequency is slower than optimal compared to a correspondingly faster stride frequency (de Ruiter et al., 2013; Högberg, 1952). Thus, it seems odd from an energy-saving perspective that runners freely choose a slightly slower than optimal stride stride frequency compared to a slightly faster one. However, the difference is relatively small and may be inconsequential.

At a given running speed, concomitant with a change in stride frequency is a change in stride length, both of which tend to increase with running speed, although proportionally greater increases in stride frequency are seen compared to the increase in stride length, at least at submaximal speeds where the measurement of E_{run} is valid (Cavanagh and Kram, 1989). When E_{run} is expressed in terms of the energy cost required to transport a unit body weight a unit distance, small animals use more energy to run a given distance than do large animals (Kram and Taylor, 1990); since small animals must take many fast strides to cover the same distance a large animal can cover in one stride. The mass-specific energy cost is highest in small animals since the muscle fibres of these animals must develop force more quickly, thus requiring greater rates of cross-bridge cycling and Ca²⁺ pumping (Bárány, 1967; Rome, 1992). In human runners, those runners with longer legs, and thus longer stride lengths should have a lower energy cost; they will take fewer strides to cover a given distance than a runner with small strides. However, the

relationship between stride length (expressed either in absolute terms or relative to height or leg length) and E_{run} in human runners is moderate at best (Cavanagh and Williams, 1982; Williams and Cavanagh, 1987).

Running is often considered a bouncing gait whereby humans literally bounce along the ground (Cavagna et al., 1964), storing and recovering kinetic and potential energy as the centre of mass rises and falls with each stride, thus closely resembling a simple spring. By having subjects hop at various speeds on a treadmill, Farley et al. (1991) were able to deduce that a range of hopping frequencies existed whereby the body behaved like a spring, storing and recovering elastic energy. However, at higher than optimal frequencies, the time available to apply force to the ground was necessarily shorter, but more contacts per unit time would be required. Average energy cost would probably relate to the integral of force over some fixed time, rather than per bounce. Below the optimal frequency, the body did not behave in a springlike manner and the recovery of elastic energy was reduced. Clearly there is a trade-off between ground contact time, and the requirement to generate force rapidly and the ability to generate large forces over a relatively long period during the stance phase, which serves to minimize E_{run} . The fact that runners tend to choose a stride frequency slightly lower than optimal frequency suggests a greater importance is placed on maintaining ground contact time (and thus allowing a slower recruitment of muscle fibres) over maximizing the storage and release of elastic energy. The self-selected stride frequency should be the one at which the metabolic cost of operating the springs is the lowest (Farley et al., 1993) since muscle metabolic energy is required in order to store and release elastic strain energy from the tendons (Alexander, 1986; Fletcher and MacIntosh, 2014).

2.5.4.2 Ground contact time

Modeling running as a simple spring-mass system can characterize the mechanics of the body's centre of mass quite well (Farley et al., 1993; McMahon and Cheng, 1990); however, it does not adequately explain the energetics of running, since theoretically a perfectly-elastic spring could supply all of the metabolic energy required to run (Arellano and Kram, 2014a). An alternative to the spring-mass model hypothesis, the 'cost of generating force hypothesis' was proposed (Taylor et al., 1980).

By measuring the metabolic cost of carrying various loads, these authors observed the metabolic cost increased in direct proportion to the added load. Therefore it was proposed that the metabolic cost of running arose in association with the cost of generating force over time, rather than generating mechanical work. The metabolic cost is proportional to the average vertical force applied to the ground and inversely proportional to the ground contact time over which the force can be applied (Kram and Taylor, 1990).

Higher running speeds are achieved with higher peak ground reaction force (F_z , (Cavanagh and Lafortune, 1980) but average F_z over a complete stride is equal to the subject's body weight (Kram and Taylor, 1990). By determining a constant 'cost coefficient', these authors were able to determine that the mass-specific metabolic cost of running could be explained by how quickly F_z could be generated during the stance phase. Since faster running speeds are associated with shorter ground contact times, the required F_z needs to be generated more quickly as speed increases, elevating the metabolic cost. Roberts et al. (1998a) later showed that 70-90% of the speed-associated increase in metabolic rate could be explained by the increase in the rate of force generation. For any tendon stiffness the velocity of fascicle shortening will be proportional to the rate of force development. To generate a given force more

muscle fibres must be recruited to that produce force (Roberts et al., 1998a). To further support the cost of generating force hypothesis, several authors have shown an inverse relationship between E_{run} and ground contact time (Chapman et al., 2012; Di Michele and Merni, 2013; Williams and Cavanagh, 1987). Di Michele and Merni (2013) estimated that an increase in ground contact time of 1 ms was equivalent to a reduction in E_{run} of approximately 0.05 J•kg⁻¹•m⁻¹, since the force to support body weight has to be generated more rapidly. Together, these results suggest the speed-associated increase in E_{run} is a result of the elevated muscle energy cost associated with generating force more rapidly.

2.5.4.3 Footstrike Pattern

The fastest marathon runners primarily use a forefoot strike pattern as opposed to heel strike (Ardigo et al., 1995; Cavanagh and Lafortune, 1980; Nilsson and Thorstensson, 1989). This seems counter-intuitive for minimizing E_{run} since ankle plantarflexor moments are larger during the first half of stance (Williams et al., 2000). Heelstrike reduces the F_z moment at the ankle because the centre of pressure resides under the heel of the foot during the first half of stance and this reduces the length of the F_z moment arm (Cavanagh and Lafortune, 1980; Williams and Cavanagh, 1987). Conversely, the centre of pressure during stance, a surrogate of the F_z moment arm length, is centered under the ball of the support foot in the forefoot landing pattern. Thus, heel strike pattern substantially reduced the EMG of the lateral gastrocnemius and soleus muscles compared to forefoot strike (Cunningham et al., 2010).

However, despite heel striking to be theoretically-optimal to minimize E_{run} for the reasons listed above, when E_{run} was measured in the same subjects adopting either a heelstrike or forefoot strike pattern during running at various speeds, no difference in E_{run} (expressed as the O_2

cost of transport) was seen between the two conditions (Cunningham et al., 2010). These results are contrary to those of Williams and Cavanagh (1987) who found the most economical runners were those with a heelstrike pattern. These authors suggested that a heelstrike pattern may reduce E_{run} because (contrary to a forefoot), the forefoot runners were not using the available cushioning in the heel of their running shoes, and thus the forefoot landing results in additional muscle activation in order to attenuate the impact associated with ground contact (Boyer and Nigg, 2004; Nigg and Wakeling, 2001). Alternatively, forefoot striking may result in a higher average gear ratio resulting in higher necessary TS force.

The main issue with examining differences in E_{run} between forefoot and heel strike patterns is that many studies artificially impose an unnatural gait to the subject. Thus, a lower E_{run} measured under one condition may be the result of runners being unfamiliar with the novel gait pattern. We have described above how runners may self-optimize movement patterns (stride length, frequency, etc) to reduce E_{run} . It is also likely that runners self-select the footfall pattern that results in the lowest possible E_{run} . To demonstrate this theory, Gruber et al. (2013) measured E_{run} in habitual forefoot and heelstrike runners and found no difference in $\dot{V}O_2$ between groups when running with their habitual footstrike pattern. Interestingly, at all running speeds, runners habituated to the heel strike pattern showed a higher $\dot{V}O_2$ when asked to forefoot strike, which was not seen when the forefoot group ran with a heel strike pattern. Only at high speeds was the heel strike pattern less economical in the habitual forefoot runners. Taken together, these results suggest a heel strike pattern might confer an advantage in endurance running events, as a result of a lower E_{run}, in both habitual heel strike and forefoot runners. When the muscletendon unit of the triceps surae was modeled to assess the muscle mechanics and energetic differences between foot strike patterns, it was shown that the forefoot strike pattern resulted in a

near-isometric contraction during stance. This allows a lower muscle energy cost for a given force compared to the heel strike pattern, where high contraction velocities during stance were demonstrated (Gruber, 2012). A significant difference in the metabolic energy cost, however, could not be shown.

2.5.4.4 Flexibility

Despite the general belief among runners and coaches that greater flexibility may result in improved E_{run} (Craib et al., 1996), there is very little evidence to support this notion. A lower flexibility (measured during a sit and reach test) is associated with a lower E_{run} (Craib et al., 1996; Gleim et al., 1990; Trehearn and Buresh, 2009). Various suggestions have been made by which a lower flexibility may decrease E_{run} : 1) reducing the trunk muscle energy cost to maintain stability (Craib et al., 1996) and/or 2) increasing the storage and return of elastic energy (Jones, 2002). The latter mechanism appears unlikely 1) given that such a small portion of the total metabolic energy (500-900 J) is stored and released as elastic energy (<90 J, Fletcher and MacIntosh, 2014; Ker et al., 1987) and 2) mechanically, a stiff AT stores less strain energy for a given force compared to a more compliant tendon. As we have previously suggested (section 2.5.3.1), an optimal tendon stiffness exists and therefore, a delicate balance between the amount of flexibility training (with the intention that stretching training will reduce AT stiffness (Kubo et al., 2002a; Morse et al., 2008)) and strength training (to increase tendon stiffness (Kubo et al., 2001a; Kubo et al., 2001b)) may result in less than optimal tendon mechanical properties in order to minimize muscle energy cost (Fletcher et al., 2013b)...

2.6 Conclusions and future directions: Muscle energetics and Erun

 E_{run} has been extensively studied in the biomechanics and exercise physiology literature and is known to be influenced by a variety of factors. However, much of the interpretation of E_{run} exists from the measurement of the steady-state $\dot{V}O_2$ at a given submaximal running speed, without calculation of the energy equivalent. It is difficult to conclude whether similar interpretations of E_{run} exist (eg. male vs female E_{run} , altitude vs sea-level) where E_{run} is expressed in terms of energy cost to run a fixed distance at a given relative intensity. It is only recently that this expression of E_{run} has been encouraged.

For the first time, we have described the influence of many of the factors which influence E_{run} in terms of the biomechanical and physiological factors which dictate the muscle energy cost during the stance phase of running. This has allowed us to consider the relative importance of the storage and release of elastic energy from tendon in reducing the energy cost, which we argue is relatively minor compared to the muscle energy cost required to store the elastic strain energy. Consideration has been given to the influence of biomechanics (limb weight and length, AT and vertical ground reaction force etc) and physiology (force-length-velocity properties of muscle) in dictating the muscle energy cost, and therefore E_{run} .

Future research in elite athletes should be aimed at the effectiveness of different training interventions (eg. strength, stretching or plyometric training) on E_{run} where it is expressed in terms of energy. Specifically, a greater understanding of the muscle and tendon interactions during running is warranted: during distance running, where does the muscle operate relative to their submaximal force-length-velocity relationships? How is this altered through training intervention (where muscle and tendon properties may be changed)? What is the impact of fatigue (mechanical or physiological) on the muscle energy cost, and on E_{run} ?
Future directions should also include the measurement of factors which dictate muscle energy cost across different pathologies (aging, disease, disuse) in order to best prescribe appropriate training and/or rehabilitation programs for elite Paralympic athletes who may have compromised muscle and/or tendon function or for individuals where exercise tolerance may be limited by an elevated energy expenditure.

2.7 Author Contribution

JRF drafted the manuscript and JRF and BRM revised it critically for important intellectual content. All authors approved the final draft of the article.

2.8 Acknowledgements

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2.9 Figures



Figure 2.1. E_{run} and muscle energy cost in male and female runners.

Whole-body energy cost per stride (solid bars) estimated across three relative running speeds in three groups: Elite males (EM), trained males (TM) and trained females (TF). Dashed bars indicate the estimated muscle energy cost (c) per stride for each group and speed, respectively. Adapted from Fletcher and MacIntosh (2014). *Used with kind permission of the American Physiological Society*.





Solid and dashed lines represent the mean and sd of the second-order polynomial (Equation F =

AdL2 + BdL

3-3) for all

groups, respectively. Used with kind permission of the American Physiological Society.



Figure 2.3. The effect of greater shortening velocity on muscle activation to achieve a target force.

The force-velocity relationship, scaled to activation (Chow and Darling, 1999). The short dashed and solid lines represent 50% and 85% of maximal motor unit activation, respectively. The long dashed line represents maximal activation. When force can be generated isometrically, target force can be achieved with minimal motor unit activation, as shown by the open square. When shortening is permitted, additional motor unit activation is required (filled square). *Used with kind permission of Springer Science+Business Media*.



Figure 2.4. Estimated Achilles tendon (AT) force from ground reaction forces (GRF) and the gear ratio, the ratio of the moment arms of the GRF and AT, respectively.





Subjects were asked to run while: swinging their arms normally (NORMAL), holding the hands with the arms behind the back in a relaxed position (BACK), holding the arms across the chest (CHEST), and holding the hands on top of the head (HEAD). During the trials, oxygen uptake was collected in order to calculate E_{run} . The data demonstrate that running without arm-swing (compared with the control, * indicates P<0.05 and ** indicates P<0.01) increases net metabolic cost, indicating that arm-swing provides a small, but significant metabolic benefit during human running. Reprinted from Arellano and Kram (2014a). *Used with kind permission of Oxford University Press*.



Figure 2.6. Allometric-scaling of body mass on submaximal \dot{VO}_2 during running.

Data are from Fletcher et al. (2013a), showing the relationship between the measured $\dot{\mathbf{VO}}_2$ (L•min⁻¹) at 95% sLT and body mass. Black lines show the linear regression (± 95% C.I.) of the relationship. The 95% C.I. for *b* in Equation $\dot{\mathbf{VO}}_2$ = **aBMb**

2-1was 0.86 to 1.42. This value was not significantly different

from 1 (p=0.553).





Measured data points are from Lutz and Rome (1994) and are shown as open and closed circles. A. During jumping, sarcomeres operate near the plateau region of the force-length relationship to maximize force production. B. The mean velocity of shortening during maximal jumping corresponds to a shortening velocity associated with maximal power production. *Used with kind permission of the American Association for the Advancement of Science*.





Data are from a representative subject from de Ruiter et al. (2013). The solid vertical arrow at the minimum of the curve indicates the stride frequency where running cost was minimal (SFopt). The dashed vertical arrow indicates the self-selected stride frequency (SFsel). This subject would reduce his running cost by about 4% if he chose to run at 91 instead of 82 strides•min⁻¹. *Used with kind permission of Taylor & Francis*.

Chapter Three: ENERGY COST OF RUNNING AND ACHILLES TENDON STIFFNESS IN MALE AND FEMALE TRAINED RUNNERS

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3.1 Abstract

The energy cost of running (E_{run}) , a key determinant of distance running performance, is influenced by several factors. Although it is important to express E_{run} as energy cost, no study has used this approach to compare similarly trained males and females. Furthermore, the relationship between Achilles tendon (AT) stiffness and Erun has not been compared between males and females. Therefore, our purpose was to determine if sex-specific differences in E_{run} and/or AT stiffness existed. E_{run} (kcal•kg⁻¹•km⁻¹) was determined by indirect calorimetry at 75%, 85% and 95% of the speed at lactate threshold (sLT) on 11 male (mean±SEM, 35±1 years, 177 ± 1 cm, 78 ± 1 kg, $\dot{V}O_2$ max = 56±1 ml·kg⁻¹·min⁻¹) and 18 female (33±1 years, 165±1 cm, 58±1) kg, $\dot{V}O_2$ max = 50±0.3 ml·kg⁻¹·min⁻¹) runners. AT stiffness was measured using ultrasound with dynamometry. Male E_{run} was 1.01 ± 0.06 , 1.04 ± 0.07 and 1.07 ± 0.07 kcal•kg⁻¹•km⁻¹. Female E_{run} was 1.05±0.10, 1.07±0.09 and 1.09±0.10 kcal•kg⁻¹•km⁻¹. There was no significant sex effect for E_{run} or RER but both increased with speed (p<0.01) expressed relative to sLT. High-range AT stiffness was 191 ± 5.1 N•mm⁻¹ for males and 125 ± 5.5 N•mm⁻¹, for females (p<0.001). The relationship between low-range AT stiffness and E_{run} was significant at all measured speeds for females ($r^2=0.198$, p<0.05), but not for the males. These results indicate that when E_{run} is measured at the same relative speed, there are no sex-specific differences in E_{run} or substrate use. Furthermore, differences in E_{run} cannot be explained solely by differences in AT stiffness.

3.2 Introduction

Several studies have been concerned with the main physiological determinants of performance in distance running. These determinants include, among other variables: maximal oxygen uptake ($\dot{V}O_2$ max), fractional utilization of $\dot{V}O_2$ max, the ability to withstand a disturbance in homeostasis, or tolerance, and the energy cost that is required to transport the body over a given distance (Di Prampero *et al*, 1993). In the case of running, this latter variable is typically defined as a subject's energy cost of running (E_{run}). In a heterogeneous group of runners with a large range of $\dot{V}O_2$ max values, a strong positive relationship exists between running performance and $\dot{V}O_2$ max (Costill *et al*, 1973). Among runners who possess similar $\dot{V}O_2$ max values, E_{run} becomes a better predictor of running performance than $\dot{V}O_2$ max alone (Pollock, 1977).

Several studies have compared E_{run} between males and females and some argue that no difference exists (Ingham *et al*, 2008, Pate *et al*, 1992). In contrast, some contest that female runners are more economical than their male counterparts (Helgerud *et al*, 2010, Helgerud, 1994). Still others argue to the contrary; males are more economical (Daniels and Daniels, 1992). Further study of this controversy may allow a better understanding of the fundamentals that affect E_{run} . As we have previously outlined (Fletcher *et al*, 2009) appropriate measurement of E_{run} should be performed at similar %sLT between individuals, in order to ensure the steadystate of $\dot{V}O_2$ is achieved and to minimize differences in substrate use (as reflected by the steadystate RER) between individuals, knowing that the energy equivalent of $\dot{V}O_2$ changes with RER. It also makes sense to test at a speed that relates to a competition distance, like a 10 km race (ie. a similar %sLT). None of the previous studies comparing E_{run} of males and females have done this. E_{run} should also be expressed as an energy cost per unit distance rather than a $\dot{V}O_2$ to allow comparison between different absolute speeds. Thus, much of the conflicting evidence previously presented regarding E_{run} between males and females may result from an inappropriate expression of E_{run} itself.

In one of the two studies to date that have compared E_{run} in male and female runners at similar relative intensities (relative to maximal oxygen uptake), E_{run} was expressed as ml•kg^{-0.75}•m⁻¹, without justification for doing so. It was reported that the lighter female runners were more economical than their heavier male counterparts (Helgerud *et al*, 2010). In the other study (Tarnopolsky *et al*, 1990) E_{run} was expressed as kcal/kg to run a given distance, and no significant difference between males and females was detected. Furthermore, no study has made direct comparison between similarly-trained male and female runners that have expressed E_{run} in terms of energy (kcal or J•kg⁻¹•m⁻¹). Could it be that sex-specific differences in E_{run} may be confounded as a result of differences in allometric scaling for body mass on metabolic rate? Since metabolic rate does not increase to the same extent as body mass (Bergh *et al*, 1991, Rogers *et al*, 1995), allometric-scaling for body mass has been used when comparing E_{run} for groups of varied body mass. The allometric scaling relationship is:

$$\dot{V}O_2 = aBM^b$$
 3-1

where BM is body mass, *a* is a constant and *b* is the scaling exponent. Where the relationship between BM and $\dot{V}O_2$ is linear, the value of *b* should be 1.

Therefore, it needs to be confirmed whether the previous use of an allometric scaling factor of b=0.75 has influenced the comparison of E_{run} between sexes, or whether this is simply a function of the cohort tested and/or of the methods used in the determination of E_{run}. As we have proposed previously, E_{run} should be measured at similar intensities expressed relative to sLT for all runners.

It has become apparent in recent years that the muscle-tendon unit mechanical properties of the lower limbs may be important as determinants of E_{run} . Specifically, it has been shown that AT stiffness is generally less than optimal and greater stiffness corresponds with a lower energy cost of running (Arampatzis *et al*, 2006, Fletcher *et al*, 2010, Kubo *et al*, 2010, Lichtwark and Wilson, 2008). Recently, we have demonstrated that it is a combination of factors, including muscle shortening, shortening velocity and level of muscle activation which dictate the energy cost of muscle contraction *in vivo (Fletcher et al*, 2013) and this may help explain the relationship between AT stiffness and E_{run} .

To date, virtually nothing is known about the muscle-tendon interactions in female runners. Female AT stiffness is generally thought to be lower than the AT stiffness in similarly trained males (Kubo *et al*, 2003). This may be a result of females having a lower isometric strength. The relationship between strength and AT stiffness is well-reported (Muraoka *et al*, 2005). Given the reported relationship between AT stiffness and E_{run} , it seems logical to hypothesize that E_{run} of females would be greater than that of the males. This hypothesis has not been tested directly to date. If E_{run} is not different between sexes, then the question exists: why are the ATs of female runners less stiff than their male counterparts? We hypothesize that this level of AT stiffness is required in the female runners, running at a slower speed than the males, in order to keep muscle shortening velocity low.

Therefore, the primary purpose of this study was to investigate E_{run} and AT stiffness between similarly trained male and female runners and to determine if sex-specific differences in E_{run} and/or AT mechanical properties exist. In order to do so, and to compare directly with current literature, the use of allometric scaling for body mass was also considered.

3.3 Methods

3.3.1 Ethical Approval

Male (11) and female (18) trained runners participated in this study (Table 3-1). The runners gave their informed written consent to participate in the experimental procedures, which were approved by the University of Calgary Conjoint Health Research Ethics Board.

3.3.2 Experimental Protocol

All subjects participated in training for running a minimum of five times per week and none of the subjects had any neuromuscular or musculoskeletal injuries at the time of the study. All subjects were following a similar periodized training plan for either the 10 km or halfmarathon road race distance. Self-reported estimates of current 10 km race time (mean \pm sd) were 39.67 \pm 4.51 minutes for the males and 47.17 \pm 6.07 minutes for the females. This difference in race time was significant (p<0.002). When compared to the current National records for the 10 km race distance, the mean male and female race times were 30.2 \pm 7.5% and 32.1 \pm 8.4% slower than National record times (http://athletics.ca/page.asp?id=66), respectively, illustrating a similar level of performance in the two groups (p=0.53)

The subjects visited the lab on two separate occasions. On the first visit, an incremental exercise test to exhaustion was performed on a treadmill (Woodway Pro, Woodway USA, Waukesha, WA) to determine the subject's maximal oxygen uptake ($\dot{V}O_2$ max) and speed associated with the lactate threshold (sLT). Prior to arriving at the lab, subjects were instructed to not consume any food or beverage, other than water, for a minimum of 12 hours prior to the testing. They were also asked to refrain from the ingestion of caffeine and avoid vigorous

physical activity for 24 hours prior to the testing. The subjects wore cool, loose clothing and their own lightweight running shoes.

 \dot{VO}_2 max and sLT were determined based on methods used previously in our lab (Fletcher et al. 2010; Fletcher et al. 2009). Following a self-selected warm-up of no more than 15 minutes of running, the subjects began running on a motorized treadmill with zero gradient at approximately 3 km•hr⁻¹ slower than the subject's self-reported 10 km race pace. Expired gases were collected by a metabolic cart (Parvomedics Truemax 2400, Salt Lake City, UT) for the determination of \dot{VO}_2 (ml•kg⁻¹•min⁻¹) and carbon dioxide output (\dot{VCO}_2 ,ml•kg⁻¹•min⁻¹). The metabolic cart was calibrated before and after each testing session, as described previously by Fletcher et al. (2009). The treadmill speed was increased by 0.48 km•hr⁻¹ every 2 minutes. After each 2 minute stage, the subjects briefly straddled the belt and a fingertip blood sample was taken for the determination of blood lactate concentration ([BLa⁻], Lactate Pro). When [BLa⁻] rose more than 1 mM from the previous sample, the treadmill belt was returned to the previous speed and the gradient was increased 2% every minute until the subject was unwilling to continue.

sLT was defined as the speed at the stage preceding that which elicited a [BLa⁻] increase of greater than 1 mM. All tests were terminated due to volitional exhaustion. $\dot{V}O_2$ max was defined as the highest 30 s average $\dot{V}O_2$ during the test and was said to have been reached if there was an increase in $\dot{V}O_2$ no greater than 2 ml·kg⁻¹·min⁻¹with an increase in treadmill gradient. 18 of the 29 subjects achieved $\dot{V}O_2$ max based on this criterion. In the other 11 subjects, $\dot{V}O_2$ max was said to have been reached if two of the following occurred: 1) RER greater than 1.15; 2) [BLa⁻] greater than 8 mM or 3) subjects reached their age-predicted

maximal heart rate (220 beats•min⁻¹ - age). All of the remaining 11 subjects achieved $\dot{V}O_2$ max based on these criteria.

Between 48-72 hours following the $\dot{V}O_2$ max testing session, the subjects returned to the lab for determination of AT stiffness and E_{run}. The subjects followed the same pre-testing instructions as the first testing session. AT stiffness was determined on the right leg as described previously by Fletcher et al. (2010). The subjects laid prone with their knee at 180° and their ankle at 90°. Before each MVC, the axis of rotation of the dynamometer (Biodex, Medical Systems Inc., Shirley, NY, USA) was carefully aligned with the axis of rotation of the ankle joint. The shank and unshod foot were affixed to the dynamometer using a series of Velcro straps. The subjects performed three isometric ramp MVC plantarflexions. Moment during the MVC was sampled at 100 Hz. The trial eliciting the highest moment was used for analysis.

During each MVC, a 12.5 MHz linear array ultrasound probe (50mm, Philips Envisor, Philips Healthcare, Eindhoven, Netherlands) was used to visualize the medial gastrocnemius muscle (MG) fascicles, close to the AT. The ultrasound probe was placed on the MG muscle belly, near the myotendinous junction, and secured using a custom-built apparatus. Ultrasound scans were recorded at 49 Hz. A clear point where a fascicle inserts into the deep aponeurosis was followed throughout the MVC and its displacement was measured using *ImageJ*, (NIH, Baltimore, MD, USA). This displacement of a fascicle-aponeurosis junction was considered tendon elongation. An external function generator (B-K Precision 3010, Dynascan Corp., Chicago, IL, USA) was manually started at the beginning of the MVC and served as a time-stamp between image and moment data collection.

3.3.3 Correction for joint rotation

Despite affixing the ankle joint to the dynamometer tightly with Velcro straps, plantarflexion during the MVC is inevitable (Magnusson *et al*, 2001). This inevitable joint rotation would result in a lower resultant torque and would contribute erroneously to the apparent tendon elongation measured during the contraction (Muramatsu *et al*, 2001, Spoor *et al*, 1990). The resultant moment and apparent tendon elongation were corrected for this motion, as described previously (Fletcher *et al*, 2010). Ankle joint motion during the contraction was imaged at 30 Hz using a portable video camera (Canon GL1, Canon Inc., Tokyo, Japan). Joint angle change was determined by following two to four small dots drawn on the medial aspect of the unshod right foot. From this, ankle joint angle could be calculated throughout the contraction using *ImageJ*. We assumed the moment about the ankle resulted in a force perpendicular to the foot. Any change in angle of the foot relative to the Biodex lever will result in an underestimation of the ankle joint moment. To estimate this error, we measured the change in angle of the foot relative to the Biodex lever, and the corrected moments were calculated as:

$$M_{\rm C} = M_{\rm M} \, \mathbf{x} \, \cos(\theta)^{-1}$$

where M_C and M_M are the corrected and measured moments, respectively, and θ , the angle of the foot during the MVC. The corrected moments were used for further calculation of plantarflexion force.

The moment arm of the AT was estimated using the tendon travel method (An *et al*, 1984) under in vivo conditions (Ito *et al*, 2000, Maganaris, 2000). The displacement of a fascicle-aponeurosis cross-point (d_L , mm) caused by passively rotating the ankle at 10°·s⁻¹ from 5° of dorsiflexion to 5° of plantarflexion (d_{θ} , rad) was measured. The AT moment arm was

calculated as the ratio d_L/d_{θ} (mm•rad⁻¹). Triceps surae force was calculated by dividing the ankle joint moment by the estimated AT moment arm.

AT stiffness was determined by fitting the Force (F) - elongation (d_L) data to a quadratic regression equation using:

$$\mathbf{F} = A\mathbf{d}_{\mathrm{L}}^2 + B\mathbf{d}_{\mathrm{L}}$$
 3-3

Where *A* and *B* are constants. AT stiffness was calculated as the slope of the F-d_L curve at three different force ranges: from 25-45% (low range stiffness), 30-70% (mid-range stiffness) and 50-100% (high-range stiffness) of MVC force (Fletcher *et al*, 2010). These force ranges were chosen because the lower force ranges may be more similar to forces experienced by the AT during submaximal running (Giddings *et al*, 2000, Scott and Winter, 1990, Gruber, 2012)

After a 10 minute warm up at 8 km•hr⁻¹ for the females and 9.6 km•hr⁻¹ for the males, the subjects ran at 75, 85 and 95% sLT for 5 minutes each, with a 5 minute standing rest period between speeds. [BLa⁻] was determined immediately prior to and following each speed. E_{run} was calculated as the O₂ cost to cover a given distance (ml O₂•kg⁻¹•km⁻¹) as well as the EC of running a given distance (kcal•kg⁻¹•km⁻¹), as described previously (Fletcher et al. 2009). The steady-state $\dot{V}O_2$, defined as the average $\dot{V}O_2$ over the final 2 minutes of each stage, was used to calculate E_{run}. In all cases, the $\dot{V}O_2$ over the final 2 minutes of each stage did not differ more than 2 ml•kg⁻¹•min⁻¹.

3.3.4 Statistics

Values are presented as mean \pm standard error of the mean (SEM), unless otherwise indicated. A two-way ANOVA (sex, speed) with repeated measures (speed) was used to test for differences in E_{run} , and for stiffness (repeated measures, sex by force range). When there was no significant interaction and a significant main effect was found, Tukey's post hoc test was used to detect significant differences between the three speeds. Pearson Product-moment correlation coefficients and simple linear regression were used to evaluate the relationship between BM and metabolic rate. Correlation and linear regression analysis were also used to evaluate the relationship between E_{run} and AT stiffness by sex. Sidak's multiple comparison tests were used to correct for more than one linear regression analysis. All analyses were performed using GraphPad Prism version 6.01 for Windows (GraphPad Software, La Jolla, CA, USA, www.graphpad.com). Statistical significance was considered *P*<0.05.

3.4 Results

Subject characteristics are listed in Table 3-1. Height and mass were significantly greater in the males compared to the females (p<0.05). The males also had a significantly greater sLT and relative $\dot{V}O_2$ max. The intensity at which sLT occurred (expressed relative to $\dot{V}O_2$ max) was not significantly different between sexes, indicating a similar level of training in these two groups.

Two-way repeated measures ANOVA revealed no significant sex by speed interaction (p=0.48) and no differences in O₂ cost (ml•kg⁻¹•km⁻¹) between sexes (Table 3-2); however O₂ cost increased significantly with speed (p<0.0001, Figure 3.1). Similarly, there was no significant sex by speed interaction for RER and RER was not significantly different between sexes (p=0.59). RER increased significantly as a function of relative speed (p<0.0001). Consistent with these observations, for O₂ cost and RER, there was no significant difference in E_{run} between sexes (Table 3-2). E_{run} also increased significantly with increasing relative speed (p<0.0001).

The use of allometric scaling to $BM^{-0.75}$ did not reduce the inter-individual variability in either O₂ cost or E_{run}. Furthermore, when our data were scaled to $BM^{-0.75}$, there was still no sex by speed interaction in E_{run} (p=0.39) and no main effect for sex (p=0.30).

When male and female data were combined, there was a significant positive relationship between $\dot{V}O_2$ max and sLT (r²= 0.568, p<0.0001), suggesting that runners with the highest $\dot{V}O_2$ max also possessed a high sLT. There was no relationship between $\dot{V}O_2$ max and E_{run} at any of the measured speeds (p=0.468, 0.790, and 0.983 at 75, 85 and 95% sLT, respectively).

Figure 3.2 shows the relationship between E_{run} and absolute speed during the E_{run} tests. E_{run} decreased significantly with increases in absolute speed at 75% sLT (r²= 0.197, p<0.02), suggesting the better runners (i.e. the runners with the highest sLT) had a lower EC of running. This relationship did not reach a significant level for the other two relative speeds (r²=0.128, p=0.056 at 85% sLT; r²=0.09, p=0.086 at 95% sLT), suggesting that runners with a high sLT were no more economical than the runners with a low sLT at high relative speeds. The E_{run}speed relationship was not affected by substrate use, as no relationship between RER and absolute speed existed (r²<0.05, p>0.238 across all measured relative speeds).

3.4.1 Tendon Mechanical Properties

Tendon mechanical properties for both males and females are shown in Table 3-3. AT stiffness of males was significantly greater than the AT stiffness of the females regardless of force range evaluated (p<0.001). There was also a significant, positive relationship between AT stiffness and body mass for all subjects (r^2 =0.295, p=0.002).

Figure 3.3 shows the relationship between AT stiffness at the highest force range and E_{run} in both males and females. There were no significant relationships between AT stiffness and the E_{run} at any force range in the males; however in the females, this relationship was significant at all force ranges at 75% sLT (corrected p<0.05), at the lowest force range at 85% sLT (p<0.05), and at the two lowest force ranges at 95% sLT (p<0.05). The relationships at the other speeds and force ranges approached statistical significance in the females (p=0.054 to p=0.084). When both male and female data were combined, and when AT stiffness was scaled to body mass, the relationship between AT stiffness and E_{run} was significant at all measured speeds (r²=0.158-0.191, p<0.033).

3.5 Discussion

The main findings of this study were three-fold. Firstly, E_{run} did not differ between sexes. This was true when E_{run} was expressed either as the energy cost (kcal•kg⁻¹•km⁻¹) or as an O₂ cost (ml O₂•kg⁻¹•km⁻¹). Secondly, expressing the energy cost relative to BM^{-0.75} did not change this conclusion. Lastly, relationships existed between AT stiffness and E_{run} in the female runners and between AT stiffness and body mass in all runners.

We are aware of only one study to date, in which E_{run} of men and women were reported at similar relative intensities (Helgerud *et al*, 2010). These authors reported that females had a lower O₂ cost than males. However, in that study, O₂ cost was scaled to BM^{-0.75}. We observed no differences in O₂ cost nor in E_{run} between the sexes when we scaled O₂ cost to BM^{-.75}. Another difference between the current study and Helgerud et al. (2010) is that our subjects ran on the treadmill at a level grade, while subjects in the Helgerud study ran on the treadmill with a grade of 1.5%. These latter authors report mean O₂ costs of 670-685 and 753-755 ml•kg^{-0.75}•km⁻ ¹ at speeds near the sLT for females and males, respectively. We have calculated the mean O_2 costs of our runners to be approximately 593 and 621 ml•kg^{-0.75}•km⁻¹ at 95% sLT. It seems unlikely that the difference in oxygen cost between our study and that of Helgerud et al. (2010) can be accounted for by this methodological difference, but this methodological difference may contribute to the different results for the between sex comparison.

It seems possible that the methodological difference (slope of the treadmill), coupled with the normalization to 0.75 of body mass are contributing factors for these discrepant results. For example, using the average mass of males and females from the study by Helgerud (2010) it can be calculated that O₂ cost expressed per kg would have been 254 and 245 ml•kg⁻¹•km⁻¹ for males and females respectively. This represents a difference of 3.7%, whereas the reported values (752 and 686 ml•kg^{-0.75}•km⁻¹) respectively, differ by 9.6%. This larger difference may have allowed reaching statistical significance. The higher values associated with running on a slope of 1.5% coupled with the allometric scaling may be the main reasons for the finding of a significant difference.

However, it is unclear whether other factors (apart from the increased work associate with greater BM of the males and expression of O₂ cost relative to BM^{0.75}) may have affected these reported differences. Although it has been found that running on a treadmill with 1% slope more accurately reflects the O₂ cost of running over ground than running on a treadmill at zero slope (Jones and Doust, 1996), the fundamental factors dictating energy cost of running on a flat surface are different from those factors dictating energy cost of running up a slope. Running up a slope at increasing speed will increase the energy cost of running in proportion to body mass, whereas running over ground at increasing speed increases energy cost of running in proportion to frontal surface area and drag coefficient. The current study demonstrates, however, that when

running on a treadmill with zero gradient, the O₂ cost of running does not differ between males and females of different body mass.

We have previously shown that in a group of highly-trained runners, those runners with a higher sLT have a lower E_{run} (Fletcher *et al*, 2009). This phenomenon is also demonstrated here in lesser-trained runners and, for the first time in female runners (

Figure 3.2). Our results are consistent with the findings of Pollock (1977) who suggest that runners with the lowest E_{run} are associated with the fastest running performance. However, in our study, the relationship between E_{run} and sLT was not statistically significant at the highest speeds tested (p=0.056 and p=0.086). E_{run} is influenced by a variety of factors, and while it is generally accepted that better distance runners are more economical when O₂ cost is measured at an absolute speed, this isn't necessarily the case when E_{run} is presented at similar relative intensities. Differences in O₂ cost at a given speed between individuals are likely a result of the runners being tested at different relative speeds, and it is clear from the current results that E_{run} increases with relative intensity. Thus, faster runners running at a given absolute speed are probably running at a lower relative intensity than the slow runners. It seems logical to compare runners at the speed they would be competing in a long distance run. Thus, in order to elucidate any differences in E_{run} between males and females, then E_{run} should be measured at the same relative intensity.

It could be argued that the current sample size is not sufficiently large to detect a difference between sexes in either RER or E_{run} and this is in fact true. To detect a between-group difference in RER of 0.03 at a given %sLT, it was estimated that a sample size of >140 per group would be required. Also, given our current data, between 63 and 252 runners per group would be needed to detect a difference in E_{run} of the magnitude presented in Table 3-2 at the measured

speeds. The magnitude of difference would be in the order of 3% and if the current results prevailed, females would have the higher E_{run} .

A secondary purpose of this study was to evaluate the relationship between AT stiffness and E_{run} in both male and female runners. It has been shown previously that a stiff AT is associated with a lower E_{run} (Arampatzis *et al*, 2006, Fletcher *et al*, 2010). Furthermore, changes in AT stiffness are associated with changes in E_{run} (Fletcher *et al*, 2010), supporting that this is likely a cause and effect relationship. Here, we show a similar stiffness- E_{run} relationship, but only in the females and not in the males, and only when AT stiffness was measured at the lowest force ranges. Further, no clear demarcation between the male and female data is visible in this relationship. The possibility exists that the small range of E_{run} values and low n in the males precludes any significant relationship between E_{run} and stiffness to be shown.

However, understanding how and why changes in AT stiffness are associated with changes in E_{run} are difficult to elucidate. We speculate that AT stiffness is finely tuned in order to minimize the shortening of the muscle in series with it. This reduces the muscle energy cost (Fletcher *et al*, 2013).

It has been previously shown that energy cost is related to the amount and/or velocity of muscle shortening (Askew and Marsh, 1998) as well as the level of muscle activation, which is necessarily higher to achieve a given force when velocity of shortening is greater (Fletcher *et al*, 2013). During the stance phase of running, the AT will stretch, and subsequent passive recoil of the AT will contribute to positive mechanical work of the muscle-tendon unit at the end of the stance phase (Biewener and Roberts, 2000) decreasing the need for work contributed by the fascicles, which can remain near isometric (Hof *et al*, 2002, Lichtwark *et al*, 2007). For the same load or force exerted by a muscle, a stiffer tendon reduces the amount of energy storage

and return, but minimizes the energy cost of the muscle contraction since it reduces the amount of muscle shortening required to effect joint rotation, thereby reducing the metabolic cost.

Ultimately, optimal AT stiffness is the stiffness which allows the maximal contribution of positive mechanical work by the tendon while keeping the muscle fascicle shortening velocity low during muscle activation. This keeps active muscle volume to a minimum (Barclay *et al*, 2008). It should be kept in mind, however, that we have only examined the mechanical properties of the tendon of one muscle group (the triceps surae), which does not solely dictate the E_{run} . Furthermore, E_{run} is influenced by a variety of factors (Saunders *et al*, 2004), tendon mechanical properties being just one of these.

3.6 Conclusion

In conclusion, the main finding of this study was that when energy cost of running is normalized to body mass, at similar relative speeds of running, that no sex-specific differences in substrate use nor E_{run} exist among similarly trained runners. Furthermore, the stiffness of the Achilles tendon of females is lower than in males, but the relationship between E_{run} and Achilles tendon stiffness is not different between the sexes.

3.7 Author Contribution

All experiments were performed at the Human Performance Lab at the University of Calgary, Calgary, Alberta, Canada. JRF and BRM were responsible for conception and design of the experimental protocol. JRF and TRP collected and analyzed the data. JRF and BRM were primarily responsible for interpreting the experimental data. JRF drafted the manuscript and JRF

and BRM revised it critically for important intellectual content. All authors approved the final draft of the article.

3.8 Acknowledgements and disclosures

The authors would like to thank the subjects for their time and effort in completing the experimental protocol. JRF was supported by NSERC Canada. TRP was supported by the Prize for Undergraduate Research Excellence (PURE), University of Calgary. None of the authors report any conflicting interests.

	NI	Age	Height	Mass	VO ₂ max	sLT	VO ₂ at sLT
Sex	IN	(years)	(m)	(kg)	(ml•kg ⁻¹ •min ⁻¹)	(m•min ⁻¹)	(% VO ₂ max
Male	11	35.3±0.8	1.77±0.04*	77.6±0.7*	55.5±0.8*	234±3*	89±1
Female	18	32.8±0.9	1.65±0.07	57.9±0.6	49.8±0.6	202±2	88±1

3.9 Tables Table 3-1. Subject Characteristics.

Sex	Ν	$O_2 \cos t$				
		ml•kg ⁻¹ •km ⁻¹				
sLT		75%	85%	95%		
Male	11	200±11	204±13	209±11		
Female	18	209±18	212±17	215±17		

Table 3-2. Running Economy and RER.

RER

sLT		75%	85%	95%
Male	11	0.91±0.03	0.93±0.03	0.97±0.03
Female	18	0.90±0.03	0.92±0.03	0.96±0.03

Energy Cost	Energy	Cost
-------------	--------	------

kcal•kg⁻¹•km⁻¹

sLT		75%	85%	95%
Male	11	1.01±0.06	1.04±0.07	1.07±0.07
Female	18	1.05±0.10	1.07±0.09	1.09±0.10

Values are mean \pm standard deviation

Sex	N	AT stiffness			
		(N•mm ⁻¹)			
Force range		25-45%	30-70%	50-100%	
Male	11	$164 \pm 8*$	$175 \pm 6*$	$191 \pm 5*$	
Female	18	97 ± 4	108 ± 5	125 ± 5	

Table 3-3. Tendon mechanical properties for males and females.

Values are mean ± standard error of the mean

* significantly different (p<0.05), males vs females

3.10 Figures



Figure 3.1. O_2 cost at the three measured relative speeds in both males and females.

Speed is expressed relative to the speed at lactate threshold (sLT). Vertical bars represent SD.



Figure 3.2 E_{run} as a function of absolute running speed.

 E_{run} was measured at 75% (A), 85% (B) and 95% (C) of sLT.





Closed and open squares represent each male and female subject, respectively. $E_{\text{run}} \ is$

represented as mean \pm SD for all measured speeds.

Chapter Four: **Tendon compliance, muscle shortening and muscle energetics** Jared R. Fletcher¹, Erik M. Groves^{1,2}, Ted R. Pfister¹ & Brian R. MacIntosh¹

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4.1 Abstract

Decreased whole-body energy cost of running has been associated with an increased Achilles tendon stiffness. It is usually assumed that this lower energy cost can be attributed to less muscle fascicle shortening with a stiffer tendon. Increased fiber shortening is an important determinant of muscle energetics in vitro. However, other factors, like increased muscle activation may be important when considering whole muscle energetics in vivo. To determine the effects of a small additional muscle shortening on skeletal muscle energy requirement, 19 subjects performed 30 plantarflexions on two separate occasions: isometric (ISO) and isokinetic (KIN, $6.98 \text{ rad} \cdot \text{s}^{-1}$), each with a target of 50% of maximum isometric torque. Medial gastrocnemius muscle fascicle length (Lf) was measured by ultrasound and rate of oxyhaemoglobin (HbO₂) desaturation was measured during blood flow occlusion using nearinfrared spectroscopy. KIN resulted in significantly greater muscle shortening (23.8±1.3 mm) than ISO (18.3 \pm 1.0 mm, p<0.001, mean \pm SEM), and greater shortening velocity (KIN=2.5 \pm 0.3 FL•s⁻¹, ISO=1.1±0.1 FL•s⁻¹, p<0.001). Rate of HbO₂ desaturation was 19±7 %, greater in KIN than ISO p<0.01), despite $19\pm2\%$ lower mean torque (p<0.001) and 9.8 ± 1.6 Nm•s lower mean impulse per contraction (p<0.001) in KIN compared to ISO. Root mean square for EMG was significantly greater (p < 0.05) during KIN (73±3%) than during ISO (63±2%). These results illustrate that muscle energy requirement is greater when muscle fascicle shortening and/or velocity of shortening is increased, and suggest that greater activation contributes to that increased energy requirement.

4.2 Introduction

The energy cost (EC) of exercise is primarily determined by the EC of muscle contraction yet little is known regarding the factors affecting the EC of generating muscular force and/or work in humans. The mechanical properties of the major force-generating muscles of the lower limbs have been well investigated, but interpretation of these muscle mechanics in terms of energetics relies on extrapolation from in vitro studies, often using amphibian muscle. It has been shown that a stiff Achilles tendon (AT) is associated with lower EC of running (Arampatzis *et al*, 2006, Fletcher *et al*, 2010). Furthermore, changes in AT stiffness have been shown to relate to changes in economy of running (Fletcher *et al*, 2010), confirming that this is likely a cause and effect relationship.

Ultimately, optimal AT stiffness is that which allows the muscles to operate relatively isometrically during contraction, while the length change of the entire muscle-tendon unit can be accommodated by the tendon alone. In keeping the muscle fascicles isometric, the force-length-velocity relationship of muscle is maximized (Askew and Marsh, 1998). Considering that during running the triceps surae muscles do not undergo substantial stretch prior to shortening (Lichtwark *et al*, 2007, Ishikawa *et al*, 2007), an optimally-tuned AT would result in less fiber shortening to achieve active joint rotation. Furthermore, the elastic energy storage and release of the AT may also contribute to reducing the energy cost of running; however, this effect is small. Given the data reported by Fletcher et al. (2010), and assuming an AT elongation during running of 10 mm (Farris *et al*, 2011) and a hysteresis of 7%, the elastic energy contribution of the AT is estimated to be between 5.4 and 5.7% of the energy cost of each stride, assuming an energy cost of running between 4.40 and 4.64 kJ·kg⁻¹·km⁻¹ and a stride length of 150% of standing height. It has typically been assumed that the lower EC of running associated with a stiff Achilles tendon
is due to reduced shortening of the fibers of the triceps surae muscles (Roberts *et al*, 1998, Alexander, 1991, Arampatzis *et al*, 2006); however, further explanation of this assertion is not given. Does this simply relate to the idea that shortening increases the energy cost of contraction as has been shown in maximally activated muscle (Hill, 1938, Fenn, 1923) or is something more involved?

It has been acknowledged for many years that the heat liberated (ie. the energy) above that required for a purely isometric contraction, *in vitro* is proportional to the work done (Fenn, 1923), which is to say any increase in muscle shortening with a given load would result in a higher EC of contraction. However, work accomplished during a contraction has a complicated relationship with total EC in whole muscle in situ (Stainsby, 1982). Since much of what we know regarding the EC of muscle contraction has been performed in vitro at non-physiological temperatures, we wanted to investigate the relationship between muscle shortening and muscle group EC in humans at physiological temperatures.

It is important to consider that during running, we are dealing with a voluntary contraction, where the force is a consequence of the controlled motion of the leg. Comparing the same movement of the leg with a more compliant tendon (where additional shortening is permitted) should reveal additional potential factors that could affect the energetics of muscle contraction. A more compliant tendon will require not only greater muscle fiber shortening but also greater velocity of fiber shortening for a given load if the leg movement is not different. Greater velocity of shortening would most likely also require increased activation or recruitment of motor units in order to achieve the same force. This increased recruitment can be illustrated by consideration of the force-velocity relationship (see Figure 4.1).

The force-length relationship could also play an important role here. If the more compliant tendon resulted in shortening of fibers on the ascending limb of the force-length relationship, additional activation would be needed to reach the required force for limb movement. By minimizing the magnitude of fiber shortening, a stiff Achilles tendon allows the muscles to operate near isometrically, and to remain near optimal length. In running, where the EC is determined mainly by the cost of producing force to support body weight (Kram and Taylor, 1990, Taylor *et al*, 1980), operating at non-optimal muscle lengths requires a greater level of muscle activation to generate the required force, and thus would elevate the EC of running (Roberts *et al*, 1998). A reduction in muscle activation, if muscle can operate close to its optimal length, should contribute to minimize the EC of contraction (Hogan *et al*, 1998, Bergstrom and Hultman, 1988, Heglund *et al*, 1982).

Near-infrared spectroscopy (NIRS) offers an affordable, portable solution to measuring muscle oxygen uptake. A number of thorough reviews have been dedicated to the use of NIRS during exercise (Ferrari *et al*, 2004, Hamaoka *et al*, 2007, Neary, 2004, McCully and Hamaoka, 2000). When blood flow is occluded to the exercising muscle, the relative rate of change in oxyhaemoglobin (HbO₂) to deoxyhaemoglobin (HHbO₂) signals is considered a reflection of the rate of muscle oxygen uptake (Ding *et al*, 2001, Im *et al*, 2001). Thus, NIRS appears to provide an effective tool in examining the link between the EC of muscle contraction and *in vivo* muscle shortening. Combined with ultrasound to simultaneously measure fascicle shortening and tendon mechanical properties, the effects of these properties on the EC of contraction can be investigated.

Despite a vast array of research examining the EC of running and/or the EC of muscle contraction in a wide range of conditions and species (Taylor *et al*, 1970, Taylor and Heglund,

1982, Sih and Stuhmiller, 2003), no studies to date have directly determined the effects of additional shortening on the EC of muscle contraction of human skeletal muscle at physiological temperatures. Therefore, the purpose of this study was to investigate the possible differences in the EC of contractions performed *in vivo* at physiological temperatures with minimal shortening, and for which extra shortening was allowed. It was hypothesized that when extra fiber shortening was permitted, a greater level of muscle activation would be required to achieve the target force and a greater EC of contraction would result. It seems logical to believe that if the hypothesis is supported, that the increased EC associated with greater activation and increased shortening can explain why optimally-tuned AT stiffness is associated with a reduced EC of contraction

4.3 Methods

4.3.1 Subjects

Characteristics of the 19 triathletes (9 males, 10 females) who participated in the study are shown in Table 4-1. These subjects were chosen because at the time of the study, all subjects were in the pre-competition phase of their run training for either the 10 km or half-marathon race distance. We also anticipated a wide range of AT stiffness and EC in this group. The subjects gave their informed written consent to the experimental procedures, which were approved by the University of Calgary Conjoint Health Research Ethics Board. None of the subjects had neuromuscular or musculoskeletal injuries at the time of the study. All subjects were familiar with the measurement of AT stiffness from previous experiments, but were further familiarized with each measurement prior to data collection. All tests were performed on the same day for each subject.

4.3.2 Tendon Mechanical Properties

The experimental set-up is shown in Figure 4.2. AT stiffness was determined according to Fletcher et al. (2010) and is briefly described here. Each subject performed ramp maximal voluntary isometric ankle plantarflexion contractions (MVC) on their right side. The subjects laid prone with their knee at 180° and their ankle at 90°. Before each MVC, the axis of rotation of the dynamometer (Biodex Medical Systems Inc., Shirley, NY, USA) was carefully aligned with the axis of rotation of the ankle joint. The shank and unshod foot were affixed to the dynamometer using Velcro straps. To further familiarize the subjects with the protocol and to locate at least one visually distinctive and persistent fascicle-aponeurosis cross-point, a warm-up consisting of 3-5 min of submaximal isometric plantarflexions, where they were instructed to gradually and continuously increase the measured torque until their voluntarily-elicited maximum torque generation. The subjects then attempted to maintain this torque for 2-3 seconds, such that the entire ramp MVC took 5-7 seconds to complete. Torque during the MVC was sampled at 100 Hz. The trial eliciting the highest torque was used for analysis.

During each MVC, a 12.5 MHz linear array ultrasound probe (50mm, Philips Envisor, Philips Healthcare, Eindhoven, Netherlands) was used to visualize the deep aponeurosis of the medial gastrocnemius (MG). The ultrasound probe was placed on the MG muscle belly and secured using a custom-built apparatus. Ultrasound scans were captured at 49 Hz. To determine if the probe moved during the contraction, a point on the ultrasound images where a muscle fascicle attaches to the deep aponeurosis was identified both before and after a test contraction for each subject. This point was always in the same position following the test contraction. An external function generator (B-K Precision 3010, Dynascan Corp., Chicago, IL, USA) was

manually started at the initiation of the contraction and acted as a time-stamp for synchronization between image, NIRS and moment data collection. Ultrasound images were recorded and a clear echo point where a fascicle inserts into the deep aponeurosis was followed throughout the contraction and its displacement was measured using publicly-available image analysis software (*ImageJ*, NIH, Baltimore MD, USA). This displacement of a fascicle-aponeurosis junction was interpreted as tendon elongation during these MVCs.

4.3.3 Correction for joint rotation

The amount of joint rotation during the MVC was measured according to Fletcher et al. (2010). This inevitable joint rotation would result in a lower resultant torque and would contribute erroneously to the apparent tendon elongation measured during the contraction (Muramatsu *et al*, 2001, Spoor *et al*, 1990). The resultant moment and apparent tendon elongation were corrected according to Fletcher et al. (2010). Ankle joint motion during the contraction was imaged at 24 Hz using a portable video camera (Canon GL1, Canon Inc., Tokyo, Japan). Joint angle change was determined by drawing two to four small dots on the medial aspect of the unshod right foot. From this, ankle joint angle could be calculated throughout the contraction using *ImageJ*. We assumed the moment about the ankle resulted in a force perpendicular to the foot. Any change in angle of the foot relative to the biodex lever will result in an underestimation of the ankle joint moment. To estimate this error, we measured the change in angle of the foot relative to the biodex lever assured the corrected moments were calculated as:

 $M_{\rm C} = M_{\rm M} \, x \, \cos(\theta)^{-1}$

4-1

where M_C and M_M are the corrected and measured moments, respectively, and θ is the angle of the foot at peak moment. The corrected moments were used for further calculation of plantarflexion force.

The moment arm of the AT was estimated using the tendon travel method (An *et al*, 1984) under in vivo conditions (Ito *et al*, 2000, Maganaris, 2000). The displacement of a fascicle-aponeurosis cross-point (d_L , mm) caused by rotating the ankle from 5° of dorsiflexion to 5° of plantarflexion (d_{θ} , rad) was calculated from the passive rotation. The AT moment arm was calculated as the ratio d_L/d_{θ} (mm·rad⁻¹). Triceps surae force was calculated by dividing the ankle joint moment by the estimated AT moment arm.

The measured force-elongation data were fitted to a quadratic equation:

$$Force = A(dL)^2 + B(dL)$$
 4-2

AT stiffness was defined as the force-elongation slope from 50 -100 % of maximal isometric plantarflexion force, calculated from the quadratic force-elongation relationship Force = $A(dL)^2+B(dL)$ 4-2).

4.3.4 Measurement of EC of contraction

To evaluate the effects of muscle fascicle shortening, during brief contractions with specific target force, on MG EC, the subjects laid prone on the dynamometer in the same position as for the testing of AT mechanical properties. The ankle was affixed to the dynamometer and the ultrasound probe placed on the MG and the subject's MVC was determined as described previously.

4.3.4.1 Testing Protocol

Following the MVC trials, the subjects performed 30 plantarflexions at a frequency of 1 Hz, attempting to reach 50% of maximum torque with each brief contraction under two conditions (see below). This load was chosen as it is similar to the force exerted on the Achilles tendon during running at 3 m•s⁻¹ (Kyröläinen *et al*, 2003), which is equivalent to approximately 84% of the speed associated with the lactate threshold for our subjects. Contractions were performed on an isokinetic dynamometer under two conditions, in random order: isometric (ISO) and isokinetic (KIN). Plantarflexion angular velocity was set at 6.98 rad•s⁻¹ during KIN. Throughout the contractions, torque, angular velocity and position angle signals were collected from the dynamometer at 100 Hz using data acquisition software (WinDaq Pro+, DataQ Instruments Inc., Akron, OH, USA). The subjects received feedback on the magnitude of contractile torque from a monitor displaying the torque signal as % MVC in front of them.

The maximal joint rotation allowed during KIN was set prior to the contractions based on an estimated additional AT elongation (d_L) of 15 mm. The estimated additional joint rotation (d_{θ} , rad) required for this elongation during KIN was estimated from the previously determined calculation of Achilles tendon moment arm (MA, mm•rad⁻¹):

$$\mathbf{d}_{\boldsymbol{\theta}} = \mathbf{d}_{\mathrm{I}} \cdot \mathbf{M} \mathbf{A}^{-1}$$

This magnitude of elongation was chosen because based on pilot testing, this magnitude of AT elongation represented an increase of 50% of the magnitude of the non-corrected AT elongation during an isometric MVC. Based on a maximum plantarflexion force of 3000 N, this increased magnitude of elongation was estimated to represent an apparent increase in AT compliance of 40%. This increase in AT compliance is consistent with a 2.7% increase in the

 E_{run} (Fletcher *et al*, 2010) and represents an increase of approximately 2 kcal•km⁻¹ for the runners in this study.

During both experimental conditions, the rate of haemoglobin (HbO₂) desaturation was measured during blood flow occlusion using spatially resolved near-infrared spectroscopy (NIRS, PortaMon, Artinis, Zetten, The Netherlands) collected at 10 Hz. Blood flow occlusion was achieved by rapidly inflating a blood pressure cuff placed around the subject's thigh. Cuff pressure was maintained at 240 mmHg for the duration of the contractions. Blood flow occlusion was confirmed by examining the change of HbO₂ saturation and desaturation signals throughout the contraction protocol. In all cases, a symmetrical change for HbO₂ saturation and desaturation existed, suggesting no change in total Hb implying that no additional saturated blood had entered the area during the contractions (Ryan *et al*, 2012).

HbO₂ desaturation was assumed to be proportional to energy use, the rate of which was expressed as AU•s⁻¹. The NIRS device was positioned medially relative to the position of the ultrasound probe. HbO₂ desaturation was calculated as the first derivative of the HbO₂ desaturation signal using Matlab (ver. R2010a, Mathworks, Natick, MA, USA).

Fascicle lengths were measured using *ImageJ* at rest and throughout the 10th, 15th, 20th, 25th and 30th contractions. Wherever possible, the same fascicle was measured throughout the contractions. Where this was not possible, a visually distinctive fascicle near the vicinity of the originally-measured fascicle was used. In a small number of cases, a complete MG fascicle could not be seen on the ultrasound image. In those cases, fascicle length was measured using linear extrapolation (Finni *et al*, 2001) by measuring the distance between superficial and deep aponeuroses and dividing this distance by the sine of resting pennation angle (Austin *et al*, 2010).

Internal muscle work (J) was calculated as the integral:

$$Work = \Sigma(F x dL_f)$$

$$4-4$$

from rest to peak force generation, where dL_f is the change in MG fascicle length, and F is the mean plantarflexion force over the time-course of dL. It was assumed that dL was the same for all triceps surae muscles during the contractions. This was considered a better assumption than estimating the MG muscle contribution to plantarflexor force based on the physiological crosssection of individual triceps surae muscles. Power (W) was calculated as muscle work divided by duration of positive work.

The level of muscle activation was assessed using surface electromyography (EMG) throughout the contractions. Prior to the contractions, a 4 cm x 8 cm area on the skin over the muscle belly of the lateral gastrocnemius (LG) and soleus (SOL) muscles, as well as over the head of the fibula were shaved and cleaned with alcohol. Two EMG electrodes (Norotrode 20 bipolar Ag-AgCl electrodes, Myotronics Inc, Kent, WA, USA, inter-electrode distance: 22±1 mm), were affixed longitudinally to the shaved area over each muscle oriented along the direction of the muscle fibers, as confirmed for each muscle by ultrasound. A single electrode over the head of the fibula served as a ground. EMG of the MG was not possible due to space limitations on the muscle as a result of the ultrasound probe and NIRS device. EMG data were recorded at 2048 Hz using the NeXus-10 Biotrace+ (version 1.16) Wireless Biofeedback System (Mindmedia, Roermond-Herten, The Netherlands). To reduce noise and signal artifact, the signal was filtered through a 5th order Butterworth filter (high and low-pass filter of 20 and 500 Hz, respectively). EMG amplitude was calculated as the root mean square (RMS) of the raw EMG signal. This RMS was interpreted as the level of muscle activation; an accumulation of recruitment and rate coding. EMG RMS of the LG and SOL were evaluated on the same

contractions as the fascicle measurements for each experimental condition. In a subset of subjects on a separate day (n=4, 26 ± 3 years 1.65 ± 0.04 m, 61.3 ± 12 kg), the EMG amplitudes of the MG and LG were evaluated, without the use of NIRS or ultrasound, in order to determine whether the EMG amplitude of LG provided an appropriate estimate of MG activation during the experimental trials where the NIRS and ultrasound probe were placed on the MG. EMG amplitude was measured and calculated as described above, during a ramp MVC, and during similar ISO and KIN trials. To confirm whether EMG amplitude increased equally as a function of load (expressed as %MVC), the EMG amplitude was also collected while the subjects attempted to maintain a constant isometric torque at 30, 40 and 50% of MVC.

4.3.5 Statistics

Data are presented as mean \pm standard error of the mean (SEM) and were analyzed using SPSS analysis software (SPSS Inc. v15.0, Chicago, IL, USA). A two-way repeated measures ANOVA was performed to examine the condition x contraction number for the following: torque, impulse, MG shortening length, MG shortening velocity and for EMG RMS. No significant interactions were found, so statistical comparison of these variables refers to main effects. Paired *t*-tests were used to test for differences between conditions for HbO₂ desaturation, Pearson product-moment correlations were used to identify relationships between HbO₂ desaturation. The *a priori* level of statistical significance was set at alpha <0.05.

4.4 **Results**

AT stiffness was $151 \pm 66 \text{ N} \cdot \text{mm}^{-1}$. dL_{ROM} was $15.5 \pm 2.2 \text{ mm}$. The calculated ankle range of motion during KIN was 23.4 ± 3.1 deg. Average torque during ISO was $56.2\pm 5.1 \text{ Nm}$

(52.6 \pm 2.1 % MVC). Average torque during KIN was 34.5 \pm 2.2 Nm (33.4 \pm 1.4 % MVC), significantly lower than during ISO (p<0.001) and substantially less than the target. Mean impulse was also significantly greater for ISO (19.6 \pm 1.9 Nm•s) compared to KIN (9.8 \pm 0.8 Nm•s, p<0.001).

Mean MG fascicle length (L_f) measured prior to the contractions was 55 ± 2 mm. L_f at peak torque during the MVC trial was 32 ± 2 mm. Mean L_f at peak torque was significantly greater (p<0.01) for ISO (38 ± 1 mm) compared to KIN (32 ± 2 mm). The ISO contractions were of significantly longer duration (0.33 ± 0.03 s) compared to KIN (0.19 ± 0.01 s). Mean shortening velocity for ISO was 1.13 ± 0.13 L_f •s⁻¹ and for KIN was 2.48 ± 0.31 L_f •s⁻¹. This difference was significant (p<0.001).

Combining the results of fascicle shortening and force to estimate internal muscle work between conditions, ISO resulted in significantly more work compared to KIN (ISO = 32.2 ± 5.3 J•contraction⁻¹, KIN = 19.9 ± 1.8 J•contraction⁻¹, p<0.05); however, the rate of performing that work (ie. power) was not different (p>0.05) between conditions (ISO = 98 ± 13 W•contraction⁻¹, KIN = 117 ± 14 W•contraction⁻¹).

Despite a lower mean torque and impulse in KIN, the mean rate of HbO₂ desaturation was significantly greater in KIN (p<0.01). KIN resulted in a 18.6 ± 6.5 % greater HbO₂ desaturation (p<0.01) and required a shorter period of time to reach the maximum rate of HbO₂ desaturation (Figure 4.3). The contraction number at which maximum rate of HbO₂ desaturation occurred was, on average, the 12th contraction (range: 6th to18th contraction) for ISO and the 9th contraction (range: 6th to 13th) for KIN. Results of the paired *t*-test revealed this contraction number to be significantly fewer for KIN than ISO (p=0.03). Taken together, these results suggest a greater rate of energy use and thus a greater EC of contraction in KIN. Results for rate of HbO_2 desaturation and mean torque are shown in Figure 4.4.

Combining these results, the energy required to maintain a given torque (HbO₂ desaturation•impulse⁻¹) was greater in KIN ($6.2 \pm 0.6 \text{ AU} \cdot \text{Nm}^{-1} \cdot \text{s}^{-1}$) compared to ISO ($2.4 \pm 0.3 \text{ AU} \cdot \text{Nm}^{-1} \cdot \text{s}^{-1}$). This represents a difference in EC approaching 160%. HbO₂ desaturation was also significantly related to the average amount of shortening during ISO and KIN conditions (Figure 4.5) and the average velocity of shortening (Figure 4.6).

In a separate series of measurements, EMG of MG and LG was measured to determine if LG EMG changed in a similar way as EMG of MG. On average, the EMG amplitude of MG was 2-fold larger than that of LG and the relationship between EMG amplitudes of the MG and LG during the submaximal steady-state and maximal contractions was significant (r^2 =0.752, p<0.0001). Furthermore, the change in EMG amplitude from 30-100% MVC (as evaluated from the slopes of the EMG amplitude-%MVC relationships) was not different between MG and LG (p=0.478). This confirms that changes in EMG amplitude in LG during the trials could be interpreted to represent changes in EMG amplitude of MG. Mean RMS amplitude during the MVC for LG and SOL was 0.570 V and 0.907 V, respectively. Figure 4.7 shows EMG RMS data, presented relative to the EMG RMS amplitude measured during the isometric MVC. Two-way repeated-measures ANOVA revealed no significant effect of contraction number on EMG RMS amplitude; however there was a significant main effect of experimental condition on EMG amplitude. EMG amplitude in KIN was significantly higher than ISO (p<0.05).

4.5 Discussion

The purpose of this study was to investigate the effects of additional MG fascicle shortening on the EC of muscle contraction. The main finding in this study was that when greater MG fascicle shortening was imposed, the rate of muscle oxygen uptake increased. We assume this measured oxygen uptake is proportional to the total EC of the muscle contractions; that is, any anaerobic energy utilization would increase in proportion with the increases in oxygen uptake. The additional shortening and EC during KIN may help to explain the reported benefit of a stiff Achilles tendon in reducing the whole-body EC of running (Arampatzis *et al*, 2006, Albracht and Arampatzis, 2006, Fletcher *et al*, 2010). Given the current data, we propose that the explanation for the increased EC *in vivo* at physiological temperatures is more complex than simply explaining EC on the basis of extra shortening.

Early reports by Fenn (1923, 1924) would suggest the EC of maximally-activated muscle is proportional to the amount of work done; however, in this situation the load was constant and work was proportional to shortening. Given that the EC of achieving a target force is greater than that of maintaining it (Russ *et al*, 2002, Foley and Meyer, 2005), we speculated that it was the additional muscle shortening in KIN which contributed to, but is not the sole factor in the elevated EC. Here, we now demonstrate that it is not the amount of work performed per se which dictates the EC of muscle contraction, since the EC during KIN was significantly higher than during ISO, despite more work performed in the latter condition. Rather, the EC of voluntary muscle contraction performed *in vivo* is determined by a combination of muscle shortening, shortening velocity and level of motor unit recruitment.

The amount and velocity of shortening are dictated by the mechanics of joint movement and the mechanical properties of the tendon. However, the muscle's in vivo force-length and

force-velocity relationships dictate the magnitude of activation required to achieve a given shortening (Praagman *et al*, 2006). The force-velocity relationship dictates that force production for a given level of activation is maximal when that force can be developed isometrically (Biewener, 1998, Gabaldon *et al*, 2008, Roberts *et al*, 1997) and decreases as shortening velocity increases.

It has been suggested that the EC of contraction in vivo should be related not only to the amount of fiber shortening and the shortening velocity but also the level of motor unit activation (Stainsby and Lambert. 1979). In fact, Stainsby and Lambert (1979) suggest that the major determinant of metabolic cost of contraction in voluntary movement should be motor unit recruitment. This notion is consistent with the observed (RMS) EMG during cycling, which has a minimum at a unique cadence associated with a given power (MacIntosh et al, 2000), and this cadence is closely related to the optimal cadence for best efficiency (Coast and Welch, 1985). Load, shortening and velocity of shortening have less impact on the magnitude of energy requirement (Stainsby and Lambert, 1979). For submaximal contractions like those imposed during the present study, the level of activation (as measured by EMG) needed to generate a given (target) force can be minimized when the fascicles are allowed to develop force isometrically. This is illustrated in Figure 4.1. In our data (Figure 4.7), 50 % of MVC was achieved in ISO with just 50% of maximal (RMS) EMG but for KIN, the required level of activation increased to above 80%. It is presumed that the EC of contraction during KIN was greater compared to ISO as a result of the increased rate and amount of MG activation required to achieve the target force in the face of increased fascicle shortening and shortening velocity during the KIN contractions.

The additional fascicle shortening during KIN also impacts force because the muscle is operating at a different place on its force-length relationship (Gordon *et al*, 1966, Maganaris, 2003). For a given muscle force required to perform the task (eg. running a particular speed and supporting body weight), the level of activation can be minimized if the muscle is operating near optimal length. In keeping the level of activation to a minimum, active muscle volume to generate the required force is minimized and so is the considerable cost of muscle activation associated with ion pumping (Heglund et al. 1982; Hogan et al. 1998). The EC of running is determined primarily by the force of supporting the athlete's body weight and the time course of generating this force (Kram and Taylor, 1990). When the speed of running is increased, the EC is elevated because the required force is developed more rapidly (Roberts et al. 1998).

It has previously been shown that in maximally activated voluntary isometric contraction, the MG muscle is on the ascending limb of the force-length relationship at anatomical ankle joint angles (Maganaris, 2003), with the highest force at $+20^{\circ}$ dorsiflexion. Assuming this joint configuration corresponds with optimal sarcomere length, sarcomere length during a MVC at a neutral ankle angle of 90° can be estimated as approximately 83% of optimal gastrocnemius sarcomere length during MVC (Maganaris, 2003). The current study measured fascicle length for maximal (31.7 mm) and submaximal (37.8 mm) contractions at an ankle angle of 90°. Assuming an optimal sarcomere length during maximal activation of 2.6 µm at the short side of the plateau (Herzog and ter Keurs, 1988), then it is estimated that the sarcomere lengths were 2.57 µm for ISO and 2.19 µm for KIN. The EC of the force impulse is increased at short muscle length (de Haan *et al*, 1986). This increase occurs because the energy for activation (ion pumping) is independent of length (Homsher *et al*, 1972, Woledge *et al*, 1985), and energy for

force development is proportional to force. However, the small differences we observed in estimated sarcomere length would indicate that this effect was minor.

Despite greater muscle work performed during the ISO contractions, KIN resulted in a significantly greater EC of contraction compared to ISO. However, the EC of contraction was directly related to the amount and rate of MG fascicle shortening. The rate of energy use has been shown previously to relate to the rate of muscle shortening (Fenn, 1924, Hill, 1938, He *et al*, 2000). However, it is clear from the above data that this is not the only factor which dictates the EC of in vivo voluntary muscle contraction.

In spite of greater muscle work in ISO, the EC was significantly reduced in this condition compared to KIN. Despite greater force per contraction in ISO, muscle shortening under this condition was less than in KIN. Heglund et al. (1982) demonstrated that the energetic cost of locomotion is related to the rate at which muscles are turned on and off, such that a faster rate of activation is associated with an elevated metabolic cost. These results suggest that short-duration contractions (such as those seen in KIN) may require a higher amount of total energy as a result of ion pumping associated with each activation cycle. The present results support this notion, since more EMG was observed, indicating more motor unit activation. This probably relates to differences in sarcomere length and the impact of the force-velocity relationship.

The contractions by the MG fascicles during running are nearly isometric (Ishikawa *et al*, 2007) thus, the results of the current study may be relevant to running, and may explain why a stiffer tendon helps to minimize the EC of whole-body locomotion. As indicated by several authors, a more compliant Achilles tendon would require a greater amount of fascicle shortening (Fletcher *et al*, 2010, Arampatzis *et al*, 2006, Albracht and Arampatzis, 2006). However, it should be acknowledged that additional related factors contribute to the increased energy cost.

This includes increased velocity of shortening for a given joint movement and increased activation of the involved muscles. We observed that with additional shortening and a similar target force, there was increased velocity of shortening and a greater level of motor unit recruitment. This increased recruitment would contribute to the elevated EC of contraction.

4.6 **Conclusions**

In conclusion, the results of the current investigation confirm previous reports that the EC of muscle contraction is related to the amount and rate of muscle shortening. Furthermore, these results may explain why the EC of running is elevated when Achilles tendon compliance is increased, since a greater amount and rate of shortening are required for force transmission under these conditions. According to the in vivo force-length and force-velocity relationships of skeletal muscle, this shortening and velocity will impact the EC, not simply because of the greater shortening, but because increased muscle activation is required to permit similar force development when shortening velocity is greater.

4.7 Author Contributions

All experiments were performed at the Human Performance Lab at the University of Calgary, Calgary, Alberta, Canada. JRF and BRM were responsible for conception and design of the experimental protocol. JRF and EMG collected and analyzed the NIRS data, while JRF and TRP collected and analyzed the ultrasound data. JRF and BRM were primarily responsible for interpreting the experimental data. JRF drafted the manuscript and JRF and BRM revised it critically for important intellectual content. All authors approved the final draft of the article.

4.8 Ethical standards

The authors declare that the experiments comply with current Canadian laws and all experimental procedures were approved by the University of Calgary Conjoint Health Research Ethics Board.

4.9 Conflict of Interest statement

The authors report no commercial involvement which may bias the process of data collection, reporting and/or interpretation.

4.10 Acknowledgements

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4.11 Tables

Sex	Ν	Age	Height	Mass	VO ₂ max
		(years)	(m)	(kg)	$(ml \cdot kg^{-1} \cdot min^{-1})$
Male	9	36.8 ± 2.9	$1.76 \pm 0.02*$	$76.5 \pm 2.6*$	53.6 ± 2.7
Female	10	33.6 ± 3.2	1.67 ± 0.03	58.4 ± 1.8	47.7 ± 2.1

Table 4-1. Subject characteristics.

Values are mean ± SEM

* significantly different between males and females (p<0.05)



Figure 4.1. The effect of greater shortening velocity on muscle activation to achieve a target force.

The force-velocity relationship, scaled to activation (Chow and Darling, 1999). The short dashed and solid lines represent 50% and 85% of maximal motor unit activation, respectively. The long dashed line represents maximal activation. When force can be generated isometrically, target force can be achieved with minimal motor unit activation, as shown by open square. When shortening is permitted, additional motor unit activation is required (filled square).



Figure 4.2. Experimental set-up.



Figure 4.3. Representative tracing for one subject of HbO₂ desaturation as measured by NIRS.

Dashed and solid lines represent ISO and KIN conditions, respectively. The rate of HbO_2 desaturation was significantly faster in KIN.



Figure 4.4. Mean maximum rate of muscle oxygen uptake (maximum HbO₂) compared to mean torque during the experimental conditions.

Values are mean \pm SEM. Despite a lower mean torque in KIN, energy use was significantly greater than ISO.



Figure 4.5. The relationship between the rate of energy use to maintain a given torque $(HbO_2 \cdot impulse^{-1}, AU \cdot Nm \cdot s^{-1})$ and magnitude of MG muscle fascicle shortening (cm). The open diamonds represent measurements made during the ISO condition. The filled squares represent those measurements made during the KIN condition. When combined together, the relationship was significant (r²=0.21, p=0.004). Because of similar values between subjects, some data points are over-lapped.



Figure 4.6. The relationship between the rate of energy use to maintain a given torque $(HbO_2 \cdot impulse^{-1}, AU \cdot Nm^{-1} \cdot s^{-1})$ and shortening velocity $(L_f \cdot s^{-1})$.

The open diamonds represent measurements made during the ISO condition. The filled squares represent those measurements made during the KIN condition. When combined together, the relationship was significant ($r^2=0.38$, p<0.001). Because of similar values between subjects, some data points are over-lapped.



Figure 4.7. EMG amplitude for LG (top) and SOL (bottom) for both experimental conditions expressed relative to EMG amplitude measured during the isometric MVC (100% MVC).

One-way repeated measures ANOVA revealed a significant effect of experimental condition on EMG amplitude, with KIN resulting in a greater EMG amplitude compared to ISO.

Chapter Five: ESTIMATES OF ACHILLES TENDON MOMENT ARM LENGTH AT DIFFERENT ANKLE JOINT ANGLES: EFFECT OF PASSIVE MOMENT

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5.1 Abstract

Estimating active muscle forces in vivo is typically done via measurement of joint moment and dividing by the muscle's moment arm (MA). The length of the MA can be estimated noninvasively using ultrasound, and the tendon excursion (TE) method. The main assumption with the TE method, however, is that the force acting on the tendon during passive rotation is constant. However, passive force changes through the range of motion, and MA length is underestimated. Therefore, we attempted to account for passive force on the measurement of Achilles tendon (AT) MA length using the TE method in 12 male and female runners. Tendon excursion (d_L) was measured using ultrasound while the ankle was passively rotated at 0.17 rad•s⁻¹. Using the TE method, MA length was calculated at 5° intervals as the ratio of d_L to joint rotation (d_{Θ} , radians) from 70° to 115°. At the same time, passive moment (M_P) was measured by a dynamometer. The d_L attributable to M_P was calculated by monitoring d_L during a ramp isometric maximum contraction. M_P was 7.6±2.7 Nm at 70° and decreased exponentially from $70^{\circ}-90^{\circ}$. d₁ attributable to M_P was 4.8 ± 3.0 mm at 70° . This resulted in M_P-corrected MA lengths that were significantly larger than uncorrected MA lengths. The coefficients of variation for uncorrected and corrected MA lengths were $11.0 \pm 3.8\%$ and $8.4 \pm 3.3\%$, respectively. Correcting for M_P yielded more reliable measures of MA length compared to the traditional TE method, where passive forces at the ankle are not considered.

5.2 Introduction

Since direct measurement of muscle forces *in vivo* is highly invasive (Fukashiro *et al*, 1993), muscle forces are typically estimated from measurement of joint moments (An *et al*, 1984, Fukunaga *et al*, 2001). Estimating muscle forces from joint moments requires knowledge of the muscle/tendon moment arm (MA) length, the perpendicular distance from the joint centre of rotation to the line of action of the muscle (Pandy, 1999). Knowledge of any change in MA with respect to joint angle (Maganaris, 2000, Rugg *et al*, 1990, Visser *et al*, 1990) allows a more precise estimate of muscle force and has significance in muscle modeling, but often, a constant MA length is used to estimate muscle forces since determining MA is difficult to measure in cadavers and hard to determine with accuracy in vivo (Buchanan *et al*, 2005). Therefore, knowing the angle-specific muscle MA is of importance.

Two non-invasive methods to estimate muscle or tendon MA length have become popular in recent years. Magnetic resonance imaging (MRI) allows a high quality image of the joint, from which an apparent centre of rotation (COR) can be estimated, and the perpendicular distance from the estimated centre of rotation to the line of action of the muscle can be measured (Rugg *et al*, 1990). The Achilles tendon MA can also be measured in three-dimensions across ankle angles using MR-imaging (Sheehan, 2012). However, due to limited accessibility and considerable time and costs associated with its use, MRI may not be a viable option in many cases. Consequently, a series of hybrid measurements based on the COR method have been developed, including measuring the perpendicular distance of the apparent COR to AT midpoint using ultrasonography and motion analysis (Manal *et al*, 2010) or simply measuring this perpendicular distance directly (Scholz *et al*, 2008). Further, the actual COR of the joint is still estimated when using this method. Consequently, no true gold standard in the measurement of ankle joint MA length exists.

The tendon excursion (TE) method uses ultrasonography to calculate MA length by measuring the displacement of the tendon (d_L) with respect to the angular rotation of the joint (d_{θ}) (Ito *et al*, 2000, Maganaris, 2000). This approach does not require knowledge of the joint's centre of rotation, approximation of which may introduce error into the estimation of MA (Klein *et al*, 1996). The TE method is based on the principle of virtual work (An *et al*, 1984), which assumes that a constant force is applied to the tendon. A constant force will allow a constant tendon length, so measured tendon excursion represents a true displacement.

In vivo, it is assumed that when no external moment acts on the joint, such as during a passive rotation of the ankle joint, the tendon MA is calculated as the ratio of d_L to d_{θ_L} . Given these conditions, the TE method may offer a better estimate of MA length compared to the COR method since estimates of the joint centre of rotation is not required.

Given that tendons are compliant, and are stretched when a force is applied, the principle of virtual work is violated when passive force changes as occurs at dorsiflexion angles (Fath *et al*, 2010). These passive forces and the corresponding elongation of the tendon could be accounted for to allow an accurate, and potentially more valid MA length determination using the TE method. This technique is likely to be more time and cost-effective compared to the COR method.

Therefore, the purpose of the current investigation was to compare the AT MA length using the TE method to a novel TE method, accounting for passive forces on the AT. We hypothesize that the calculated MA would be considerably larger than when passive forces are not accounted for, over the range of motion where passive force is exerted. This occurs because

any change in passive force during the measurement would prevent detection of tendon translation, making the MA appear smaller. Furthermore, a secondary objective of this study was to quantify the test-retest reliability of the TE method, using the corrected measurement of d_L .

5.3 Methods

All subjects (4 males and 8 females) were distance runners, training at least five times per week. Mean (\pm sd) age, height and weight for all subjects were 33.8 \pm 10.2 years, 168.6 \pm 11.3 cm, 63.1 \pm 15.6 kg, respectively. We chose distance runners as subjects as they had previously participated in studies involving the measurement of AT moment arm using the TE method (Fletcher *et al*, 2013). While we recognize that the AT mechanical properties of this cohort were likely different than the general population, it has been previously shown that the passive stiffness of the ankle joint is independent of the AT mechanical properties (Kubo *et al*, 2001). Thus, we felt our results are generalizable to lesser-trained populations.

All subjects gave their informed written consent to participate in the experimental procedures, which were approved by a University of Calgary Research Ethics Board. None of the subjects had neuromuscular or musculoskeletal injuries. The estimate of each subject's Achilles tendon (AT) MA length was performed using a dynamometer (Biodex Medical Systems Inc., Shirley, NY, USA) while the subjects laid prone with their knee fully extended. The shank and unshod foot were affixed to the dynamometer using Velcro straps, with the ankle at 90°. Ankle angle was defined as the angle of the foot relative to the long axis of the tibia. The axis of rotation of the dynamometer was carefully aligned with the assumed axis of rotation of the ankle

joint; the dynamometer axis aligning with the midpoint of the axis aligning the medial and lateral malleoli.

The MA of the AT was estimated using the TE method (Ito et al, 2000, Maganaris, 2000). While the subject's ankle joint was passively rotated at 0.17 rad \cdot s⁻¹ from maximum ankle plantarflexion to maximum ankle dorsiflexion, a 12.5 MHz linear array ultrasound probe (50mm, Philips Envisor, Philips Healthcare, Eindhoven, Netherlands) was used to visualize the medial gastrocnemius muscle (MG) fascicles close to the myotendinous junction. Ultrasound scans were recorded at 49 Hz. A clear fascicle-aponeurosis intersection near the myotendinous junction was followed throughout the passive trials and its displacement was measured using ImageJ, (NIH, Baltimore, MD, USA). A fascicle-aponeurosis intersection was chosen in favor of the myotendinous junction since it offered better contrast resolution on the ultrasound images (and thus a more spatially distinct point to manually track, compared to the relatively large, hyperechoic myotendinous junction). The displacement of a fascicle-aponeurosis junction was assumed to represent tendon translation (d_L). In order to familiarize the subject with the procedure and to minimize the effect of time-dependent deformation, including conditioning effects (Hoang *et al*, 2007), the ankle was moved through the range of motion at least four times prior to beginning data collection.

To monitor muscle activation during the passive trial, two pairs of surface electromyography (sEMG) electrodes (Norotrode 20 bipolar Ag-AgCl electrodes, Myotronics Inc, Kent, WA, USA, inter-electrode distance: 22±1 mm), were affixed over the soleus and lateral gastrocnemius muscles, along the direction of the muscle fibers, as confirmed by a longitudinal ultrasound scan. An electrode over the head of the fibula served as ground. EMG of the MG was not possible due to the space occupied by the ultrasound probe. EMG data were

recorded at 2048 Hz using the NeXus-10 Biotrace+ (version 1.16) Wireless Biofeedback System (Mindmedia, Roermond-Herten, The Netherlands). Muscle activation (or lack thereof) was considered by evaluating the raw EMG signal throughout the passive trial. It was intended that if at any point during the trial the EMG amplitude rose 3 SDs above resting EMG amplitude, the passive trial would be repeated until EMG amplitude remained at baseline. Based on this criterion, no trials were rejected.

For each subject, a series of MA measurements using the TE method were made throughout the ankle range of motion, from maximum plantarflexion to maximum dorsiflexion. All d_L measurements were done in this direction to avoid variability due to hysteresis effects. MA lengths were calculated over several 7-8° intervals (d_0), from maximum dorsiflexion to maximum plantarflexion, in order to allow consideration of AT MA length estimates where passive forces could be considered. In all cases, the number of total MA estimates per subject was between 6 and 8. Passive moment and joint angular displacement data were sampled at 100 Hz. The d_L/d_0 data for each subject were fitted to a 3rd -order polynomial equation across the measured range of motion:

$$d_{\rm L} = a\theta^3 + b\theta^2 + c\theta + d$$
 5-1

where θ is joint angle and *a*, *b*, *c* and *d* are constants. A typical example for one subject is shown in Figure 5.1.

5.3.1 Correction for passive force

The moment measured by the dynamometer (M_M) during the passive rotation trial is shown in Figure 5.2 and consists of the moment due to the force of gravity (M_G) , on the dynamometer footplate, the moment due to the weight of the foot (M_F) and any passive muscle moment (M_P) :

$$M_{\rm M} = M_{\rm G} + M_{\rm F} + M_{\rm P}$$
 5-2

 M_G was measured by passively rotating the footplate alone, prior the start of data collection. M_M measured from maximum plantarflexion to maximum dorsiflexion was fitted to a third-order polynomial equation, similar to $d_L = a\theta^3 + b\theta^2 + c\theta + d$

5-1. M_G was subtracted from M_M at equivalent ankle joint angles. Thus, subtracting M_G from $M_M = M_G + M_F + M_P$

5-2 gives:
$$M_{M}-M_{G} = M_{F}+M_{P}$$
 5-3

There is no easy way to accurately estimate M_F , so an alternative method was used to isolate M_P . In a subset of the original subjects (n=4), M_P was calculated while the ankle was rotated throughout the subject's range of motion in a horizontal position where gravity would have no effect on the measured moment. M_P was then calculated from 90° to end range of motion in dorsiflexion. This range was used because there was no apparent M_P at angles greater than 90°. In this horizontal position, it was assumed gravity was not affecting the measured moment. However, it was found that passively rotating the footplate through the range of motion gave a small constant offset moment. This moment was subtracted from the measured moment to obtain the true M_P . This M_P is referred to as the 'horizontal' measure. Considering that M_P in the vertical position should be equal to M_P in the horizontal position, any difference between these measures should be equal to M_F . To estimate M_P with the subject lying prone, we used linear regression of horizontal Mp vs Mp+Mf calculated from $M_M-M_G = M_F+M_P$

5-3 with the data of the 4 subjects. This equation was then

used to estimate M_P across ankle angles of 70° to 90° for all subjects.

Tendon elongation attributable to M_P was estimated by having the subject perform two 7s isometric maximal voluntary contractions (MVC) immediately following the passive trials. While we recognize only a weak voluntary contraction above M_P is required to correct for M_P , the MVC was performed as part of other tendon mechanical testing (eg. Fletcher *et al*, 2013, Fletcher *et al*, 2010). The MVCs were performed with the knee fully extended and the ankle angle at 90°. The MVC eliciting the greatest isometric moment was used for further analysis. Throughout the MVC, a clear fascicle-aponeurosis cross point was tracked on the ultrasound image throughout the MVC. Moment and d_L data were fitted to a quadratic regression equation, after correcting for ankle joint rotation (Fletcher *et al*, 2010):

$$Moment = Ad_{L}^{2} + Bd_{L}$$
 5-4

where *A* and *B* are constants. Since any M_P present during the TE method would contribute to tendon elongation, the d_L attributable to M_P was estimated from Moment = $Ad_L^2 + Bd_L$

5-4 and was added to the measured $d_{\rm L}$ during the

passive trial. This was considered the M_P-corrected d_L for calculation of the corrected MA.

To compare corrected and uncorrected MA lengths at a given ankle angle, MA lengths were calculated at 5° intervals, from 70-115° by fitting each subject's corrected and uncorrected d_L -ankle joint angle relationship, using a third-order polynomial equation, an example of which is shown in Figure 5.1.

Test-retest reliability as well as the coefficient of variation for both corrected and uncorrected MA lengths were assessed by having all subjects repeat the study protocol and all analyses were repeated, using both approaches (corrected and uncorrected). To assess the validity of the tendon travel method, 8 (6 males, 2 females) of the 12 subjects later returned to the laboratory. A retest of the Achilles tendon moment arm length using the tendon travel method was done as described above. Achilles tendon moment arm length was also determined with the ankle joint at 90° by measuring the mean of the lateral and medial horizontal distances from the most prominent tip of the malleolus to the posterior aspect of the Achilles tendon using measurement calipers, to the nearest 0.25 mm. A similar method has been well described by Scholz et al. (2008).

5.3.2 Statistics and data analysis

Values are presented as mean \pm standard deviation, unless otherwise indicated. A twoway repeated measures analysis of variance (ANOVA) was used to assess differences between corrected and uncorrected MA lengths (condition) across ankle angles. A one-way repeatedmeasures ANOVA was used to assess differences in M_P and d_L as a function of ankle angle. Where a significant effect was found, Tukey's post hoc multiple comparisons test was used to assess differences in MA length across ankle angles.

Test-retest reliability for corrected and uncorrected MA lengths were assessed via intraclass correlation coefficient (ICC) and coefficient of variation (CV) according to Hopkins (2000). Specifically, CVs for test-retest data were obtained for both conditions at each measured angle for each subject and averaged across all subjects in order to assess CVs across angles and conditions. All statistical analyses were performed using GraphPad Prism version 6.01 for Windows (GraphPad Software, La Jolla, CA, USA, www.graphpad.com). Statistical significance was considered P<0.05.
5.4 **Results**

The vertical M_P , calculated from the linear regression equation of the vertical and horizontal protocols is shown in Figure 5.3. Vertical M_P was not significantly different than horizontal M_P up to 12 Nm (the greatest M_P measured in our subjects). The typical error of vertical ($M_P + M_F$) compared to horizontal M_P , measured across 80 estimates of M_P was 0.58 Nm (95% C.I. = 0.49 to 0.70 Nm), suggesting a negligible effect of M_F on M_P .

The mean passive moment data for all subjects are shown in Figure 5.4. One-way ANOVA revealed a significant difference in M_P across measured ankle angles (p<0.001), indicating that passive moment increased significantly at angles less than 90° (r²=0.989, p<0.0001). The tendon elongation attributable to passive moment is also shown in Figure 5.4. As elongation was calculated from passive moment, elongation only increased at angles less than 90°.

Figure 5.5 shows the apparent and M_P-corrected MA length measurements as a function of ankle joint angle. A two-way repeated measures ANOVA revealed a significant condition x angle interaction (p<0.0001). The corrected MA lengths were 41%, 38%, 25%, 8% and <1% higher at each of the measured angles from 70° to 90°, respectively.

The measurement of MA length at 90° using the TE method (35.2 ± 4.8 mm) was not significantly different from the MA length measured using calipers (35.0 ± 4.6 mm, p=0.344). Comparing CVs across angles and conditions revealed no significant angle x condition interaction and no angle main effects; however, there was a significant main effect of condition (p<0.03); the CVs were lower in the corrected condition ($8.4\pm3.3\%$) compared to the uncorrected condition ($11.0\pm3.8\%$).

5.5 Discussion

In estimating the AT MA length non-invasively, the TE method may be preferred as it is less costly and less time-consuming compared to the COR method that uses magnetic resonance imaging. However, the main assumption to the TE method in estimating AT MA length is that any moment acting to stretch the tendon during passive rotation remains constant. At ankle angles greater than 90°, the TE assumption appears valid since no appreciable M_P is present during plantarflexion. However, at ankle angles less than 90°, it is obvious that passive forces change during the passive rotation (Fath et al, 2010, Kubo et al, 2001). By measuring the moment-d_L relationship during an MVC, this study is the first to account for tendon stretch during the passive rotation and therefore we show here how passive forces affect the estimation of AT MA length using the TE method. It is also important to recognize that an MVC is not required in order to account for tendon translation during this corrected TE method. However, an MVC is routinely performed as part of additional measurements of AT mechanical properties or muscle energetics, so our novel method of determining AT MA length can be performed from data which are already routinely collected. In scenarios where knowledge of the AT MA length is required in order to assess muscle mechanics or energetics, we feel that by developing a method which does not require additional measurements, equipment or data collection is valuable.

It has been previously demonstrated using both the TE and COR methods that MA length changes as a function of ankle angle (Fukunaga *et al*, 1996, Maganaris *et al*, 2000, Maganaris *et al*, 1998, Fath *et al*, 2013, Fath *et al*, 2010). Specifically, MA length is thought to increase as the ankle is rotated from dorsiflexion to plantarflexion, as a result of the anatomical configuration of

the calcaneus. During plantarflexion, there is significant postero-anterior and distal translation of the calcaneus (Leardini et al, 1999), which has been used to explain the apparent increase in moment arm length. However, the calcaneus also rotates as it translates to the posterior, so the length of the MA may not change. Thus, the possibility exists, at least from an anatomical perspective, that AT MA lengths remain relatively constant throughout the ankle joint range of motion. Here we show that when accounting for the effects of M_P on d_L, AT MA remains constant over the joint range of motion (see Figure 5.5). The relationship between MA length and ankle angle we show here is also similar to the MA lengths determined in three dimensions by Hashizume et al. (2012), who demonstrate that when AT MA lengths are determined in three dimensions, the MA length remains constant across ankle angles. These authors demonstrate that determining AT MA length using the COR method from 2D MR-images results in an overestimation of the AT MA length, a result of the difference in the antero-posterior position of the lateral and medial malleoli. 2D estimates of AT MA length using the COR method has also been shown to be greater than AT MA length estimates using the TE method (Fath et al, 2010, Maganaris, 2004), thus making the TE method more in line with estimates using 3D MRimaging.

We estimated the 'additional' d_L attributable to M_P from the Moment- d_L relationship measured during each subject's MVC (Moment = $A d_L^2 + B d_L$

5-4). The relevant passive moments (shown in Figure 5.4) develop within the toe region of the moment- d_L curve, where a small change in M_P results in a large change in d_L . Thus, a small error in M_P would result in a larger error in d_L , and therefore corrected MA length estimate. From our data, an over-estimation in M_P of 1 Nm would result in an additional

 d_L of 0.6 mm. At angles near maximal dorsiflexion, where uncorrected d_L is relatively small, this would contribute substantially to the estimated corrected MA length.

The effect of underestimating AT MA length at large angles of dorsiflexion could have important implications for muscle force estimates from joint moments, particularly during the gait cycle where forces are developed in the range of ankle angles where M_P is present and AT MA length is substantially underestimated. For example, Cavanagh et al. (1977) have demonstrated the ankle joint angle during the stance phase of running is in a dorsiflexed position. Since large forces are developed during stance (Giddings *et al*, 2000), underestimating the AT MA length will substantially overestimate the required muscle forces during this portion of the running stride. This effect would have a profound impact on the estimates of muscle energetics during running.

We have to consider that our AT MA lengths are smaller than those previously reported using the TE method. This may be the result of our chosen subject pool of endurance runners. These runners are short, compared to subjects in previous studies (Fath *et al*, 2010, Sheehan, 2012), which may account partially for the smaller AT MA lengths measured here. A short AT MA may also confer an advantage to distance runners in particular, since a short AT moment arm is associated with a lower energy cost of running (Scholz *et al*, 2008). Shorter moment arms provide an advantage to runners by requiring smaller muscle shortening velocity to achieve a given joint angular velocity (Nagano and Komura, 2003). This effect may be substantial in theory, given the relatively large angular velocities at the ankle joint during submaximal running (KyroLAINEN *et al*, 2001).

 M_P is significantly higher during AT loading compared to unloading (Fath *et al*, 2010). Although this effect would be small (1-3 Nm at ankle angles where M_P is present), we wanted to

avoid the between-subject variability in tendon hysteresis and therefore, only measured M_P during AT loading. Because of the viscoelastic nature of the tendon, as the AT is unloaded, the displacement of the tendon-aponeurosis junction is reduced for a given joint rotation. These effects would lead to an under-estimation of MA length and would be dependent on joint position; hysteresis effects being greatest late in the unloading phase. Thus, in measuring tendon displacement during loading, we have avoided this source of error. From a practical standpoint, to avoid this source of error, it may be most appropriate to estimate MA length using the TE method in the same direction at which the tendon mechanical behaviour is to be evaluated. For example, in estimating AT MA length for the purposes of evaluating AT stiffness, tendon translation should be measured while increasing AT load. Further, since we show MA length does not change significantly as a function of ankle angle, estimates of M_P can be ignored if MA length is measured where no passive moments are present (ie. at and beyond the 90° ankle angle).

It has been previously reported that the CV of MA length using the TE method is 4.5-9.7% (Fath *et al*, 2010). The range of CV is primarily dependent on the differentiation technique: differentiating over a large d_{θ} produces smaller CV than differentiation over a small d_{θ} . Furthermore, larger CVs are reported at large dorsiflexion angles. These authors found the most reliable MA length measurements were when the d_{L} - d_{θ} data were differentiated over 20-30° increments. The current study's MA length CVs were $8.4 \pm 3.3\%$ for the M_P-corrected condition and $11.0 \pm 3.8\%$ in the apparent condition. These CVs are larger than those previously reported, since in the current study MA length was differentiated over a relatively small d_{θ} of between 7-10°. When differentiating d_L - d_{θ} , the estimated MA length is the average MA length over the differentiated d_{θ} . Thus finding the MA length associated with a specific joint angle is not

possible when differentiating over a large d_{θ} . It is only by sacrificing reliability and differentiating over a small d_{θ} is it possible to assess average MA lengths at a specific ankle joint angle using the TE method. In fitting our d_L/d_{θ} data to a 3rd order polynomial equation, we are able to reliably assess MA length at a specific joint angle. This would be particularly important when estimating the in vivo force-length relationship from moment data, where knowing the MA length at a specific joint angle is warranted. However, since in this study we show MA length does not change appreciably as a function of ankle angle, measurement of AT MA length need only be done once, at an angle where M_P is not present. Also, since MA length does not change appreciably, dL-d₀ can be differentiated over relatively large d₀, thus minimizing the error of AT MA measurements.

If the correction for M_P during the TE method was accurate, our estimated MA lengths should represent the horizontal MA length. Given no difference in estimated and directlymeasured MA lengths at 90° in a subset of our subjects, and the reported values over a wide range of subjects (Fath *et al*, 2013, Fath *et al*, 2010, Maganaris, 2004, Fukunaga *et al*, 1996, Spoor *et al*, 1990), we believe the uncorrected MA lengths were underestimated at dorsiflexion angles and by accounting for M_P, resulted in a reliable estimate of AT MA lengths., Therefore, we believe the M_P-corrected TE method presented here represents a quick, reliable and accurate estimation of AT moment arm lengths across the ankle joint range of motion.

5.6 Conclusion

In conclusion, the TE method offers a quick, affordable alternative to the COR method when estimating MA length; however, it is only when no passive forces are acting to stretch the tendon is the TE method valid, unless this is quantified and accounted for. Here, we have corrected for these passive forces non-invasively, through-out the range of motion. Since we show that MA length does not change as a function of ankle angle, it seems sufficient to measure MA length using the TE method at an ankle angle where M_P is negligible, or measuring the MA length manually. Both methods avoid the considerable time and financial costs associated with magnetic resonance imaging.

5.7 Author Contributions

All experiments were performed at the Human Performance Lab at the University of Calgary, Calgary, Alberta, Canada. JRF and BRM were responsible for conception and design of the experimental protocol. JRF collected and analyzed the data. JRF and BRM were primarily responsible for interpreting the experimental data. JRF drafted the manuscript and JRF and BRM revised it critically for important intellectual content. All authors approved the final draft of the article.

5.8 Conflict of Interest statement

None of the authors report any conflicting interests.

5.9 Acknowledgements

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5.10 Figures



Figure 5.1 Calculation of the uncorrected and corrected Achilles tendon MA length from joint angle and tendon displacement.

Data are from a representative subject. Uncorrected and corrected displacement during tendon loading is shown as closed and open circles, respectively. Uncorrected and corrected MA lengths were calculated as the first derivative of d_L/d_{θ} at all measured intervals, the limits of which are shown as open and closed circles, respectively. Additionally, a third order polynomial was fitted to these d_L/d_{θ} data, and differentiated at 5° intervals from 70°-115°. Solid and dashed lines show the third-order polynomial fit to uncorrected and corrected MA lengths, respectively. Arrows show the angle at which MA lengths were differentiated.



Figure 5.2. Calculation of passive moment influencing the estimate of Achilles tendon MA length.

Data are from the same subject as shown in Figure 5.1. Vertical line shows anatomical-neutral ankle angle (90°). Negative moment shown on the y-axis represents plantarflexion moment (dorsiflexed position). Measured moment (M_M) is shown in red, along with the 3rd-order polynomial fit of M_M (solid line). M_F+M_P (long dashed line) was developed by subtracting M_G from M_M . The short dashed line represents M_P .



Figure 5.3. Estimates of T_P from practical and criterion experimental approaches.

 M_F was significantly lower at 70° and 75° using the criterion approach compared to the practical approach. This resulted in a higher estimated M_P using the criterion vs practical approach.



Figure 5.4. Passive Moment (A) and corrected elongation (B) for all subjects as a function of ankle joint angle.

Values are mean \pm sd. Ankle angles less than 90° indicate dorsiflexion angles. Passive moment shown on the y-axis indicates passive moment in the plantarflexion direction.



Figure 5.5. Corrected (solid line) vs uncorrected (dashed line) Achilles tendon MA

estimates as a function of ankle angle.

Values are mean \pm sd. Two-way repeated measures ANOVA revealed a significant condition x angle effect (p<0.01). *Significantly different UN vs COR.

Chapter Six: Achilles tendon strain energy in distance running: consider the muscle energy cost

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6.1 Abstract

The return of tendon strain energy is thought to contribute to reducing the energy cost of running (E_{run}). However, this may not be consistent with the notion that increased Achilles tendon (AT) stiffness is associated with a lower E_{run} . Therefore, the purpose of this study was to quantify the potential for AT strain energy return relative to E_{run} for male and female runners of different abilities. A total of 46 long distance runners (18 elite male (EM), 12 trained male (TM) and 16 trained female, (TF) participated in this study. E_{run} was determined by indirect calorimetry at 75, 85 and 95% of the speed at lactate threshold (sLT) and energy cost per stride at each speed was estimated from previously reported stride length (SL)-speed relationships. AT force during running was estimated from reported vertical ground reaction force (F_z)-speed relationships, assuming an AT:F_z moment arm ratio of 1.5. AT elongation was quantified during a maximal voluntary isometric contraction using ultrasound. Muscle energy cost was conservatively estimated on the basis of AT force and estimated crossbridge mechanics and energetics. Significant group differences existed in sLT (EM>TM>TF, p<0.001). A significant group x speed interaction was found in the energy storage/release per stride (TM>TF>EM, p < 0.001), the latter ranging from 10-70 J•stride⁻¹. At all speeds and in all groups, estimated muscle energy cost exceeded energy return (p < 0.001). These results show that during distance running, the muscle energy cost is substantially higher than the strain energy release from the AT.

6.2 Introduction

It has been generally accepted that a primary role of the muscle-tendon unit in the lower limbs during running is the storage and release of tendon strain energy (Alexander, 1991, Alexander, 1984). This storage and release of tendon strain energy is thought to be an important factor in keeping the energy cost of running (E_{run}) at a low value. During running, the Achilles tendon (AT) is stretched, storing strain energy. A portion of this strain energy is returned during the subsequent shortening phase thereby reducing the work required by the muscle (Cavagna *et al*, 1968, Voigt *et al*, 1995). There have been several suggestions that the elastic recoil provided by the AT contributes a significant portion of the energy for propulsion (Arampatzis *et al*, 2006, Hof *et al*, 2002, Lichtwark and Wilson, 2008); however, no study to date has considered the energy cost of muscle contraction for the muscle in series with the tendon releasing energy. This energy cost would be necessary for the tendon strain energy storage to occur.

Estimates of strain energy storage of the AT are typically performed by either directly or indirectly measuring AT elongation as a function of AT force (Shadwick, 1990). The area under the force-elongation curve is considered AT energy storage. Considering estimates of tendon hysteresis, or measuring the tendon translation as a function of force during force decline provides a measure of AT energy release. AT energy storage can be estimated in vivo by combining simultaneous measurements of AT elongation and torque using ultrasonography and dynamometry, respectively.

The amount of AT strain energy storage/release varies as a function of AT stiffness. For a given AT force, energy storage would be proportional to elongation; a stiffer tendon will store less energy. Considering that it has been demonstrated that AT stiffness is higher in trained compared to untrained runners (Hobara *et al*, 2008, Albracht and Arampatzis, 2006) and

changes in AT stiffness are associated with changes in E_{run} (Fletcher *et al*, 2010, Arampatzis *et al*, 2006), more highly trained runners should have a lower capacity for AT energy storage/release and yet this deficiency is associated with a lower E_{run} . These recent results seem difficult to rationalize with the notion that the AT serves to store and release energy. Thus, it appears as though the energy-saving contribution of the stiffer Achilles tendon cannot solely be a result of stored strain energy return.

Ker et al. (1987) have estimated that the strain energy stored in the Achilles tendon during running at 4.5 m \cdot s⁻¹ was 35 J•step⁻¹ and this was considered to contribute a substantial proportion of energy to the total E_{run}. However the estimated AT forces required (4700 N) to store this amount of energy is near published estimates of maximum isometric force of 5000-6000 N (Fletcher et al, 2010, Albracht and Arampatzis, 2013). A similar amount of energy storage has been reported in human hopping (38 J), contributing approximately 16% of the total mechanical work of the hop (Lichtwark and Wilson, 2005). Using a buckle-type transducer to measure AT force directly, Fukashiro et al. (1995) found a smaller amount of tendon strain energy was stored (6-7 J•jump⁻¹) in squat and countermovement jumps, and this represented 17-23% of total calf muscle work; a result of a lower measured AT force (<2300 N) during the jumps. It would be expected that AT energy storage during running would be smaller than that found in hopping, as a result of lower vertical ground reaction forces (Cavanagh and Lafortune, 1980) and therefore lower AT force. AT force can be estimated during running from ground reaction forces, and the ratio of the resultant ground reaction forces and AT moment arms (Ker et al, 1987).

Considering E_{run} of trained and elite male and female distance runners measured in our lab (Fletcher *et al*, 2010, Fletcher *et al*, 2013) and others (Shaw *et al*, 2013) is within the range

of 4.2-4.6 J·kg⁻¹·m⁻¹, the energy storage/release of the AT would appear to be a small proportion of the total E_{run} . To date however, no study has evaluated estimates of the AT energy storage and release during running from measurements of AT elongation and moment arm using ultrasound. Furthermore, no study has considered that in order to store energy in a tendon, the muscle in series with that tendon must contract, using additional energy. This muscle energy cost would be a portion of the E_{run} , effectively reducing the value of storing energy in the tendon and may be the reason that improved economy is associated with a stiffer Achilles tendon.

A recently proposed alternative view of the role of tendons is in reducing the energy cost of muscle contraction by minimizing the amount and/or velocity of muscle shortening (Fletcher et al, 2013). The tendon, if optimally stiff, may allow the muscle fascicles to remain nearly isometric during running, while muscle-tendon unit lengthening and shortening can be accommodated primarily by the tendon (Lichtwark et al, 2007, Ishikawa et al, 2007). In keeping the muscle fascicle shortening velocity low, the muscle can operate near its optimal sarcomere length (Askew and Marsh, 1998), and the required level of muscle activation is minimized (Fletcher et al, 2013). Considering that during running the triceps surae muscle fibers do not undergo substantial stretch prior to shortening (Lichtwark et al, 2007, Ishikawa et al, 2007), an optimally-tuned AT would result in less fiber shortening to achieve active joint rotation. As a result, the joint range of motion can be accommodated primarily by the tendon, keeping the energy cost of the contraction low (Roberts et al, 1997). However, in order to do so, AT stiffness must be appropriately tuned to minimize the amount of fascicle shortening. With less than optimal stiffness, the amount and/or velocity of muscle fiber shortening is increased and this shortening will increase muscle energy cost (Fletcher et al, 2013), elevating E_{run}

Therefore, the purpose of this study was three-fold:

- 1. To estimate the proportion of strain energy storage/release of the AT relative to total E_{run}.
- 2. To estimate the energy cost of muscle contraction required by the muscle in series with the AT.
- To determine whether the contribution of strain energy storage/release differed between runners of different abilities and/or of different sexes, because it is known that the mechanical properties of the AT would differ.

6.3 Methods

A total of 46 distance runners participated in this study. Some of these runners had previously participated in studies performed in our lab (Fletcher *et al*, 2013, n=18, Fletcher *et al*, 2010, n= 8). Those data were combined with new data to address the specific research questions posed here. The runners gave their informed written consent to participate in the experimental procedures, which were approved by the University of Calgary Conjoint Health Research Ethics Board.

Runners were divided into either "elite" or 'trained' based on the highest level of competition achieved. Subjects were considered to be elite male runners (n=18) if they had competed at a National Championship (1500m or longer, including marathon and cross country race distances) within 6 months of the study and running a minimum of 6 times per week, with a minimum average training volume of 70 km·wk⁻¹ of running for at least six weeks prior to participating in the study. Trained runners (n=28, 16 males and 12 females) were those runners not meeting the inclusion criteria for the elite group, but participating in run training a minimum of five times per week, with a minimum average training volume of 40 km·wk⁻¹, during the six

weeks prior to the beginning of the study. All runners were following a similar periodized training plan for either the 10 km or half-marathon race distance and were free of any neuromuscular or musculoskeletal injuries at the time of the study. Subject characteristics are shown in Table 6-1.

6.3.1 Experimental Protocol

The subjects visited the lab on two separate occasions. On the first visit, an incremental exercise test to exhaustion was performed on a treadmill (Woodway Pro, Woodway USA, Waukeshka, WA) to determine the subject's maximal oxygen uptake ($\dot{V}O_2$ max) and speed associated with the lactate threshold (sLT). $\dot{V}O_2$ max and sLT were determined based on methods used previously in our lab (Fletcher *et al*, 2010, Fletcher *et al*, 2009). sLT was defined as the speed at the stage preceding that which elicited a [BLa⁻] increase greater than 1 mM. All tests were terminated due to volitional exhaustion. $\dot{V}O_2$ max was defined as the highest 30 s average $\dot{V}O_2$ during the test. All subjects attained $\dot{V}O_2$ max based on primary or secondary criteria previously reported from our lab (Fletcher *et al*, 2013).

Between 48-72 hours following the $\dot{V}O_2$ max testing session, the subjects returned to the lab for determination of AT stiffness and E_{run}. AT stiffness of the right AT was determined on a dynamometer (Biodex System 3, Shirley NY USA) as described previously (Fletcher *et al*, 2010, Fletcher *et al*, 2013). The subjects performed three isometric ramp maximal voluntary contractions (MVC) of the right plantarflexors. Moment elicited during the MVC was sampled at 100 Hz. The trial eliciting the highest moment was used for analysis. During each MVC, a 12.5 MHz linear array ultrasound probe (50mm, Philips Envisor, Philips Healthcare, Eindhoven, Netherlands) was used to visualize the medial gastrocnemius muscle (MG) fascicles at 49 Hz,

close to the AT. The ultrasound probe was placed on the MG muscle belly, near the myotendinous junction, and secured using a custom-built apparatus. AT tendon elongation was estimated by the displacement of an insertion of a fascicle into the deep aponeurosis, measured using *ImageJ*, (NIH, Baltimore, MD, USA) during the MVC. Measured moments and AT elongations were corrected for joint rotation (Fletcher *et al*, 2010, Fletcher *et al*, 2013) and AT moment arm length at the 90° ankle angle was estimated using the tendon travel method (Ito *et al*, 2000). AT force was calculated by dividing the ankle joint moment by the estimated AT moment arm. AT Force (F) - elongation (d_L) data were fitted to a quadratic regression equation using:

$$\mathbf{F} = A\mathbf{d}_{\mathrm{L}}^2 + B\mathbf{d}_{\mathrm{L}}$$

where *A* and *B* are constants. AT stiffness was defined as the slope of the fitted $F-d_L$ equation from 50-100% of maximum isometric plantarflexion force.

AT force during running was estimated from the assumed vertical ground reaction forces (F_z) during running, estimated as a function of running speed and body mass (Keller *et al*, 1996), assuming the F_z moment arm at peak F_z was 1.5x greater than the AT moment arm (Giddings *et al*, 2000). The F_z represents the major component (>90%) of the resultant ground reaction forces during steady-state running (Kram *et al*, 1998); however, we acknowledge that by not considering the horizontal forces, we have underestimated the required AT force during running. AT energy storage during running was estimated from the area under the measured AT force-d_L curve Figure 6.1. AT energy release was estimated assuming an AT hysteresis of 10% (Finni *et al*, 2012).

Immediately following the measurement of AT force and elongation, E_{run} was measured. After a 10 minute warm up at 8 km•hr⁻¹ for the females and 9.6 km•hr⁻¹ for the males, the subjects ran at 75, 85 and 95% sLT for 5 minutes each, with a 5 minute standing rest period between speeds. The steady-state $\dot{V}O_2$, defined as the average $\dot{V}O_2$ over the final 2 minutes of each stage, was used to calculate E_{run} . $E_{run}(J \cdot stride^{-1})$ was calculated similar to Fletcher et al. (2010):

$$E_{run} = \dot{V}O_2 \times (5.1583 \times RER + 15.972) \times S^{-1} \times SL$$
 6-2

where $\dot{V}O_2$ is measured in mL·min⁻¹, speed (S) is measured in m·min⁻¹, and stride length (SL) is measured in m. SL, defined as the distance between successive contacts of the same foot, was estimated for each subject at each speed based on the previously reported SL-speed relationship for both males and females (Cavanagh and Kram, 1989). Consequently, the estimate of AT energy storage and release is double that measured for one tendon because it includes two footstrikes per stride.

6.3.2 Muscle energy cost

In order for the AT to store and release strain energy during ground contact, active muscle contraction must occur because the triceps surae muscles are in series with the Achilles tendon. The energy cost of this muscle contraction was calculated by first estimating the number of active in-parallel crossbridges (per half-sarcomere) required to produce the AT force and this was multiplied by the number of cross-bridge cycles expected for the estimated shortening during each stance phase and the number of half-sarcomeres in series.

To estimate the number of crossbridges required in parallel, AT force was divided by the estimated force per crossbridge of 3 pN, (Rome *et al*, 1999, Finer *et al*, 1994). The number of half-sarcomeres in series was estimated as the ratio of medial gastrocnemius fascicle length during ground contact to half-sarcomere length. The MG fascicle length during ground contact

was calculated for each subject based on the fascicle length-force relationship for each subject measured during the MVC, as described previously by Fletcher et al (2013). Sarcomere lengths at rest and during running were calculated based on force-fascicle length values from Maganaris (2003) and assuming an optimal sarcomere length during maximal activation of 2.6 µm at the short side of the plateau region of the force-sarcomere length relationship (Herzog and ter Keurs, 1988). The number of crossbridge cycles was estimated from the amount of shortening within each half-sarcomere. This was estimated from the magnitude of MG fascicle shortening from rest to stance and accounting for shortening due to anticipated joint rotation. To estimate the number of cross-bridge cycles required to accommodate this magnitude of shortening, we assumed the filaments move 12 nm with each crossbridge cycle (Barclay *et al*, 2010). The amount of joint rotation during stance was considered 10° (Lichtwark et al, 2007). The estimated fascicle shortening due to joint rotation was calculated for each subject as the product of AT moment arm length (mm) and joint rotation (rad), based on the tendon travel method, as described by Fletcher et al. (2010). The shortening due to joint rotation was added to the shortening due to force-dependent stretch of the tendon to calculate total shortening during the stance phase of running. We further assumed an ATP cost to crossbridge cycle ratio of 1:1 and an energy release of 48 kJ·mol⁻¹ ATP (Homsher and Kean, 1978).

6.3.3 Statistics

Values are presented as mean \pm standard deviation (sd), unless otherwise indicated. A two-way ANOVA (group x speed) with repeated measures (speed) was used to test for differences in E_{run}, energy storage/return, sLT, and muscle energy cost. When there was no significant interaction and a significant main effect was found, Tukey's post hoc test was used to detect significant differences between the three speeds. Pearson product-moment correlation analysis was used to examine the relationship between AT stiffness and strain energy contribution to E_{run} . All analyses were performed using GraphPad Prism version 6.01 for Windows (GraphPad Software, La Jolla, CA, USA, www.graphpad.com). The *a priori* level of statistical significance was set at alpha <0.05.

6.4 **Results**

As shown in Table 6-1, sLT was significantly different between groups (p<0.001). Consequently, since we estimated SL from running speed, a significant group x speed interaction existed in SL (p<0.001). Mean SL ranged between 2.34 ± 0.23 m at 75% sLT and 2.86 ± 0.30 m at 95% sLT in EM. SL in TM and TF across speeds was 2.11 ± 0.10 m to 2.57 ± 0.31 m and 1.93 ± 0.16 m to 2.34 ± 0.21 m, respectively. E_{run} for all groups at all measured speeds is shown in Figure 6.2. There was no significant group x speed interaction; however, there was a significant main effect of speed on E_{run} (p<0.001); E_{run} increased with relative speed.

Maximum isometric force was 5180 ± 1998 N in EM, 3528 ± 1196 N in TM and 2151 ± 759 N in TF. One-way ANOVA revealed a significant effect of group for maximum isometric force (p<0.001). Furthermore, AT stiffness was significantly different between groups (p<0.001); AT stiffness was 408 ± 128 N•mm⁻¹ in EM, 188 ± 62 N•mm⁻¹ in TM and 135 ± 57 N•mm⁻¹ in TF.

Figure 6.3 shows the mean (\pm sd) F-d_L curves for all groups across the force range expected for running, Mean F_z, estimated from running speed and body mass increased as a function of running speed and was significantly lower at all speeds in TF compared to TM and EM (Table 6-2. F_z (N) as a function of % sLT. Despite running speeds being higher in EM compared to TM, significant differences in body mass between EM and TM resulted in no differences in estimated F_z during running.

Since AT force was estimated from previously reported F_z -speed relationships and known body mass, AT force increased as a function of running speed. AT force across all measured speeds was lower in TF (range: 1520 to 1877 N) compared to either EM (2200 to 2734 N) or TM (2200 to 2724 N). The highest estimated AT force (ie. at 95% sLT) was 59±20% of maximum calculated isometric force in EM. This was lower than either TM (84±28%) or TF (97±34%). In five of the 16 subjects (31%) in TF, estimated AT force was greater than the maximum calculated isometric force. Estimated AT force was greater than maximum isometric force in 3 of 12 subjects in TM (25%). In none of the subjects in EM was estimated AT force greater than maximum isometric force.

Resting fascicle length was 55.1±10.9 mm in EM, 53.9±4.8 mm in TM and 57.2±7.9 mm in TF. Assuming a common sarcomere length of 2.60 μ m at the short side of the plateau during maximal activation, these fascicle lengths corresponded to an average resting sarcomere length of 3.35±0.90 μ m in EM, 4.11±1.07 μ m in TM and 3.94±1.01 μ m in TF. Estimated sarcomere lengths at rest were significantly shorter in EM (p<0.03) compared to either TM or TF.

Fascicle length at MVC was 37.4 ± 10.1 mm in EM. This was significantly longer (p<0.05) than the measured fascicle lengths at MVC of either TM (32.4 ± 10.7 mm) or TF (31.2 ± 7.7 mm), respectively. Estimated fascicle shortening during running increased as a function of speed, but was significantly less (p<0.001) in EM (14.0 ± 2.8 to 15.9 ± 3.3 mm across speeds) compared to either TM (23.3 ± 6.6 to 27.3 ± 8.0 mm) or TF (23.9 ± 6.1 to 28.0 ± 7.5 mm).

Estimated sarcomere lengths during running were not different between groups at any of the measured speeds (p=0.48), but decreased as a function of relative speed (p<0.001); a result of

higher estimated AT forces, and thus fascicle shortening as a function of relative running speed. Average running sarcomere lengths across groups was $2.74\pm0.53 \mu m$, $2.62\pm0.52 \mu m$ and $2.50\pm0.52 \mu m$ at 75, 85 and 95% sLT, respectively.

Combining the results for E_{run} and SL, the estimated energy cost per stride revealed a significant group x speed interaction (p<0.001). These results are shown in Figure 6.4 and suggest that across all speeds, the energy cost per stride was lowest in TF and highest in EM.

Given the estimated AT forces during running, we estimated the AT energy release $(J \cdot stride^{-1})$ from the area under the measured F-d_L curve. The amount of energy released is shown in Figure 6.5. AT energy release increased as a function of relative running speed (p<0.001) and was significantly higher in TM compared to either EM or TF (p<0.02). AT energy release was not related to sLT (Figure 6.6).

A portion of the energy cost per stride consists of the energy cost of triceps surae muscle contraction, which is necessary to allow energy storage in the AT to occur. Estimating this energy cost from AT forces and sarcomere shortening during the stance phase of running revealed a significant group x speed interaction (<0.001). Values for muscle energy cost are presented in Figure 6.5.

6.5 Discussion

Based on our above-mentioned aims and assumptions, we show here that:

 The amount of strain energy release from the AT was 10-70 J•stride⁻¹ across male and female runners of different performance capabilities.

- The range of energy cost of muscle contraction in order for this AT energy storage/release to occur across all subjects and running speeds is estimated to be 90 to 525 J•stride⁻¹.
- AT energy release was significantly higher in TM compared to either EM or TF; however the estimated muscle energy cost required for this energy release to occur was lowest in EM.

6.5.1 E_{run} between groups

We were previously unable to detect a difference in E_{run} between similarly-trained male and female runners (Fletcher *et al*, 2013) and here we also were unable to detect a significant difference between trained and elite male and female runners at similar relative intensities of running. These results are consistent with the notion that when expressed at the same relative intensity of running (ie. %sLT), male and female runners show similar values of E_{run} . Our results also further confirm that E_{run} increases as a function of relative speed (Fletcher *et al*, 2009, Shaw *et al*, 2013).

6.5.2 Tendon strain energy release

The amount of tendon strain energy storage in each stride was calculated from the measured tendon stiffness and the estimated AT force during running, which in turn was based on estimates of F_z , at the speed at which each subject ran. In order to estimate AT force from F_z , we assumed a fixed moment arm length for F_z as a function of AT moment arm length. It is well-established, however that this length is not fixed during ground contact and is different between rear and mid/forefoot strikers (Cavanagh and Lafortune, 1980). However, without having directly-measured the magnitude and location of ground reaction

forces of our subjects during running, we have used previously-reported values for the average F_z moment arm length during ground contact. We specifically chose a fixed F_z :AT moment arm ratio of 1.5 since in most cases, a ratio larger than this resulted in AT force greater than the maximum isometric force for TM and TF. This ratio is also consistent with previous literature (Giddings *et al*, 2000). Since some fascicle shortening would have occurred during ground contact, it seems unlikely that AT force during running would be near the measured maximum isometric force and certainly would not exceed it. The force-velocity relationship would preclude this possibility. As a result, we have very likely over-estimated the AT force during running, particularly in TF, where 5 of 16 subjects' AT force was greater than maximum isometric force. Over-estimating AT force would result in both an over-estimate of the AT energy release per stride as well as the muscle energy cost for this AT release to occur.

It has previously been shown that the Achilles tendon releases 1.3 J•step⁻¹ during walking (Maganaris and Paul, 2002), and up to 38 J per jump during continuous, one-legged hopping (Lichtwark and Wilson, 2005). In the latter case, estimated AT forces during hopping approached 5000 N, which explains the larger AT energy storage compared to the estimates made here, where AT forces at the highest measured speeds were <2800 N. In spite of these high forces during hopping, tendon strain energy return represented a small portion of the total mechanical work (<16%), and energy would have been required for muscle contraction in order to achieve this energy return.

Although many studies have presented estimates of energy storage and release from the Achilles tendon, none have considered the need for simultaneous muscle contraction to allow the tendon stretch and energy release to occur. Here, we provide an estimate for that muscle energy

cost. This muscle energy cost represents a portion of the total metabolic cost of running and is necessary for tendon strain energy storage and release to occur. The energy cost of this muscle contraction is directly proportional to muscle shortening, so a compliant tendon increases this energy cost.

6.5.3 Estimates of muscle energy cost to allow for tendon strain energy storage

In accounting for the energy cost of muscle contraction to allow for AT energy storage/release, these results demonstrate that the return of elastic strain energy is less than the muscle energy cost required for storage/release to occur. This brings into question the relevance of tendon strain energy return alone contributing significantly to reducing the metabolic cost of running.

As an extreme, assuming (unrealistically) that maximum isometric force is generated during each stride and a conservative estimate of AT hysteresis of 10% (Finni *et al*, 2012), the average maximum possible amount of energy released from the AT is 34 J·stride⁻¹ in TF to 71 J·stride⁻¹ in EM; clearly a small portion of the total metabolic cost (500-900 J·stride⁻¹). Any shortening during F_z would contribute to elevate muscle energy cost above our estimate, as a result of the muscle's force-length-velocity relationships and subsequent increases in the required level of muscle activation (Fletcher *et al*, 2013) and due to shortening-induced increase in cross-bridge turnover (Barclay *et al*, 2010). Presumably, this shortening-induced energy cost would be higher in the lesser-trained subjects (Sano *et al*, 2013), given their relatively compliant tendons, and greater relative force required to generate F_z . Here we have calculated this shortening-induced energy cost to be on average more than 8-fold higher than the energy

released from the stretched AT. Therefore, a considerable muscle energy cost can be saved if muscle-tendon unit shortening can be accommodated by the AT alone and MG fascicle shortening is minimized as would be the case with a stiff AT.

It seems unlikely that sarcomere length is longer than we have estimated, particularly for those who activate near maximum. If the true sarcomere length is shorter, then more sarcomeres will be needed in series and this will increase the estimate of the energy required. To further highlight our conservative estimate of the shortening-induced muscle energy cost, our estimated force per crossbridge (3 pN) is the force associated with the isometric crossbridge force. The force per crossbridge would be considerably lower during shortening (Barclay et al. 2010). For example, if the force per cross-bridge was actually 2 pN, 30% more cross-bridges would need to be engaged and the energy cost would be 30% higher. A similarly-conservative estimate of the muscle energy cost is the estimated crossbridge step size (12 nm), which is the stepsize at which efficiency is maximal. Stepsize decreases at slow shortening velocities (Barclay *et al*, 2010), thus any error associated with the reduction in stepsize from 12 nm would result in a proportional increase in the estimated muscle energy cost.

We also recognize some variability in our estimated AT forces during running as a result of estimating these forces from the F_z -speed relationship (Keller *et al*, 1996). The variability associated with estimating F_z from running speed is in the order of 10-20%. Using mean values for our subjects, overestimating F_z by 10% would result in both an over-estimated muscle energy cost and AT strain energy release of 15%. A similar underestimation in F_z would result in a 17% lower muscle energy cost and tendon strain energy release; however, in our subjects, it is likely we have overestimated F_z in some and underestimated F_z in others. Furthermore, by considering

only the F_z moment arm length, rather than the resultant ground reaction force moment arm in the estimated AT force during running, we have further underestimated the required AT force. Given the magnitude of the horizontal ground reaction force represents <10% of the F_z (Kram *et al*, 1998), we feel this error is relatively minor. However, we acknowledge that we may have underestimated the required AT force during running. This results in an underestimate of both the tendon strain energy and the muscle energy cost. Therefore, we acknowledge a very conservative estimate of the muscle energy cost to allow tendon strain energy storage to occur.

It is well established that much of the required length change of the muscle-tendon unit during running can be taken up by stretch of a relatively compliant in-series tendon, thus allowing the active muscle fascicles to shorten to a lesser extent and at a slower velocity (Fukunaga *et al*, 2001, Ishikawa *et al*, 2007). This minimizes the required level of muscle activation to achieve the target force as a result of the muscle's force-velocity relationship. In so doing, the muscle energy cost is reduced considerably (Fletcher *et al*, 2013). Relative to the total metabolic cost per stride, the amount of tendon strain energy that is stored and released per stride is small. Our estimates shown here demonstrate that this energy release is on average less than 42 J per stride. Therefore, we argue that the energy savings in reducing the muscle energy cost more than makes up for decreased energy storage and release when the tendon's mechanical properties are optimal.

If mechanical properties are optimal, the role of a tendon connected in-series with the muscle is to minimize muscle fascicle shortening by taking up much of the length change required by the whole muscle-tendon unit (Roberts, 2002). This effect serves to minimize muscle metabolic cost to a much greater extent than the storage and release of tendon strain

energy; by keeping fibre shortening to a minimum, the muscle's force-length-velocity properties and muscle activation can be optimized (Fletcher *et al*, 2013). The range of sarcomere lengths used during locomotion corresponds to the plateau region of the relevant force-length curves (Ishikawa *et al*, 2007). This maximizes the amount of force for a given level of activation, or conversely as would be the case in steady-state submaximal running, minimizing the required level of activation for a given force. The fact that we saw no difference in estimated sarcomere lengths between groups suggests similar sarcomere lengths are achieved to run at a common relative speed.

6.6 Conclusion

From our estimates of tendon strain energy storage and release and muscle energy cost for this storage/release to occur, we conclude that the amount of tendon strain energy released represents a very small portion of the total metabolic cost to run a given speed. Furthermore, this energy return comes at a considerable muscle energy cost. Therefore, reducing muscle energy cost through reductions in muscle fascicle shortening during running even if this means less energy return from the tendon, contributes to an improved economy of running.

6.7 Author Contributions

All experiments were performed at the Human Performance Lab at the University of Calgary, Calgary, Alberta, Canada. JRF and BRM were responsible for conception and design of the experimental protocol. JRF collected and analyzed the data. JRF and BRM were responsible for interpreting the experimental and theoretical data. JRF drafted the manuscript and JRF and

BRM revised it critically for important intellectual content. All authors approved the final draft of the article.

6.8 Acknowledgements and disclosures

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6.9 Tables

C	N	Age	Height	Mass	VO ₂ max	sLT
Group		(years)	(m)	(kg)	$(ml \cdot kg^{-1} \cdot min^{-1})$	$(\text{m} \cdot \text{min}^{-1})$
TF	16	31.4 ± 8.9	$1.66 \pm 0.08^{\circ}$	$58.1\pm7.0^{\text{a}}$	50.3 ± 6.3	206 ± 22^{a}
TM	12	35.8 ± 8.5	1.76 ± 0.06	$\textbf{76.0} \pm \textbf{9.0}$	54.8 ± 8.5	230 ± 32
EM	19	$24.6\pm5.1^{\text{b}}$	1.78 ± 0.07	67.8 ± 7.2	$66.3 \pm 5.8^{\mathrm{b}}$	260 ± 31
					1 1 51 6 11	

Table 6-1. Subject Characteristics.

Values are mean \pm sd. TF = trained female; TM = Trained male; EM = elite male

^a significantly different (p<0.05) between groups

^b significantly different (p<0.05) EM vs TF and TM

^c significantly different (p<0.05) TF vs EM and TM

	75%	85%	95%
TF	1013 ± 121	1131 ± 136	1251 ± 151
TM	1467 ± 206	1641 ± 232	1816 ± 259
EM	1468 ± 233	1646 ± 262	1823 ± 262

Table 6-2. F_z (N) as a function of % sLT.

Values are mean \pm sd. Two-way ANOVA revealed no group x speed interaction. A significant main effect of speed existed (p<0.001).



Figure 6.1. Estimated Achilles tendon energy storage and release during running.

Short dashed line represents the average EM force-AT elongation curve measured during the isometric MVC. Solid and short-dashed lines represent the $F-d_L$ of loading and unloading during running, respectively. The area under the unloading curve represents the AT energy release. The area enclosed by the loading and unloaded curves represents the estimated AT energy lost as heat (assuming AT hysteresis = 10%).



Figure 6.2. E_{run} at the three measured relative speeds for all groups.

No group x speed interaction existed; however, there was a significant main effect of speed on E_{run} (p<0.001).


Figure 6.3. Average F-d_L curves for all groups during running.

Solid and dashed lines represent the mean and sd of the second-order polynomial (F = AdL2 +

BdL

6-1) for all groups,

respectively.



Figure 6.4. Comparison of the energy cost per stride for all groups and across relative speeds.

A significant group x speed interaction existed in the energy cost per stride (p<0.001).



Figure 6.5. AT energy release (filled bars) relative to estimated muscle energy cost required to allow AT energy storage to occur (hashed bars) for all groups and all measured running speeds.

AT energy release and muscle energy cost increased across speeds in all groups (p<0.001).



Figure 6.6. Relationship between AT energy release and 95% sLT.

Filled, grey and open squares represent EM, TM and TF subjects, respectively. When assessed across all groups the relationship was not significant (dashed line, $r^2 = 0.01$). The relationship was similar when assessed across the other two measured speeds (75% and 85% sLT).

Chapter Seven: Changes in Achilles tendon stiffness and energy cost following a prolonged run in trained distance runners

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7.1 Abstract

The Achilles tendon (AT) has viscoelastic properties; thus during prolonged running the magnitude of AT length change may increase over time. Assuming the same AT force and hysteresis during running, additional lengthening should increase tendon strain energy return. However, AT elongation might also affect the magnitude of triceps surae (TS) muscle shortening. Any additional muscle shortening for the same duration at the same force would increase velocity of shortening and require greater activation and elevate the energy cost contraction. Therefore, we aimed to quantify the tendon strain energy return and muscle energy cost necessary to allow energy storage to occur prior to and following prolonged running. 14 trained male (n=10) and female (n=4) distance runners (24±4 years, 1.72±0.09 m, 61±10 kg, $\dot{V}O_2$ max 64.6±5.8 ml·kg⁻¹·min⁻¹) ran 90 minutes (RUN) at approximately 85% of lactate threshold speed (sLT). Immediately prior to and following RUN, AT stiffness and running energy cost (E_{nun}) at 85% sLT were determined. AT energy return was calculated from dynamometry and ultrasound. TS energy cost was estimated on the basis of AT force and assumed crossbridge mechanics and energetics. Following RUN, AT stiffness was reduced from 332 ± 168 N•mm⁻¹ to 304 ± 151 N•mm⁻¹ (p<0.012). E_{run} increased from 4.56 ± 0.32 J•kg⁻¹•m⁻¹ to 4.62±0.32 J•kg⁻¹•m⁻¹ (p=0.044). Estimated AT energy return was not different following RUN (p=0.061). Estimated TS muscle energy cost increased significantly by 11.8±12.3 J•stride⁻¹, (p=0.0034), accounting for the post-RUN increase in E_{run} (8.6±14.5 J•stride⁻¹). These results demonstrate that a prolonged, submaximal run can significantly reduce AT stiffness and increase E_{run} in trained runners, and it appears that the elevated TS energy cost contributes substantially to the elevated E_{run} .

7.2 Introduction

The energy cost of running (E_{run}) is one of the key determinants of distance running performance (Di Prampero, Atchou, Brückner et al. 1986). E_{run} is primarily determined by the energy cost of generating the force needed to support body weight for the duration of the stride (Taylor, Heglund, McMahon et al. 1980, Kram and Taylor. 1990) and there is growing evidence to suggest that mechanical properties of the tendons of the major force-generating muscles that are active during running greatly influence E_{run} . It has been suggested that the mechanical properties of tendon allow the muscle to operate near-isometrically (Lichtwark, Bougoulias and Wilson. 2007, Ishikawa, Pakaslahti and Komi. 2007), thus minimizing the magnitude and velocity of muscle shortening during contraction (Askew and Marsh. 1998). This minimized shortening magnitude and shortening velocity will minimize the required level of muscle activation needed to generate the necessary force, due to the muscle's force-velocity relationship (Fletcher, Groves, Pfister et al. 2013, Roberts. 2002).

The Achilles tendon (AT) undergoes rapid lengthening and shortening during the stance phase of running, taking up much of the length change of the whole muscle-tendon unit, preventing the need for muscle fibre lengthening and keeping to a minimum the triceps surae (TS) muscle fascicle shortening (Lichtwark, Bougoulias and Wilson. 2007, Ishikawa, Pakaslahti and Komi. 2007, Roberts. 2002). Hypothetically then, the muscle energy cost is lowest when the AT is mechanically 'tuned' to allow near-isometric muscle fascicle force generation during the stance phase and the whole length change of the muscle-tendon unit can be accommodated by the AT alone, greatly reducing the amount of shortening required by the muscle fascicles (Alexander. 1991). However, the AT demonstrates viscoelastic properties; when cyclically loaded, such as during running of sufficient duration, the AT may develop dynamic creep (Hawkins, Lum, Gaydos et al. 2009), resulting in greater length change for a given load. During running, assuming similar kinematics at the same running speed, a reduction in AT stiffness would be expected to move the mechanical properties away from the energetically-optimal AT properties; the reduction in AT stiffness may result in increased fascicle length change. This additional fascicle shortening would require additional muscle energy (Fletcher, Groves, Pfister et al. 2013). This increased muscle energy cost would contribute to an elevated E_{run} .

Bouts of long distance running of 60-90 minutes have been shown to acutely elevate E_{run} in moderately-trained runners (Hunter and Smith. 2007, Xu and Montgomery. 1995). Increases in E_{run} have also been shown following half-marathon and marathon runs (Peltonen, Cronin, Stenroth et al. 2012). Repetitive contractions, like those performed during running, have been shown to decrease AT stiffness (Kay and Blazevich. 2009, Kubo, Kanehisa, Kawakami et al. 2001a, Kubo, Kanehisa, Kawakami et al. 2001b). Furthermore, we have previously shown that changes in AT stiffness correlate with a changes in E_{run} , (Fletcher, Esau and Macintosh. 2010). Thus, it seems logical to hypothesize that a submaximal run of sufficient duration will cause a decrease in AT stiffness and a corresponding increase in E_{run} . No study to date, however, has related any change in E_{run} to changes in estimated muscle energy cost.

Farris et al. (2011) measured AT stiffness in recreational runners before and following a 30 minute run at 12 km•hr⁻¹. At this speed, the average stride length is approximately 2.25 m (Cavanagh and Kram. 1989a), representing approximately 2667 strides over the course of the run. A single bout of running did not elicit a change in AT stiffness in these runners, but the duration (# of AT loading cycles) may not have been sufficient to elicit a change in AT stiffness.

Similar results have been shown in lesser-trained runners following half-marathon (21.2 km) and marathon (42.2 km) runs whereby AT stiffness measured 1 hour following the half or fullmarathon run was not changed (Peltonen, Cronin, Stenroth et al. 2012). However, an average post-run increase in E_{run} of 6% was observed, suggesting the post-run increase in E_{run} was not dependent on changes in AT stiffness. It is unknown if a similar lack of change in AT stiffness can be demonstrated in a run of sufficient duration (ie. greater than 30 mins) in trained runners, where it was anticipated the run would be as fast or faster than that which can be sustained during a marathon (Peltonen, Cronin, Stenroth et al. 2012). It seems likely that the combination of a sufficiently fast run and a high number of loading cycles is required to elicit a significant change in AT stiffness. This hypothesis is consistent with the results of Arampatzis et al. (2007) who provided evidence that a threshold of applied AT strain existed in order to induce any mechanical changes in the tendon.

If significant dynamic creep does occur during/following a prolonged run, this implies that for any muscle-tendon unit length during stance, AT elongation would be greater, necessitating greater TS fascicle shortening. This additional fascicle shortening is expected to be accompanied by an elevated energy cost and it is unclear whether this elevated muscle energy cost is sufficient to contribute to the expected increase in whole-body E_{run}. To date, the change in AT energy storage and release relative to the change in the associated muscle energy cost has not been described. Therefore, the purpose of this study was to investigate the changes in AT stiffness and muscle energetics prior to and immediately following a typical long-distance training run of 90 minutes.

7.3 Methods

14 trained male (n=10) and female (n=4) distance runners participated in this study. It was anticipated that the male and female runners would demonstrate a range of values for E_{run} and AT stiffness. Subject characteristics are shown in Table 1. All runners were training regularly at least 6 times per week and following a similar, periodized training plan. None of the runners had any neuromuscular or musculoskeletal injuries at the time of the study. The runners gave their informed written consent to the experimental procedure, which was approved by the University of Calgary Conjoint Health Research Ethics Board.

The subjects visited the lab on two separate occasions. The experimental protocol is shown in Figure 7.1. During the first visit, an incremental test to exhaustion was performed on a treadmill (Woodway Pro, Woodway USA, Waukeshka, WA) to determine the subject's maximal oxygen uptake ($\dot{V}O_2$ max) and speed associated with the lactate threshold (sLT). The methods used to determine $\dot{V}O_2$ max and sLT were similar to ones used previously in our lab (Fletcher, Pfister and MacIntosh. 2013, Fletcher, Esau and Macintosh. 2010, Fletcher, Esau and MacIntosh. 2009). All incremental tests were terminated due to volitional exhaustion and all subjects attained $\dot{V}O_2$ max based on primary or secondary criteria (Fletcher, Pfister and MacIntosh. 2013).

Between 48 and 96 hours following the first laboratory visit, the subjects returned to the lab for determination of AT stiffness and E_{run} before and following the prolonged, 90 minute run (RUN). RUN was performed on an outdoor road surface over level ground under similar weather conditions (14-19° C, 38-54% relative humidity) in the subject's own running shoes at a pace corresponding to approximately 85% of sLT. The speed and heart rate associated with 85% sLT were determined from the incremental test to ensure consistent relative pacing between

subjects. Each subject wore a heart rate monitor (Suunto t6c, Oy, Finland) during RUN and was instructed to maintain the pre-instructed heart rate throughout. Since these were experienced runners, they were also given information prior to RUN regarding the approximate target speed (minutes•km⁻¹ and minutes•mile⁻¹). Following RUN, heart rate data were downloaded to a computer using the manufacturer's software (Suunto Team Manager, Suunto, Oy, Finland). The average (± 2 sd) heart rate was calculated and only those runs where the average heart rate was within \pm 3 beats•min⁻¹ of the target heart rate were used for subsequent analysis for AT stiffness and E_{run} following RUN. In total, five RUN trials (4 men and 1 woman) had to be repeated 7 days later based on the above exclusion criterion. Average heart rate during RUN was used to estimate approximate run speed, from the heart rate-speed (below sLT) relationship determined during the incremental test.

7.3.1 Determination of AT Stiffness

Prior to and immediately following RUN, AT stiffness was determined on the subject's right leg as described previously (Fletcher, Pfister and MacIntosh. 2013, Fletcher, Esau and Macintosh. 2010). Three isometric ramp maximal voluntary contractions (MVC) of the right plantarflexors were performed, the highest of which was used for subsequent analysis. The first MVC was performed less than 2 minutes following the completion of RUN in all subjects. During the MVC, a 12.5 MHz linear array ultrasound probe (50mm, Philips Envisor, Philips Healthcare, Eindhoven, Netherlands) was used to visualize the medial gastrocnemius muscle (MG) fascicles near the myotendinous junction. This was done to reduce any impact of aponeurosis compliance on the measurement of tendon elongation; however, we acknowledge the measured elongation may represent (some) aponeurosis elongation along with tendon

elongation. It should be noted that Arampatzis et al. (2005) demonstrated that measurements of tendon elongation can be made at any point along the aponeurosis without compromise. The probe was secured using a custom-built apparatus. AT tendon elongation was estimated by the displacement of an insertion of a fascicle into the deep aponeurosis as close as possible to the myotendinous junction, measured using *ImageJ*, (NIH, Baltimore, MD, USA) during the MVC. Measured moments and AT elongations were corrected for joint rotation detected by video analysis and the known passive joint angle-tendon translation relationship (Fletcher, Esau and Macintosh. 2010, Fletcher, Groves, Pfister et al. 2013). AT moment arm length at the 90° ankle angle was estimated using the tendon travel method (Ito, Akima and Fukunaga. 2000). AT force was calculated by dividing the ankle joint moment by the estimated AT moment arm. AT Force (F)-elongation (d₁) data were fitted to a quadratic regression equation using:

$$\mathbf{F} = A\mathbf{d}_{\mathrm{L}}^{2} + B\mathbf{d}_{\mathrm{L}}$$
 7-1

where *A* and *B* are constants. In order to account for any difference in MVC force prior to and following RUN, AT stiffness was defined as the slope of the fitted $F-d_L$ equation from 50-100% of maximum isometric plantarflexion force prior to RUN.

7.3.2 AT energy storage/release and muscle energy cost

AT energy storage and release, as well as muscle energy cost to allow AT energy storage to occur were estimated according to Fletcher and MacIntosh (2014). Briefly, AT energy storage was calculated as the area under the F-d_L curve during running, where F was estimated from the assumed average peak vertical ground reaction forces and running speed (Keller, Weisberger, Ray et al. 1996) and assuming the moment arm of F_z during the stance phase was 1.5x greater than the AT moment arm (Giddings, Beaupre, Whalen et al. 2000). We

acknowledge that this length is not fixed during the stance phase and is different between rear and midfoot strikers (Cavanagh and Lafortune. 1980). However, we chose a fixed F_z :AT moment arm ratio since in some cases, a ratio larger than this resulted in an estimated AT force during running which was greater than the maximum isometric force. It seems unlikely that the AT force during running would be near the maximum isometric force, we likely have overestimated the AT force during running. This over-estimation of the AT force would result in both an over-estimation of the AT energy release as well as of the estimated muscle energy cost.

The corresponding tendon length change (d_L) during running was estimated from the measured tendon stiffness and expected joint rotation. AT energy storage was calculated and AT energy release (2 footstrikes per stride), was estimated assuming an AT hysteresis of 10% (Finni, Peltonen, Stenroth et al. 2012).

TS muscle energy cost for AT storage/release to occur was calculated from the estimated MG force and fascicle shortening during two sequential footstrikes (one stride). The energy cost corresponds to the energy associated with cross-bridge cycling, which was estimated from the number of in-parallel crossbridges required to produce the AT force, assuming 3 pN per crossbridge (Finer, Simmons and Spudich. 1994, Rome, Cook, Syme et al. 1999), and the number of cross-bridge cycles required to accommodate the half-sarcomere shortening. This value was multiplied by the estimated number of half-sarcomeres in series and doubled to represent a complete stride. The number of half-sarcomeres in series was estimated by dividing an assumed sarcomere length into the known fascicle length. A linear increase in force as a function of fascicle length, with no evidence of a plateau (Maganaris. 2003) suggests that during maximal contractions, the medial gastrocnemius operates on the ascending limb of the force-length relationship. Thus, the longest sarcomere length could only be at the short end of the

plateau. We assumed this sarcomere length to be 2.6 μm at MVC (Herzog, Read and ter Keurs. 1991).

7.3.3 Measurement of E_{run}

Immediately following the measurement of AT stiffness, the measurement of E_{run} was performed by having the subjects run at 85% sLT for five minutes on a motorized treadmill (Woodway Pro, Woodway USA, Waukesha, WI). Prior to RUN, a 5 minute warm up at 133 m•min⁻¹ for the females and 160 m•min⁻¹ for the males was performed. No cool-down was performed following RUN. \dot{VO}_2 was measured throughout the 5 minute run using a metabolic measurement cart (TrueMax 2400, Parvomedics, Salt Lake City, UT). The cart was calibrated before and after each session with a two-point calibration using room air and a gas mixture of known composition (4% CO₂, 16% O₂) and a manual 3-L syringe. According to the manufacturer, the accuracy of this system is 0.03% and 0.1% for O₂ and CO₂, respectively and ±2% for volume.

 E_{run} was calculated from the steady-state $\dot{V}O_2$ and respiratory exchange ratio (RER) over the final 2 minutes of the 5 minute stage. E_{run} was expressed in units of energy (J•kg⁻¹•m⁻¹), as described previously (Fletcher, Pfister and MacIntosh. 2013, Fletcher, Esau and Macintosh. 2010, Shaw, Ingham, Fudge et al. 2013, Shaw, Ingham and Folland. 2014).

7.3.4 Statistics

Values are presented as mean \pm sd unless otherwise indicated. Two-tailed paired t-tests were used to test for differences between pre and post-run values for E_{run} , MVC force, AT stiffness, AT energy release and muscle energy cost. One-way ANOVA was used to test for

differences in d_L across absolute force levels. Linear regression analysis was used to examine the relationship between AT stiffness and E_{run} prior to and following the run as well as to examine the relationships between the change in AT stiffness, and the change in E_{run} , and the change in AT energy release and muscle energy cost following RUN. All analyses were performed using GraphPad Prism version 6.04 for Windows (GraphPad Software, La Jolla, CA, USA, www.graphpad.com). The *a priori* level of statistical significance was set at alpha <0.05.

7.4 Results

Mean heart rate during RUN was 139 ± 9 b•min⁻¹, equivalent to heart rate at 83.6 ± 4.1 % sLT. This corresponds to an approximate run speed of 214.1 ± 13.7 m•min⁻¹. This speed was not different than the anticipated speed associated with 85% sLT (218.2 ± 17.9 m•min⁻¹, p=0.175). The approximate run distance was 19.3 ± 1.2 km.

 E_{run} prior to and following RUN is shown in Figure 7.2. E_{run} increased following RUN in 10 of the 14 subjects. Following RUN, E_{run} was significantly higher (p=0.044) compared to E_{run} measured prior to RUN. This represents a mean increase in E_{run} of 0.06±0.10 J•kg^{-1•}m⁻¹ (1.3%) following RUN.

MVC force was reduced by $3.0\pm5.7\%$ following RUN, from 4489 ± 2013 N to 4333 ± 1917 N. The 95% C.I. for the difference (POST-PRE) was -312 to 1 N. This difference was not significant (p=0.0512). The F-d_L relationship prior to and following RUN is shown in **Error! Reference source not found.** AT stiffness prior to and following RUN are shown in Figure 7.4. AT stiffness was reduced following RUN by 28.5 ± 36.5 N•mm⁻¹. This reduction in AT stiffness was significant (p=0.009). The relationship between the change in E_{run} and the change in AT stiffness as a result of RUN is shown in Figure 7.5. This relationship was significant (r²=0.430, p=0.011), suggesting that the increase in E_{run} was associated with a decrease in AT stiffness.

Any change in d_L and/or force following RUN would result in a change in the amount of AT energy storage/release. Prior to RUN, we estimated this amount of strain energy release to be 21.6 ± 9.0 J•stride⁻¹. Following RUN, AT energy release appeared to be higher (23.5 ± 8.0 J•stride⁻¹), but this increase was not significant (p=0.061, 95% C.I. for the difference = -0.1 to 2.8 J•stride⁻¹). The estimated TS muscle energy cost to allow AT energy storage/release to occur was significantly elevated (p=0.0034) following RUN, from 163.5 ± 61.1 J•stride⁻¹ to 175.2 ± 62.7 J•stride⁻¹. This change in estimated TS muscle energy cost was significantly related to the change in E_{run} following RUN (r²=0.368, p=0.023), suggesting that nearly 40% of the variance in E_{run} can be accounted for by variability in muscle energy cost. This relationship is shown in Figure 7.6.

7.5 **Discussion**

The results of the current study demonstrate that a prolonged, submaximal run similar to that regularly performed in training by distance runners can elicit a small but significant increase in E_{run} and a reduction in AT stiffness. The changes in E_{run} and AT stiffness as a result of this RUN were significantly related, confirming previous reports that a change in AT stiffness is associated with a change in E_{run} in highly-trained distance runners (Fletcher, Esau and Macintosh. 2010). Considering the expectation for decreased stiffness in the current paper, combined with our attempt to manipulate AT stiffness via a prolonged run, there is justification for assuming that this is a cause and effect relationship.

Changes in E_{run} following prolonged running have been demonstrated in highly-trained runners previously (Petersen, Hansen, Aagaard et al. 2007). Specifically, it is reported that runners had a 5.2% (Brueckner, Atchou, Capelli et al. 1991) increase in E_{run} following a marathon run at 273 m•min⁻¹. Here, we observed a smaller change in E_{run} (1.3%) following a 90 min run at a slower speed (214 m•min⁻¹). It seems logical to consider that the change in E_{run} should be dependent on speed and distance run. Brueckner et al. (1991) have previously estimated that E_{run} increases as a function of distance run, by approximately 0.08 %•km⁻¹. Here, we show a similar change of 0.07±0.12 %•km⁻¹. Our slightly smaller observed change in E_{run} may have been a result of the familiarity to the task, as this is a run typically performed during training by these runners.

It cannot be overlooked, however, that the observed change in E_{run} is small. The magnitude of difference between pre and post-RUN values for E_{run} (1.3%) is smaller than the typical error in measurement of E_{run} of highly-trained runners reported in our lab (Fletcher, Esau and MacIntosh. 2009) and others (Shaw, Ingham, Fudge et al. 2013). Furthermore, the smallest worthwhile change (SWC) in E_{run} in highly-trained runners has been reported to be 2.7% when E_{run} is expressed in terms of energy (Shaw, Ingham, Fudge et al. 2013). However, data from our laboratory for trained and elite male and female distance runners suggest the SWC in E_{run} is between 0.8% and 1.1% (Fletcher and MacIntosh. 2014); this SWC is smaller than the magnitude of change we report in E_{run} following RUN. Although we must be cautious in the interpretation that the changes seen post-RUN are 'real' and 'worthwhile' and not simply related to testing error and typical variation of E_{run} , the fact that the difference did reach significance, probably because of the consistency of measurement, is evidence that it is real.

It is interesting to consider the context of a progressive increase in E_{run} and its impact on performance of a record-breaking marathon performance. There was a recent Viewpoint (Joyner, Ruiz and Lucia. 2011) that presented the question: "Who and When" with reference to breaking that record. In the associated Discussion, there was a general consensus that current physiological measures of elite runners are consistent with performance of a 2 hour marathon. However, if there is a progressive increase in E_{run} in these runners, then clearly a slow-down would be required if a constant energy cost was the requirement for elite performance. Also, the estimate of heat generation did not consider a progressive increase in E_{run} (Fletcher, Esau, Holash et al. 2011). A similar change in E_{run} of 0.07% per km over the course of a world-class marathon (eg. 2 hours 5 minutes) would equate to a near 4 minute (3 minute and 41 seconds) increase in race time. It may be more difficult than we think to break the 2 hour barrier.

We specifically chose to perform the runs when the outdoor ambient temperature and relative humidity were relatively low in order to prevent any heat-associated increases in E_{run} . Body temperature increase would rise primarily from metabolic heat generation, for which we estimate 90±10 kJ/kg based on our mean E_{run} values. This amount of additional heat would necessitate 2.2±0.6 L of sweat in order to lose heat by evaporation alone (for subjects ranging in mass from 45 to 73 kg). Much of this heat loss would also occur via conduction and convection, so this is an overestimate. Our subjects may have had a reduction of body mass over the course of the run so their E_{run} should have been divided by a smaller body mass. If we assume a weight change of 0.75 kg following the run, then the post- E_{run} is 2.5% higher than the E_{run} measured prior to RUN.

We also observed a significant decrease in AT stiffness following RUN which was larger than the technical error in AT stiffness we have previously reported (Fletcher, Esau and

Macintosh. 2010). The reduced AT stiffness was a result of a greater AT elongation at any given absolute force post-RUN without a significant reduction in MVC force (p=0.0512). Even if this had reached significance, the difference was only 3%. This finding is contrary to previous prolonged running studies which report a significant decline in MVC following prolonged running. These reductions include a 17% reduction following two hours of treadmill running at $75\% \dot{V}O_2$ max (Saldanha, Nordlund Ekblom and Thorstensson. 2008) and a 30% reduction in plantarflexor force after a run of 24 hours, with no significant reduction in plantarflexor force after 4-hours of running (Martin, Kerherve, Messonnier et al. 2010). It is likely that two or more mechanisms were responsible for the non-significant reduction in MVC force with our runners. First, contrary to previous reports which measured MVC force following runs of longer duration, our run was of a shorter duration and at a constrained submaximal intensity; the run may not have been of sufficient duration and/or intensity to elicit a significant reduction in MVC force. Therefore, a reduction in force as a consequence of central fatigue mechanisms would not have been expected. Furthermore, as these runners were highly-trained and RUN was typically performed (weekly) as part of their regular training, they may have adapted to perform a run of this duration without fatigue.

We also observed a significant increase in d_L at all absolute forces following RUN. Given the same AT forces throughout the run, an increased d_L is indicative of dynamic creep of the tendon. We estimate, based on the duration of RUN and the average stride length at the RUN speed (Cavanagh and Kram. 1989b), approximately 7500 AT loading cycles during RUN. This should be sufficient to elicit dynamic creep in the AT (De Zee, Bojsen-Moller and Voigt. 2000, Hawkins, Lum, Gaydos et al. 2009) and therefore a significant reduction in AT stiffness post-RUN. Previous studies which could not demonstrate a significant reduction in AT stiffness post-

run may not have been of sufficient duration (Farris, Trewartha and McGuigan. 2011) or speed (Peltonen, Cronin, Stenroth et al. 2012) to cause dynamic creep since a minimum threshold may need to be achieved in order to elicit any change in AT mechanical properties (Arampatzis, Karamanidis and Albracht. 2007, Lichtwark, Creswell and Newsham West. 2013). Based on previous studies on dynamic creep of the AT (De Zee, Bojsen-Moller and Voigt. 2000, Hawkins, Lum, Gaydos et al. 2009), the increased d_L should occur early on in the run and reach a steady-state after approximately 30-35 minutes (De Zee, Bojsen-Moller and Voigt. 2000). It would appear that when the contractions are of a sufficient magnitude to cause dynamic creep, this occurs early, but when the contractions are only of marginal magnitude, it may require more loadings to impact the stiffness. This may help explain the apparently-contrary observations in AT stiffness following RUN between the current study and previously published work.

In the only other study to examine the change in E_{run} and AT stiffness following a prolonged run, Peltonen et al. (2012) could not show a significant change in AT stiffness despite a 7% increase in E_{run} (measured as O₂ cost) following a marathon run. However, upon reexamination of the data presented in that study, and in removing one potential outlier, a similar significant relationship (r²=0.441, p=0.036) emerges between the change in E_{run} and the change in AT stiffness. This relationship is similar in magnitude to our current results (shown in Figure 5) as well as those previously reported from our lab in highly-trained runners (r2=0.523, Fletcher, Esau and Macintosh. 2010). In previous studies (Farris, Trewartha and McGuigan. 2011, Lichtwark, Creswell and Newsham West. 2013, Peltonen, Cronin, Stenroth et al. 2012), the RUN may not have been of sufficient speed and/or duration to elicit a significant reduction in AT stiffness. This suggestion has been proposed previously (Lichtwark, Creswell and Newsham West. 2013). A greater d_L without a change in AT force would result in a greater storage of AT strain energy during ground contact. However, in order to compensate for the additional d_L , and assuming similar kinematics pre and post-RUN (Nicol, Komi and Marconnet. 1991b), an increase in muscle fascicle shortening is necessary. This additional shortening would come at a significant muscle energy cost, which we have estimated following a prolonged run here for the first time based on cross-bridge kinetics and energetics, similar to our previous estimates during steady-state running (Fletcher and MacIntosh. 2014).

Our estimates of the storage/release of tendon strain energy revealed a small (3.7 J•stride ¹) but non-significant (95% CI: -0.1 to 2.8 J•stride⁻¹) increase. This lack of significant difference may relate to the subtraction of 10% of the stored energy to estimate energy return. The additional $d_{\rm L}$ resulted in a significant increase in the estimated muscle energy cost of nearly 12 J•stride⁻¹. Therefore, we conclude that the storage and release of tendon strain energy by the AT is relatively less important. Rather, the limited lengthening of the AT during running serves to minimize muscle fascicle shortening. Less shortening should minimize the muscle energy cost (Fletcher, Groves, Pfister et al. 2013, Alexander. 1991, Roberts. 2002). From our correlation analyses, we show the elevated E_{run} post-RUN was associated with a post-RUN decrease in AT stiffness (Figure 5). This relationship is similar in magnitude and direction to the one we have previously shown following 8 weeks of isometric training in highly-trained runners, further suggesting a change in AT stiffness is associated with a change in E_{run} (Fletcher, Esau and Macintosh. 2010). We acknowledge, however, that other factors such as changes in kinematics and/or kinetics may also contribute to an elevated E_{run} (Hunter and Smith. 2007, Candau, Belli, Millet et al. 1998, Nicol, Komi and Marconnet. 1991a).

It has recently been demonstrated, in attempts to simulate running muscle mechanics (in frog muscle) that the energy cost of shortening contractions was nearly triple the energy cost of isometric force production (Holt, Roberts and Askew. 2014). These results were obtained in spite of the fact that the force during the shortening was substantially less than the force during the isometric contraction. Considering the need to have similar force production during running, additional motor unit recruitment would be needed in the shortening condition as suggested by the results of Fletcher et al. (2013), resulting in even higher energy cost difference.

To further support the relationship between AT stiffness and E_{run} , we demonstrate here that the change in E_{run} is also associated with a change in the estimated muscle energy cost. This additional muscle energy cost is a result of the predicted increase in muscle fascicle shortening following RUN. This additional shortening is a consequence of the mechanical fatigue of the AT.

7.6 Conclusion

In conclusion, the current results demonstrate dynamic creep of the AT during a prolonged run below the lactate threshold in trained male and female distance runners. The change in AT mechanical properties following RUN appears to have a small but significant effect on muscle energy cost and the associated E_{run} . Calculations suggest that this effect is greater than the additional tendon strain energy release from the AT. These results further support the notion that a mechanically-optimal AT minimizes E_{run} in trained distance runners by minimizing muscle fascicle shortening.

7.7 Author Contributions

All experiments were performed at the Human Performance Lab at the University of Calgary, Calgary, Alberta, Canada. JRF and BRM were responsible for conception and design of the experimental protocol. JRF collected and analyzed the data. JRF and BRM were primarily responsible for interpreting the experimental and theoretical data. JRF drafted the manuscript and JRF and BRM revised it critically for important intellectual content. All authors approved the final draft of the article.

7.8 Acknowledgements and disclosures

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7.9 Tables

N	Age	Height	Mass	VO ₂ max	sLT
1	(years)	(m)	(kg)	(ml•kg ⁻¹ •min ⁻¹)	(m•min ⁻¹)
14	24.4±5.8	1.72±0.09	61.1±10.0	64.6±5.8	257±21

Values are mean \pm standard deviation

7.10 Figures



Figure 7.1. Experimental protocol.



Figure 7.2. E_{run} prior to (PRE) and following (POST) RUN.

Black lines represent individual subject responses.



Figure 7.3 AT force-elongation curve prior to and following RUN.

 d_L is shown at the same relative force level (shown at 20% MVC-PRE force increments). At all relative forces, assessed, d_L was significantly higher POST-RUN (p<0.001). 50% MVC-PRE is also shown to demonstrate the change in AT stiffness (slope from 50-100% MVC).



Figure 7.4. AT stiffness measured prior to (PRE) and following (POST) RUN.

Solid lines represent individual subject responses. AT stiffness was significantly lower

(p=0.009) following RUN.



Figure 7.5. Relationship between the change in E_{run} and AT stiffness following RUN.

The solid line represents the linear relationship between E_{run} and stiffness (r²=0.430, p=0.011). Dashed lines represent the 95% confidence interval for the relationship. Note that the relationship crosses the abscissa at 0% change in stiffness.



Figure 7.6. Relationship between the change in E_{run} and TS muscle energy cost following RUN.

The solid line represents the linear relationship between E_{run} and muscle energy cost (r²=0.368, p=0.0213). Dashed lines represent the 95% confidence interval for the relationship. Note that the relationship crosses the abscissa at 0 % change in muscle energy cost.

Chapter Eight: Conclusions and Future Directions

8.1 Conclusions

The main findings from chapter 3 were that when energy cost of running is normalized to body mass, at similar relative speeds of running, no sex-specific differences in substrate use nor in E_{run} existed among similarly trained runners. Furthermore, the stiffness of the AT of women is lower than in men, but the relationship between E_{run} and AT stiffness was not different between the sexes.

The results of the chapter 4 confirm previous reports that the EC of muscle contraction is related to the amount and rate of muscle shortening. Furthermore, these results may explain why the EC of running is elevated when Achilles tendon compliance is increased, since a greater amount and rate of shortening are required for force transmission under these conditions. According to the in vivo force–length and force–velocity relationships of skeletal muscle, this shortening and velocity will impact the EC, not simply because of the greater shortening, but because increased muscle activation is required to permit similar force development when shortening velocity is greater.

Investigation into consideration for passive forces during the measurement of AT moment arm revealed that the tendon excursion method to estimate AT moment arm offers a quick, affordable alternative to the COR method when estimating MA length; however, it is only when no passive forces are acting to stretch the tendon is the TE method valid, unless this is quantified and accounted for. We have corrected for these passive forces non-invasively, throughout the range of motion. Since we show that MA length does not change as a function of ankle angle, it seems sufficient to measure MA length using the TE method at an ankle angle where passive

moments are negligible, or measuring the moment arm length manually. Both methods avoid the considerable time and financial costs associated with magnetic resonance imaging. Furthermore, these data have allowed us to reconsider the shape of the in vivo force-fascicle length relationship as a result of us considering the effect of AT moment arm length change (or lack thereof) and passive forces throughout the ankle range of motion.

From our estimates of tendon strain energy storage and release and muscle energy cost for this storage/release to occur, we conclude that the amount of tendon strain energy released represents a very small portion of the total metabolic cost to run a given speed. Furthermore, this energy return comes at a considerable muscle energy cost. Therefore, reducing muscle energy cost through reductions in muscle fascicle shortening during running even if this means less energy return from the tendon, contributes to an improved economy of running.

Lastly, measurements of E_{run} and AT stiffness made immediately prior to and following a prolonged run demonstrated significant mechanical fatigue of the AT in trained male and female distance runners during the run. The change in AT mechanical properties following RUN have a significant impact on the change in E_{run} and, using methods employed in Chapter 6, the associated muscle energy cost. This additional muscle energy cost following the run was greater than the estimated additional tendon strain energy release from the AT.

Taken together, results from this thesis support the notion that a relatively stiff AT minimizes E_{run} in trained distance runners by reducing muscle fascicle shortening. Further, changes in AT mechanical properties away from the apparently optimal mechanical properties significantly elevate the muscle energy cost and $E_{run,}$. This elevated energy cost is primarily associated with an elevated muscle shortening and associated increases in the level of motor unit recruitment to achieve the required force during running.

8.2 Future Directions

8.2.1 How quickly does AT stiffness get 'tuned' to be optimal?

We have proposed here that the AT stiffness is optimally-tuned in order to reduce muscle fascicle shortening and/or shortening velocity. In reality, whether the AT reaches an optimal level during running remains unclear. What is more clear though is that this additional muscle shortening is associated with a higher in vivo rate of energy use and level of activation (Chapter 4) and greater estimated triceps surae muscle energy cost (Chapter 6). Departure from this apparently-optimal AT stiffness further results in an elevated TS muscle energy cost and E_{run} (Chapter 7). To date, however, very little is known regarding *how* the AT stiffness is tuned in order to optimize muscle function across a range of walking and running speeds.

Lichtwark and Wilson (2008) have proposed that there exists an optimal combination of muscle fascicle length and AT stiffness which reduces the estimated energy cost of contraction. We further know that fascicle length and tendon stiffness differ between individuals of different sporting histories (Arampatzis *et al*, 2007, Muraoka *et al*, 2005, Kongsgaard *et al*, 2005), yet it is unclear whether these optimal combinations defines the sport or the combination of optimal muscle architecture and tendon mechanical properties are a result of long-term training. Therefore, further longitudinal examination of how these properties are adapted to training is required in order to support the hypothesis that muscle and tendon properties are tuned to reduce muscle energy cost.

8.2.2 Validation of muscle energetics model

Another area of investigation involves validation of the muscle energetics model presented in Chapter 6. This model was developed using previously reported values for similarly trained runners as those in our study. However, very little running kinetics or energetics data are available for elite runners like those assessed in Chapter 6. Thus, future research may focus on the direct measurement of muscle fascicle and tendon length change during running. Combined with kinematic and kinetic analysis of the stance phase, and level of muscle activation, a more reliable and potentially valid model of muscle energetics during running can be described.

8.2.3 Direct measurement of force-length-velocity relationship

It has been suggested that variables that alter muscle force production are probably more suitable for explaining variation in E_{run} (Martin and Morgan, 1992). These variables include the muscle force-length and force-velocity relationships as well as the level of activation of the muscles involved in running. In isolated muscles, the force-length and force-velocity relationships are well-investigated, fundamental properties of skeletal muscle, but little is known about how these relate to in vivo contractile responses and in particular how they relate to E_{run} . Within this thesis, we have attempted to expand upon this suggestion. Future research then could be concerned with measuring the force-length and force-velocity relationships of the triceps surae, in combination with estimates or direct measurement of fascicle length change during running. In doing so, a greater understanding on the optimal conditions for economical force generation can be considered.

Furthermore, results from Chapter 7 confirm that an acute change in AT stiffness as a result of a prolonged run can occur and this affects the estimated muscle energy cost. This

mechanical fatigue could result in the muscle operating at a less-than-optimal portion of the force-length and/or force-velocity relationships. This effect has not been investigated in trained runners.

The shifts in the force-length-velocity relationships as a result of skeletal muscle fatigue have been well-examined in situ (Rijkelijkhuizen et al, 2005, Butterfield and Herzog, 2005, De Ruiter et al, 1999, Curtin and Edman, 1994, Ameredes et al, 1992, Devrome and MacIntosh, 2007, Jones et al, 2006); however, to date these properties have not been fully examined under in vivo conditions of skeletal muscle fatigue. Maganaris et al. (2002) showed that following a series of just ten ramp contractions up to 80% MVC, MG fascicle length had shortened from 34 mm to 30 mm. The authors contest that this fascicle shortening would correspond to a reduction in the average operating range of individual sarcomeres. If the MG muscle operates on the ascending limb of the force-length relationship (Herzog et al, 1991, Maganaris, 2003), the contraction-induced shortening would shift the average operating length of the sarcomeres further down the ascending limb and away from the optimal myofilament overlap on the nonfatigued, maximally-activated force-length curve, thus reducing the contractile force generated for a given level of activation. The effect of shorter fascicles as a result of repetitive contractions further compounds the force-generating capacity of the muscle. Presumably, these contractions would cause fatigue, resulting in a shift down and to the right of the force-length curve. Thus, the level of activation required to generate the necessary active force at short fascicle lengths becomes even greater. This has important implications as it relates to skeletal muscle function and energetics in vivo.

8.2.4 Estimates of role of tendon stiffness in other muscles relevant during running

It has previously been shown that in a group of trained distance runners, the most economical runners displayed a higher stiffness of the triceps surae (TS) tendon compared to the less economical runners (Arampatzis *et al*, 2006, Fletcher *et al*, 2010). The former study demonstrated the opposite to be true in the vastus lateralis (VL) tendon – that the most economical runners had a lower VL tendon stiffness compared to the less economical runners (Arampatzis et al., 2006). This suggests to us that the roles of these two muscles in minimizing the energy cost during running are different. The reason for these apparently contrary observations with respect to the impact of tendon stiffness on E_{run} is not obvious, however.

The lengthening of a tendon for energy storage is relevant in stretch-shortening cycles where a substantial pre-stretch of the tendon occurs early in a contraction. A compliant tendon allows more energy conversion of either kinetic or gravitational energy to potential energy. This energy can subsequently be released upon shortening so the muscle shortening can be reduced. A compliant tendon may also help by allowing the tendon to lengthen during the stretch phase of the SSC thereby keeping fascicle shortening velocity low. This permits high active force to be generated with relatively little muscle activation. If tendon compliance is optimal, muscle energy cost can be reduced because the fascicles are shortening at the appropriate velocity (Askew and Marsh, 1998, Gabaldon *et al*, 2008). It still remains unclear whether this appropriate velocity is one associated with optimal power production (0.2-0.3 V_{max}). It is hypothesized that these mechanisms operate in the VL tendon, which would lend support to previous evidence suggesting a more compliant VL tendon might decrease E_{run} (Arampatzis *et al*, 2006, Albracht and Arampatzis, 2006). This hypothesis requires further testing however.
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