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Determining characteristics of the Achilles tendon properties on metabolic cost and 3000 m running performance

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The thesis presented to the School of Physical Education, Physiotherapy and Dance Graduation Committee of the Federal University of Rio Grande do Sul as a partial requirement for approval in the teaching activity: TCC II.

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EXAMINER

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I dedicate this thesis to my family who has always supported me in the realization of my dreams. Thanks to them, I see myself getting closer to my goals.

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I am very happy to be part of this team and to be able to see myself grow as a scientist. Thank you.

Dedicated to Marta Machado dos Santos and Sthefano Machado dos Santos.

ABSTRACT

Tendons play a fundamental role in storing and releasing elastic energy, minimizing metabolic cost (C_{MET}) in distance running. This behavior is related to the tendon's ability to resist deformation (e.g. stiffness), which is controlled by changes in the tendon morphological [cross-sectional area (CSA)], and material (e.g. Young's modulus) properties. However, the relationship between Achilles tendon properties, C_{MET}, and running performance is still uncertain. This study aimed to correlate the Achilles tendon properties, C_{MET} and 3000 m running performance. 7 trained male long-distance runners (31 ± 8 years) participated in this study (Ethics Committee approval number: 2.437.616). Ultrasound was used to determine the Achilles tendon CSA, length, and elongation as a function of plantar flexion torque during voluntary plantar flexion. Tendon force-elongation and *stress-strain* relationships were determined by maximum voluntary isometric contractions on a dynamometer. Then, the maximal incremental test was performed until exhaustion on a treadmill. After 24 hours, C_{MET} was measured in the running economy test for 5 minutes at 12 and 16 $km.h^{-1}$ on a treadmill. After 10 minutes at rest, the 3000 m running performance test on an athletics track was performed. The oxygen uptake was measured by spirometry. Correlations between Achilles tendon properties, C_{MET} (12 and 16 $km.h^{-1}$), and 3000 m running performance were obtained through Pearson's test ($p<0.05$). Correlation coefficient was classified as null (0), low (0-0.3), moderate (0.3-0.6), high (0.6-0.9), very high (0.9-1), and perfect (1). C_{MET} at 16 $km.h^{-1}$ correlated with CSA (r=-0.834, p=0.02), *stress* (r=0.901, p=0.006) and Young's modulus (r=0.880, p=0.009). Moreover, *stress* also correlated with CSA (r=-0.886, p=0.008) and Young's modulus (r=0.878, p=0.009). Tendon stiffness showed a very high correlation with *strain* (r=-0.931, p=0.002). Finally, 3000m running performance correlated with vVO_{2MAX} (r=-0.781, p=0.038). There was no correlation with TL or TL-SL, plantar flexor force, C_{MET} at 12 $km.h^{-1}$, VO_{2MAX}, first and second ventilatory threshold. We concluded that runners with lower Young's modulus, mainly due to greater CSA (related to lower stress) and greater stiffness (related to lower strain), presented better RE at 16 $km.h^{-1}$ due to the greater tendon work at this speed, minimizing the C_{MET}. Moreover, RE at 16 $km.h^{-1}$ is indirectly related to the 3000 m running performance, due to the high correlation between vVO_{2MAX} and 3000 m running performance.

Keywords: Running economy, Stiffness, Young's modulus, speed associated with the maximal oxygen consumption, long-distance runners.

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Equation 1. Achilles tendon cross-sectional area (CSA) equation.

Equation 2. Achilles tendon moment arm equation.

Equation 3. *Stress* equation.

Equation 4. *Strain* equation.

ABBREVIATIONS LIST

CSA	Cross sectional-area
C_{MET}	Metabolic cost
EMG	Surface electromyography
MA	Moment arm
MJ	Myotendinous junction
MVIC	Maximal voluntary isometric contraction
RE	Running economy
SL	Shank length
TL	Tendon length
TL-SL	Tendon length normalized by shank length
US	Ultrasound
VO_2	Oxygen uptake
VO_{2MAX}	Maximal oxygen uptake
$VO_{2SUBMAX}$	Submaximal oxygen uptake
vVO_{2MAX}	Speed associated with maximal oxygen uptake

SUMMARY

CHAPTER I

GENERAL INTRODUCTION

Chapter I comprises four sections: General Presentation, Problem Statement, Aims and finally the Literature Review.

GENERAL PRESENTATION

Contextualization and Delimitation of Study

This thesis is a partial requirement for approval at the teaching activity, TCC II (Undergraduate's Thesis). It is an outcome of the postdoctoral project "Effects of photobiomodulation therapy on the performance of runners in different athletic tests", which was focused on the Photobiomodulation Therapy effects on the running performance, running economy, mechanical efficiency, muscle recovery, and vertical jumps performance. This project was conceived at the Exercise Research Laboratory (LAPEX) of the School of Physical Education, Physiotherapy and Dance (ESEFID) at the Federal University of Rio Grande do Sul (UFRGS). The project is supervised by Prof. Dr. Leonardo Alexandre Peyré Tartaruga, wrote and developed by Prof. Dr. Fábio Juner Lanferdini. The Research Ethics Committee of the University approved this work. One article was already published with the project data, entitled "Physiological predictors of maximal incremental running performance", which I am co-authorship.

I started to study Terrestrial Locomotion in 2018 when I got in the Locomotion group. I was taught to read scientific articles, to create presentations, to talk in public and to do research. During this time, I wrote an abstract to present in Brazilian Congress of Biomechanics, 2019, entitled "Relationship between plantar flexors muscle architecture and the power of the vertical jump", in which I found that the lateral gastrocnemius pennation angle predicts 51% of the squat jump power. I loved this Congress. Then, I took the opportunity to make other abstracts like that to present at other events. In the UFCSPA Congress: Connecting health and society and in the II Symposium of PhysioMechanics of Terrestrial Locomotion, I presented an abstract entitled "Effects of photobiomodulation therapy on running economy", and in this second one Congress, my abstract was awarded the best poster presentation. I was so excited about my results and, therefore, I decided to deepen my research and investigate something that interests me: tendon properties and your relationships with running. Therefore, researchers like Prof. Ms. Edson Soares da Silva, Prof. Dr. Jeam Marcel Geremia, Prof. Dr. Fábio Juner Lanferdini and Prof. Dr. Leonardo Alexandre Peyré Tartaruga are involved in this project to help me to build beautiful theories.

Structure of Monograph

This project was developed at Biodynamic and Neuromuscular Plasticity Sectors in the ESEFID at UFRGS. This thesis is divided into five chapters. The first chapter presents the general introduction and the general presentation of the project, besides that the problem statement, the aims and the hypothesis of the study. Then, the second chapter provides a systematic literature review regarding running performance, running economy and Achilles tendon morphological, mechanical and material properties. The third chapter presents the scientific article about the relationship between running performance, running economy and Achilles tendon morphological, mechanical and material properties. Afterward, the fourth chapter presents the general discussion and the general conclusion of the project. Finally, the fifth chapter lists the abstracts and studies published during the Physical Education Degree.

PROBLEM STATEMENT

Running is a mode of locomotion that is decisive in human evolution (SAIBENE; MINETTI, 2003). At the organism level, human beings have undergone adaptations that allowed their bodies to travel greater distances at a lower energy cost. These adaptations were called energy-saving mechanisms, in the running represented by the spring-mass model (SAIBENE; MINETTI, 2003). This model represents the behavior of the body center of mass when running, which moves forward (BLICKHAN, 1989).

The tendons play a fundamental role in the spring-mass model. This structure can transmit forces from the muscles to the bones (KVIST, 1994), but also to transfer gravitational potential, kinetic and elastic energies during a running stride (SAIBENE; MINETTI, 2003). Therefore, tendons store and release elastic energy during the running stride allowing economical movements (CAVAGNA, 2017).

During a stride, 50% of the running propulsion energy comes from the storage and release of elastic energy from the tendons (CAVAGNA; SAIBENE; MARGARIA, 1964). Thus, tendons seem to have a fundamental role in the running economy (RE), generating less muscle work and more tendon work, explaining the greater efficiency of the entire muscle-tendon unit and the better RE (CARDINALE, 2010). Better REs indicate a lower energy cost during running at submaximal speeds (FLETCHER; MACINTOSH, 2018; TARTARUGA et al., 2012; CONLEY; KRAHENBUHL, 1980).

Therefore, the optimization of the elastic energy storage and release system can positively influence RE (CARDINALE, 2010; ROBERTS, 2002). During the landing phase in the run, there is tendon stretching which is crucial for elastic energy storage (CARDINALE, 2010). Such behavior is related to the tendon stiffness (WIESINGER et al., 2015). Arampatzis et al. (2006) observed better RE in long-distance runners with greater tendon stiffness, as well as a greater elastic energy storage capacity at low speeds.

However, Cavagna (2006) observed that at low and medium running speeds, muscle activation tends to be moderate, causing a rapid stretching and slow shortening of the muscles, demonstrating high energy expenditure. At speeds above 14 km.h^{-1} the muscles will have greater activation acting almost isometrically; thus, stretching and shortening will be determined by the tendons without time differences and with less energy expenditure (MONTE et al., 2020b; CAVAGNA, 2006). Furthermore, Cavagna (2006) demonstrated that at high speeds the tendon work is greater than at lower speeds, but does not specify whether these different running speeds influence the relationship between tendon properties and RE.

Another important variable is the Young’s modulus, which normalizes tendons stiffness and deformation by their initial dimensions. Tendon stiffness is mainly controlled by adaptations in the tendon in the tendon morphological (e.g. cross-sectional area) and material properties, obtained by Young's Modulus (BOHM et al., 2015; SINGER et al., 2016). These adaptations occur mainly during growth and physical training periods (GEREMIA et al., 2018; NEUGEBAUER; HAWKINS, 2012; WAUGH et al., 2012). However, studies show minor variations in Young's Modulus values between athletes from different sports and non-athletes (PELTONEN et al., 2010; WIESINGER et al., 2016), making it difficult to understand their interactions with RE and running performance.

The best long-distance running performance can be achieved through changes in biomechanical (e.g. stride length and frequency) and physiological (e.g. RE) factors (TARTARUGA et al., 2012). Studies have compared different running performances using maximal oxygen consumption ($\dot{V}O_{2MAX}$) as a predictor of performance (POLLOCK, 1977). However, homogeneous long-distance athletes present different performances and similar $\dot{V}O_{2MAX}$. For this reason, RE has been become a crucial physiological determinant of distance running performance, since oxygen consumption (VO_2) is measured submaximally, showing an effective variation among athletes with similar $\dot{V}O_{2MAX}$ (MORGAN et al., 1989). Therefore, the most economical runners have a lower energy cost at a given submaximal running speed (SAUNDERS et al., 2004). However, Bragada et al. (2010) demonstrated that the RE was not related to the performance of 3000 m of running, due to the short event duration.

Therefore, although tendon stiffness may be related to RE (CAVAGNA, 2017; ALBRACHT; ARAMPATZIS, 2013; FLETCHER et al., 2010; CARDINALE, 2010; ARAMPATZIS et al., 2006; ROBERTS, 2002; CAVAGNA; SAIBENE; MARGARIA, 1964), the findings on the relationship between stiffness and running performance are contradictory (DA ROSA et al., 2019; BRAGADA et al., 2010). Studies evaluating the association between 5000 m running and Achilles tendon stiffness indicate that less rigid tendons are related to better running performance (KUBO et al., 2015; KUBO et al., 2010). On the other hand, Ueno et al. (2017) observed that the passive stiffness of the plantar flexor muscle-tendon unit demonstrates a positive relationship with running performance. A possible justification for this discrepancy is that athletes with great performance can present different RE (FOSTER; LUCIA, 2007).

Therefore, the relationship between Achilles tendon properties, physiological parameters and running performance remains unclear.

AIMS

This project aimed to correlate the Achilles tendon morphological, mechanical and material properties, metabolic cost at 12 and 16 km.h^{-1} and 3000 m running performance in trained long-distance runners.

HYPOTHESIS

1. RE at 16 km.h^{-1} will correlate positively with Achilles tendon mechanical properties (stiffness), mainly due to the changes in stiffness that happen practically together with the RE changes, according to the literature;
2. RE at 16 km.h^{-1} will not correlate with Achilles tendon material (Young's modulus) and morphological (cross-sectional area and tendon length) properties, due to the minor variations in Young's Modulus values found in the literature.

CHAPTER II

LITERATURE REVIEW

Running performance

Long-distance running performance is determined by several anthropometric, physiological and biomechanical factors. Studies demonstrate several aspects that can influence long-distance running performance in an integrative way (SAUNDERS et al., 2004; PRAMPERO, 2003; COYLE, 1999; PRAMPERO et al., 1986; PRAMPERO, 1986; CONLEY; KRAHENBUHL, 1980). Changes in aerobic capacity, muscle mitochondrial density, availability of carbohydrate and fat seem to affect directly long-distance running performance (COYLE, 1999). Besides, cardiorespiratory, muscular and enzymatic factors can also influence aerobic behavior (COYLE, 1999). Saunders et al. (2004) explain that variations in running performance can also be caused, in addition to anthropometric, physiological and biomechanical factors, by training and environment factors, according to their conceptual model (Figure 1).

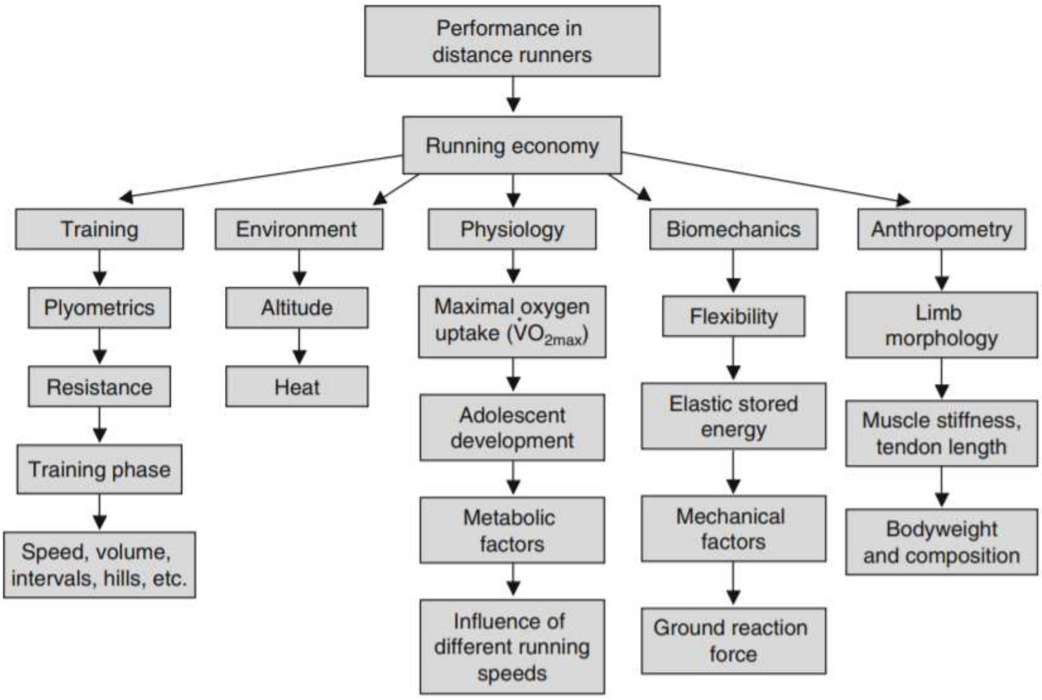

Figure 1. Factors affecting running performance. From Saunders et al. (2004).

$\dot{V}O_{2MAX}$ is one of the factors that can explain long-distance running performance, as it demonstrates the athlete's maximal aerobic capacity to sustain a high $\dot{V}O_2$ fraction for a long period. Lanferdini et al. (2020) showed that 3.000 m running performance is determined by $\dot{V}O_{2MAX}$ and RE at 16 km.h^{-1}. Thus, to differentiate the great homogeneity that $\dot{V}O_{2MAX}$ presents in athletes with different performances, RE has been considered as the determining running performance factor in long-distance events (PRAMPERO, 2003; CONLEY; KRAHENBUHL, 1980; PRAMPERO et al., 1986; PRAMPERO, 1986; SAWYER et al., 2010). RE can distinguish runners with similar maximal aerobic capacities and different performances.

Differences in RE are often attributed to biomechanical and physiological factors, contrary to the VO_{2MAX}, according to Figure 1.

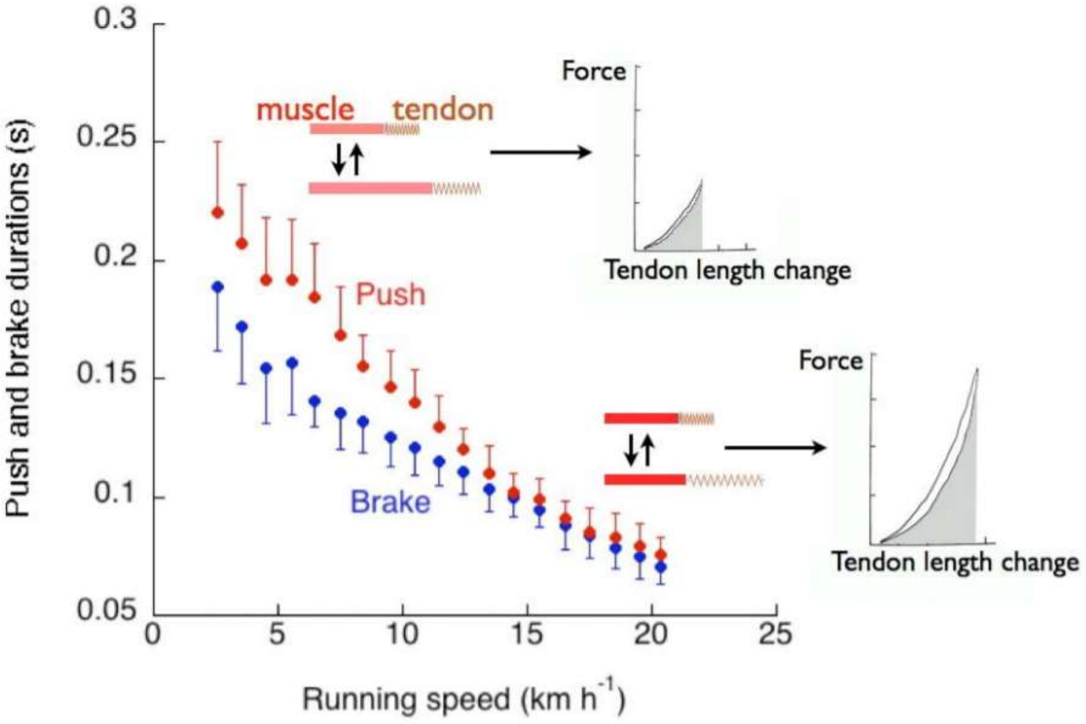

Figure 2. The positive work (Push, red circles) and negative work (Brake, blue circles) durations (s) are plotted as a function of running speed (km.h^{-1}). Hypothesis: at low speeds, muscle (red bars) contributes with changing length of muscular-tendon unit, while at high speeds (>13 km.h^{-1}) the tendon (brown spring) sustained the length changes on muscular-tendon unit, and muscle contracts quasi isometrically with higher force. From Cavagna (2010) and adapted from Ker et al. (1987).

Furthermore, the stretch-shorten cycle of muscle and tendon is directly related to the running speed (Figure 2) (MONTE et al., 2020b; CAVAGNA, 2006). Analyzing different speeds, the energy loss in the muscle is greater than the energy loss in the stretch-shorten cycle of the tendon (CAVAGNA, 2010). Cavagna (2006) demonstrated that at high speeds the lower limb muscles acted almost isometrically and the tendon work was greater (Figure 2). Thus, at high speeds, running mechanics is better, probably due to the greater elastic energy stored of the tendon (Figure 1), although the metabolic cost tended to increase due to muscle contraction (FLETCHER; MACINTOSH, 2015). Besides, the ability to quantify mechanical work is understood as efficiency (PEYRÉ-TARTARUGA; COERTJENS, 2018; CAVAGNA 2010). Efficiency is characterized as the fraction of the amount of metabolic energy that can be transformed into mechanical work (PEYRÉ-TARTARUGA; COERTJENS, 2018). According to this possible deterministic relationship between efficiency and RE, the association between efficiency and running performance is inevitable (PEYRÉ-TARTARUGA; COERTJENS, 2018). This suggestion can be considered true due to the high relationship between RE and running performance (LANFERDINI et al., 2020; PRAMPERO, 2003; PRAMPERO et al., 1986; PRAMPERO, 1986). However, efficiency includes measurement of mechanical work and it is very difficult to quantify in running, and improved efficiency may not relate to performance.

Running economy

Long-distances runners need to move economically, saving a high amount of energy for long periods. This is essential to maintain a constant speed throughout a long-distance race, to achieve better results. Moving economically is about RE. This is typically measured as the steady-state rate of submaximal oxygen consumption ($\dot{V}O_{2SUBMAX}$) at a given speed (TARTARUGA et al., 2012; DANIELS, 1985). Therefore, a better RE is directly related to the best long-distance running performance results, being determinant for running performance (LANFERDINI et al., 2020; TARTARUGA et al., 2012; FOSTER; LUCIA, 2007; SAUNDERS et al., 2004; PRAMPERO et al., 1993; CONLEY; KRAHENBUHL, 1980; POLLOCK, 1977). The RE may be evaluated by the metabolic rate ($J.kg.^{-1}.min^{-1}$) or metabolic cost ($J.kg^{-1}.m^{-1}$). The latter has an advantage over the former due to the possibility of a comparison between different running speeds.

The running performance estimation through other physiological factors can be impaired due to the individual's homogeneity (e.g. $\dot{V}O_{2MAX}$) (SAUNDERS et al., 2004). Athletes with similar VO_{2MAX} can present variations in $VO_{2SUBMAX}$, between 15 and 30% (SAUNDERS et al., 2004; WILLIAMS; CAVANAGH, 1987; MAYHEW, 1977). For this reason, RE has been commonly used by coaches and researchers as a safe tool to measure running performance and it is generally used as a dependent variable in biomechanics studies (SAUNDERS et al., 2004; WILLIAMS, 1990; POLLACK, 1977; TARTARUGA et al., 2012).

In addition, numerous factors and interventions seem to influence RE, such as nutritional strategies, physical training and motor education in trained athletes, decreasing energy expenditure at the same running speed (BALSALOBRE-FERNÁNDEZ et al., 2016; HAUSSWIRTH; BRISSWALTER, 2008; SAUNDERS et al., 2006; 2004). Prampero et al. (1993) determined that a 5% improvement in RE would induce a 3.8% improvement in running performance. Hoogkamer et al. (2016), inducing an increase in the metabolic cost of long-distance runners by adding loads as percentages of body mass have noticed a drop in the 3000 m running performance proportional to the mass added, is directly attributable to the decline in RE.

Therefore, using the RE as a performance predictor is more reliable than using other factors, such as VO_{2MAX}. However, it is necessary to be careful about the level of athletes during measurements, as relative speeds can represent different absolute speeds for athletes during a treadmill test, thus requiring different mechanical and energetic demands (TARTARUGA et al., 2012; DANIELS, 1985). As previously mentioned, RE measured as C_{MET} can be a good alternative because it is an independent variable of the running speed in indoor tests (ARELLANO; KRAM, 2014).

C_{MET} is calculated by dividing metabolic power by the speed in $m.s^{-1}$ (PRAMPERO et al., 1986). Net metabolic power ($W \cdot kg^{-1}$) is considered the difference between the VO_2 measured during exercise and the resting VO_2. This value is multiplied by the energy coefficient (20.9 $J.ml^{-1}$), due to the unit measure used in Watts, and divided by the time in seconds (60 s).

Achilles tendon morphological, mechanical and material properties

The human body has the Achilles tendon as the largest and strongest tendon (MAFFULLI; ALMEKINDERS, 2007). Tendons are connective tissues, composed of dense fibrous tissues that connect muscle to bone (NIGG; HERZOG, 1999). They contain 30% collagen, 2% elastin (which characterizes the tendon flexibility) and approximately 68% water (CARDINALE et al., 2010). Besides, they are responsible for transmitting muscle forces to the bone levers of the human body, producing movement and joint stability (KVIST, 1994). All of these elements will structure the Achilles tendon, morphological properties (tendon length, cross-sectional area), and changes in these structures may generate adaptations in the mechanical and material Achilles tendon properties.

Achilles tendon mechanical and material properties can be measured through the force-deformation and *stress-strain* relationship, respectively, which demonstrate the mechanical tendon behavior (SHARMA; MAFFULLI, 2005; MAFFULLI; ALMEKINDERS, 2007). The force-deformation relationship in a quantified way informs the tendon deformation as a function of an applied force. The normalization of this relationship by the tendon dimensions generates the *stress-strain* relationship (SHARMA; MAFFULLI, 2005), which contributes to differentiate the mechanical tendon behavior between species. *Stress* is obtained using the ratio between the Achilles tendon force and the cross-sectional area of this structure and the *strain* is considered the percentage of tendon deformation relative to the tendon length. Tendon stiffness and Young's modulus, therefore, can be obtained respectively by the force-deformation and *stress-strain* relationship.

The evaluation of these tendon mechanical properties can be performed through passive joint mobilizations, measuring the myotendinous junction (MJ) tracking, using ultrasound (US), and estimating the force generated by the tendon by a dynamometer (NAKAMURA et al., 2011; MIZUNO et al ., 2011; ABELLANEDA et al., 2009; MORSE et al., 2008). During isometric muscle contractions, it is also possible to measure these mechanical tendon properties, through the force-deformation and *stress-strain* relationship (MAGNUSSON et al., 2001; GEREMIA et al., 2015). For that, synchronization equipment is required. US was used to track the MJ during isometric contraction in real-time (MAGANARIS et al., 2008). Muscle force is calculated from dynamometry, knowing joint torque and muscle moment arm. However, it is necessary to take some care and make some corrections, because even with the footwell fixed, there may be ankle joint rotations during contraction, overestimating the tendon deformation due to the unwanted plantar flexion movement (MAGNUSSON et al., 2001). Also, the measurement of agonist strength (plantar flexor) can be underestimated, due to antagonistic activation during contraction (MADEMLI; ARAMPATZIS, 2005). Studies perform the agonist torque correction by estimating antagonist torque (GEREMIA et al., 2015; ARYA; KULIG, 2010; MADEMLI; ARAMPATZIS, 2005; MADEMLI et al., 2004), through a direct relationship between antagonistic muscle activation and torque (ARYA; KULIG, 2010). Therefore, longitudinal tendon deformation is measured as a function of the applied agonist muscle strength only.

Studies demonstrate relationships between tendon stiffness and RE through the stretching and shortening cycle and the elastic energy storage and release (ALBRACHT; ARAMPATZIS, 2013; FLETCHER et al., 2010; CARDINALE et al., 2010; ARAMPATZIS et al., 2006; ROBERTS, 2002; CAVAGNA; SAIBENE; MARGARIA, 1964). Arampatzis et al. (2006), for example, found better RE values in long-distance runners with greater tendon stiffness, as well as a greater capacity to store elastic energy. Fletcher et al. (2010) found that the triceps surae tendon stiffness and the energy cost during running seem to suffer inversely and conjunct variations during training periods. Albracht and Arampatzis (2013) observed an increase in tendon stiffness, after a training protocol, and a consequent improvement in RE. Moreover, Monte et al. (2020b) showed that greater tendon work is correlated with better RE.

Kubo et al. (2015; 2010) found in plantar flexors a negative relationship between running performance and tendon stiffness. On the other hand, Ueno et al. (2017) observed that the passive stiffness of the plantar flexor muscle-tendon unit demonstrates a positive relationship with running performance, however, this study demonstrates the muscle as the main responsible for this positive relationship. In addition, Monte et al. (2020a) demonstrated that Achilles tendons with greater lengths at rest were related to the lowest energy cost of running and the best performance of long-distance runners. According to CAVAGNA et al. (1988), a greater tendon length can optimize the elastic energy storage and release compared with shorter Achilles tendons. Then, the muscle work is reduced, as well as their metabolic demands. Kovács et al. (2020) found that Achilles tendon thickness was positively related to the marathon running performance.

Furthermore, it is important to note that tendon stiffness is mainly controlled by adaptations in the tendon morphological (e.g. cross-sectional area) and material properties, obtained by Young's Modulus (BOHM et al., 2015; WIESINGER et al., 2015). These adaptations occur mainly during growth and in initial periods of physical training (GEREMIA et al., 2018; NEUGEBAUER; HAWKINS, 2012; WAUGH et al., 2012). However, other studies have not found significant variations in Young's Modulus values between athletes of different sports and non-athletes (PELTONEN et al., 2010; WIESINGER et al., 2016). Few studies have focused on these issues with RE and running performance.

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CHAPTER III

Determining characteristics of the Achilles tendon properties on physiological parameters and 3000 m running performance

ABSTRACT

Introduction: Tendons play a fundamental role in storing and releasing elastic energy, minimizing metabolic cost (C_{MET}) in distance running. This behavior is related to the tendon's ability to resist deformation (e.g. stiffness), which is controlled by changes in the tendon morphological [cross-sectional area (CSA)], and material (e.g. Young's modulus) properties. However, the relationship between Achilles tendon properties, C_{MET}, and running performance is still uncertain. This study aimed to correlate the Achilles tendon properties, C_{MET} and 3000 m running performance. **Methods:** 7 trained male long-distance runners (31 ± 8 years) participated in this study (Ethics Committee approval number: 2.437.616). Ultrasound was used to determine the Achilles tendon CSA, length, and elongation as a function of plantar flexion torque during voluntary plantar flexion. Tendon force-elongation and *stress-strain* relationships were determined by maximum voluntary isometric contractions on a dynamometer. Then, the maximal incremental test was performed until exhaustion on a treadmill. After 24 hours, C_{MET} was measured in the running economy test (5min) at 12 and 16 km.h^{-1} on a treadmill. After 10 minutes at rest, the 3000 m running performance test on an athletics track was performed. The oxygen uptake was measured by spirometry. Correlations between Achilles tendon properties, C_{MET} (12 and 16 km.h^{-1}), and 3000 m running performance were obtained through Person's test ($p<0.05$). Correlation coefficient was classified as null (0), low (0-0.3), moderate (0.3-0.6), high (0.6-0.9), very high (0.9-1), and perfect (1). **Results:** C_{MET} at 16 km.h^{-1} correlated with CSA (r=-0.834, p=0.02), *stress* (r=0.901, p=0.006) and Young's modulus (r=0.880, p=0.009). Moreover, *stress* also correlated with CSA (r=-0.886, p=0.008), Young's modulus (r=0.878, p=0.009). Tendon stiffness showed a very high correlation with *strain* (r=-0.931, p=0.002). 3000 m running performance correlated with vVO_{2MAX} (r=-0.781, p=0.038). There was no correlation with TL or TL-SL, plantar flexor force, C_{MET} at 12 km.h^{-1}, VO_{2MAX}, first and second ventilatory threshold. **Conclusion:** Therefore, runners with lower Young's modulus, mainly due to greater CSA (related to lower stress) and greater stiffness (related to lower strain), presented better RE at 16 km.h^{-1} due to the greater tendon work in this speed, minimizing the C_{MET}. Moreover, RE at 16 km.h^{-1} is indirectly related to the 3000 m running performance, due to the high correlation between vVO_{2MAX} and 3000 m running performance.

Keywords: Running economy, stiffness, Young's modulus, speed associated with the maximal oxygen consumption, long-distance runners.

INTRODUCTION

Running is a decisive means of locomotion in human evolution (SAIBENE; MINETTI, 2003). During the evolutionary process, adaptations at the organism level were materialized so that human beings could running greater distances with a lower energy cost (SAIBENE; MINETTI, 2003). Thus, energy-saving mechanisms have been developed, which can be represented by the spring-mass model (SAIBENE; MINETTI, 2003). In this model, tendons play a fundamental role, once they store and release elastic energy, allowing more efficient and economical movements (CAVAGNA, 2017), which can improve running performance.

According to the conceptual model proposed by Saunders et al. (2004), running performance is mainly influenced by Running Economy (RE), which can be influenced by secondary factors, such as anthropometric and biomechanics factors. Better RE indicates a lower metabolic cost (C_{MET}) during running at submaximal speeds (FLETCHER; MACINTOSH, 2018; TARTARUGA et al., 2012; CONLEY; KRAHENBUHL, 1980). Plantar flexors tendons appear to have a fundamental role in RE, once 50% of the propulsion energy during running comes from the elastic energy storage and release from these tissues (CAVAGNA; SAIBENE; MARGARIA, 1964). Thus, this behavior can generate a better RE due to lower muscle work and greater tendon work, with less energy expenditure, explaining the greater efficiency of the entire muscle-tendon unit (CARDINALE et al., 2010; CAVAGNA, 2006).

The optimization of the elastic energy storage and release system can influence positively the RE (CARDINALE et al., 2010; ROBERTS, 2002). During running, in the landing phase (e.g., tendon stretching) there is elastic energy storage, while in the propulsion phase there is energy release (CARDINALE et al., 2010). Thus, the tendon stretching capacity seems to be decisive for elastic energy storage. This behavior is related to the tendon stiffness (WIESINGER et al., 2015). Arampatzis et al. (2006), for example, found better RE in long-distance runners with greater stiffness in the triceps surae tendon, as well as a greater capacity to store elastic energy at low speeds.

However, Cavagna (2006) observed that at low and medium running speeds, muscle activation tends to be moderate, causing a fast stretching and slow shortening of the muscles, demonstrating high energy expenditure. At speeds above 14 km.h^{-1} the muscles having greater activation and acting almost isometrically (CAVAGNA, 2006). Thus, the stretch and shortening will be determined by tendons with less energy expenditure (MONTE et al., 2020b; CAVAGNA, 2006). In this case, Monte et al. (2020b) and Cavagna (2006) demonstrate that at high speeds tendon work is greater than at lower speeds, decreasing C_{MET}. However, they do not specify whether different running speeds can influence the relationship between tendon stiffness and RE.

Another important variable is the Young's modulus, which normalizes tendons stiffness and deformation by their initial dimensions. Studies indicate that tendon stiffness is mainly controlled by adaptations in the tendon material properties, obtained by Young's Modulus (WIESINGER et al., 2015; BOHM et al., 2015). These adaptations happen mainly during growth and physical training periods (GEREMIA et al., 2018; NEUGEBAUER; HAWKINS, 2012; WAUGH et al., 2012). However, studies show insignificant variations in Young's Modulus values between athletes from different sports and non-athletes (PELTONEN et al., 2010; WIESINGER et al., 2016), making it difficult to understand their relationships with RE and running performance.

Although the greater tendon stiffness may be related to the best RE (ALBRACHT; ARAMPATZIS, 2013; FLETCHER et al., 2010; CARDINALE et al., 2010; ARAMPATZIS et al., 2006; ROBERTS, 2002; CAVAGNA; SAIBENE; MARGARIA, 1964), we cannot say the same for running performance, once this is not always related to RE. Bragada et al. (2010) demonstrated that the RE is not related to the 3000 m running performance due to the short event duration. Studies that analyzed 5000 m running performance, without evaluating the RE, indicated that the best performance is related to the less tendon stiffness of the plantar flexors, as they store greater elastic energy (KUBO et al., 2015; KUBO et al., 2010). However, athletes with good performance can demonstrate different RE (FOSTER; LUCIA, 2007).

Therefore, this study aimed to correlate the Achilles tendon properties, metabolic cost at 12 and 16 $km.h^{-1}$ and 3000 m running performance in trained long-distance runners.

METHODS

Participants

The study was approved by the Research Ethics Committee of the Federal University of Rio Grande do Sul (number: 2.437.616). 7 trained male long-distance runners (body mass 66.2 ± 9 kg, height 175 ± 8.1 cm and age 31.4 ± 8.1 years), all with 2 years of competitive running experience at least, all having regularly participated in regional and national competition in middle and long-distance events, training volume of at least 30 km $week^{-1}$ and reaching a minimum speed of 19 $km.h^{-1}$ in the incremental test were included in the present study. Individuals with any medical restriction to the performance of maximal tests, any musculoskeletal injury of the lower limb, physical, cognitive and/or psychological limitations to the execution and understanding of the tests were excluded. All participants were informed about the aims of the study, risks and benefits of their participation in the study and the participants gave their written consent to the experimental procedure complying with the rules of the local scientific board.

Experimental Design

All athletes completed two visits. In the first visit, Achilles tendon morphological, mechanical and material properties were collected. Ultrasound was used to determine the Achilles tendon cross-sectional area, length, and elongation as a function of plantar flexion torque during voluntary plantar flexion. Tendon force-elongation and *stress-strain* relationships were determined by maximal voluntary isometric contractions (MVIC) on an isokinetic dynamometer. Then, maximal oxygen uptake ($\dot{V}O_{2MAX}$), first and second ventilatory thresholds (VT_1 and VT_2, respectively) were determined in the maximal incremental test. In the second visit, C_{MET} was measured in submaximal treadmill tests for 5 minutes at 12 and 16 $km.h^{-1}$, approximately 60 and 80% of the maximal speed of the incremental test respectively, considering that all athletes reached 19 km.h^{-1} in this test. After 10 minutes at rest, the runners performed a 3000 m running performance test on an athletics track. The oxygen uptake ($\dot{V}O_2$) was measured using a Quark metabolimeter (Cosmed, Rome, Italy).

Achilles tendon morphological evaluation

Achilles tendon morphological properties were determined from ultrasound (US) images (MURAMATSU et al. 2001; ARYA; KULIG 2010; GEREMIA et al. 2015; GEREMIA et al., 2018). The images were collected at rest in a reference position, hip flexed at 85° (0° = hip fully extended) and the knee fully extended. The ankle axis of rotation was aligned with the dynamometer's axis of rotation (Biodex System 3 Pro, 2.000 Hz, Biodex Medical Systems, USA) and the foot was fixed on it by velcro strips, which were also used to fix the leg on the dynamometer chair and stabilize the trunk and hips. Thus, with the ankle in the neutral position (90º), Achilles tendon morphological properties were assessed by a linear array probe (60 mm; 7.5 MHz) connected to an US system (SSD 4000, 51 Hz, Aloka Inc., Tokyo, Japan).

Tendon length (TL) was obtained with the US probe positioned longitudinally along the tendon. Achilles tendon insertion at the calcaneus (e.g. the most distal point of the tendon on the calcaneus in a sagittal view) was identified on the US images and the respective point was marked on the skin. Afterward, the transducer was moved to the medial gastrocnemius myotendinous junction (MJ), this point also was marked on the skin. The distance between these two points marked on the skin was measured using a metric scale, representing TL at rest (ARYA; KULIG, 2010; GEREMIA et al., 2018). To exclude differences in leg length among subjects, TL was normalized by shank length (TL-SL) (distance from proximal fibula head to the lateral malleolus tip), which was measured using a metric scale (UENO et al., 2017).

Achilles tendon cross-sectional area (CSA) was obtained with the US probe positioned perpendicular to the tendon, with the subject in the prone position. Three CSA images were obtained at 2 cm, 4 cm and 6 cm from the Achilles tendon insertion at the calcaneus (ARYA; KULIG, 2010; GEREMIA et al., 2015; GEREMIA et al., 2018). Achilles tendon CSA was measured five times in each US image and the average value was determined for each position. The average of the three positions was considered as the Achilles tendon CSA (ARYA; KULIG, 2010; GEREMIA et al., 2015; GEREMIA et al., 2018). After this procedure, the Achilles tendon CSA was obtained according to the following equation:

$$CSA = \frac{CSA2cm + CSA4cm + CSA6cm}{3}$$

Equation 1

Equation 1. Calculation of the Achilles tendon cross-sectional area (CSA). Where CSA2cm, CSA4cm and CSA6cm indicate the distance from the Achilles tendon insertion at the calcaneus.

Tendon elongation during plantar flexor ramp contractions

Tendon deformation was obtained during MVICs, with the subjects in the reference position and the ankle in the neutral position. The ramp protocol consists of the subject gradually reaching its maximal torque production. The US probe was fixed to the subject leg, positioned longitudinally to the tendon mechanical axis at the gastrocnemius medialis MJ. A marker was placed on the skin to detect possible movements of the US probe (Figures 1). When any movement was detected, the correction was carried out according to the magnitude of the movement.

The subjects performed three-ramp plantar flexor MVICs for familiarization and tendon pre-conditioning (MAGNUSSON et al., 2001). Then, subjects performed two-ramp flexor MVICs lasting 10 seconds each (MAGNUSSON et al., 2001; GEREMIA et al., 2018), with two minutes interval between contractions. During the MVICs, torque, US images, tibialis anterior electrical activation signal (EMG) and ankle kinematics were reported. US images were recorded by an external DVD recording unit (R130/XAZ, 32 Hz, Samsung Seoul Inc., South Korea) with a sampling frequency of 30 frames per second. The MVIC with the highest peak torque was used for data analysis. A synchronization system (HORITA, Video Stop Watch VS-50; HORITA Company Inc., USA) was used to synchronize torque, US images, EMG and kinematic parameters.

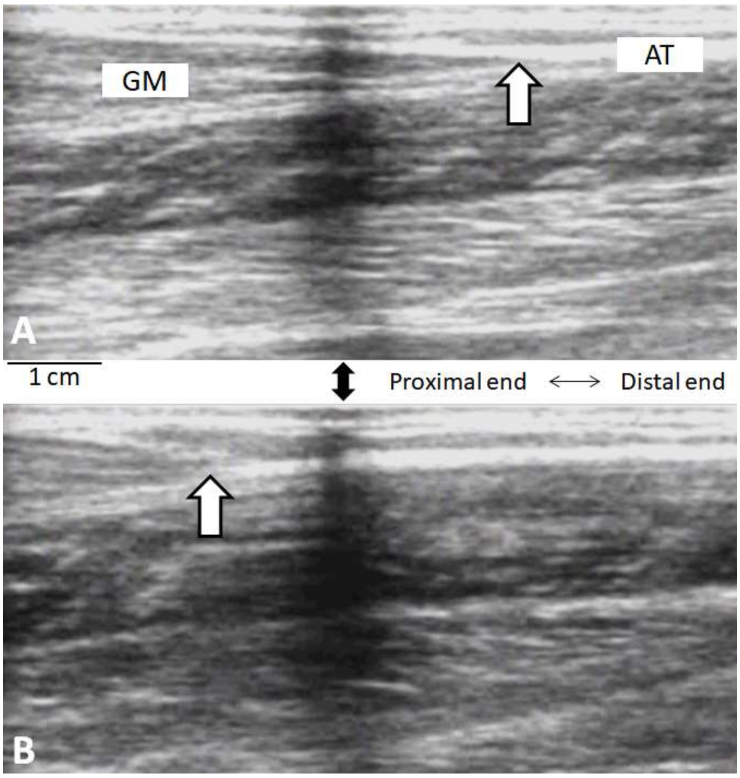

Figure 3. Location of gastrocnemius medialis (GM) myotendinous junction and Achilles tendon (AT). A = resting state, B = 100% of maximal isometric contraction. The white arrow in each scan points to the AT origin. The black double arrow point to the shadow generated by an echo-absorptive marker glued on the skin to identify any displacements of the scanning probe during muscle contraction-relaxation.

Plantar flexor torque correction through muscle electrical activity evaluation

The torque recorded by the dynamometer corresponds to the final torque of the plantar flexion, which is different from the torque produced by the plantar flexors if an antagonistic activation occurs (MAGNUSSON et al., 2001; GEREMIA et al., 2015; GEREMIA et al., 2018). The final torque during plantar flexion is less than the torque achieved by plantar flexors. For this reason, the plantar flexion torque was corrected through the relationship between the tibialis anterior EMG signal and the corresponding dorsiflexor torque (ARYA; KULIG, 2010; WAUGH et al., 2012).

Therefore, tibialis anterior EMG was obtained by surface electrodes (bipolar configuration; 20 mm inter-electrode distance; Ag/AgCl, Meditrace, Kendall, Canada) in three different conditions (MADEMLI et al., 2004;

GEREMIA et al., 2015; GEREMIA et al., 2018): (a) at rest, (b) at a lower and (c) at a higher activation than that produced during the ramp plantarflexion contractions. The EMG signals were amplified (AMT-8, Bortec Biomedical Ltd., Canada), and registered (2000 Hz) simultaneously with the dorsal flexion torque performed on the dynamometer (Windaq data collection system, DATAQ Instruments, Akron, 16-bit, USA). The EMG signals were band-pass filtered (Butterworth, 20-500 Hz), rectified and smoothed with a low-pass filter (Butterworth, 4 Hz). A linear relationship was established between the EMG signal and the obtained dorsiflexor torque at the three different activation levels. Linear regression was performed between the three different values of dorsiflexor torque and activation, allowing to estimate the torque value corresponding to the tibialis anterior co-activation during MVIC (GEREMIA et al., 2015; GEREMIA et al., 2018; MADEMLI; ARAMPATZIS, 2005). From this relation, the co-activation estimated was added to the torque measured by the dynamometer during MVIC, determining only the plantar flexors activation.

Tendon displacement correction through evaluation of ankle joint rotation

Regardless of external fixation during MVICs, joint rotations can occur causing undesired plantarflexion movements, overestimating tendon deformation (MAGNUSSON et al., 2001). Therefore, reflective markers were placed at the middle third of the leg, the malleolus, the hallux, the calcaneus and the upper and lower extremities of the isokinetic dynamometer footplate, to correct this effect. The position of these markers was monitored by a video camera (HDR-CX, 60Hz, Sony, Japan), during MVICs (MURAMATSU et al., 2001), which was synchronized with the isokinetic dynamometer using a LED ligth signal captured by the video camera and a square-wave electric signal triggered into the isokinetic dynamometer allowing the synchronization of the video camera, torque and US images.

A two-segment bi-dimensional planar model (foot = hallux marker, calcaneus marker and malleolus marker; leg = leg marker to malleolus marker) was used to calculate the plantarflexion angle during rest and along MVIC. During passive movements, the Achilles tendon displacement was corrected on the US images the gastrocnemius medialis MJ (MAGNUSSON et al., 2003).

These movements were performed at a constant angular speed of 5 degrees s^{-1} from 90 degrees (tibia perpendicular to the foot line with the knee fully extended) to 125 degrees. Three passive plantarflexion motion cycles were performed for the analysis. If the EMG activity of the gastrocnemius medialis, soleus and tibialis anterior was detected, the cycles were invalidated and repeated.

The gastrocnemius medialis MJ tracking was performed using the SkillSpector software (1.3.2, Video4Coach, Denmark). The MJ displacement was obtained for each joint angle in the three passive movement cycles. Thus, the displacement mean of each angle was used for the analysis. The MJ displacement obtained during MVICs was corrected by subtracting the MJ displacement caused by joint rotation (MURAMATSU et al., 2001; MAGNUSSON et al., 2003).

Achilles tendon moment arm evaluation

According to Kubo, Kanehisa and Fukunaga (2005) and Muraoka et al. (2005), the moment arm (MA) was estimated using the shank length (SL). The subjects were placed in the supine position, with the knees extended, to measure the SL. This parameter was considered as the distance between the proximal fibula head to the lateral malleolus tip. The MA was obtained according to the equation below:

$$MA = SL * 0.11$$

Equation 2

Equation 2. Calculation of the Achilles tendon moment arm (MA), where SL is the shank length of the subjects.

Achilles tendon mechanical and material properties

Achilles tendon force was estimated by the ratio between the corrected plantarflexion torque and Achilles tendon MA. Tendon elongation was obtained from the MJ displacement corrected during the ramp MVIC. Virtual Dub software (Avery Lee, USA) was used to screen the images desired frame by frame. After selecting the images, the MJ displacement was determined by the

SkillSpector software (1.3.2, Video4Coach, Denmark). Achilles tendon force and elongation were estimated at intervals of 10% of MVIC (from 0 to 100%). The slope of the force-elongation curve obtained between 50% and 100% of the MVIC (ARAMPATZIS et al., 2007; BOHM et al., 2014; GEREMIA et al., 2018) was considered as the Achilles tendon stiffness.

Stress was obtained by dividing the Achilles tendon force by the tendon CSA (Equation 3).

$$Stress = \frac{F}{CSA}$$

Equation 3

Equation 3. Stress Calculation, where F is the Achilles tendon force and CSA is the tendon cross-sectional area.

The *strain* was considered as the tendon deformation percentage to the tendon length at rest (Equation 4).

$$Strain = \frac{L_f - L_i * 100}{L_i}$$

Equation 4

Equation 4. Strain Calculation, where Lf is the Achilles tendon final length (length during the ramp MVIC) and Li is the tendon length at rest.

The *stress* and *strain* values were obtained at intervals of 10% of MVIC (from 0 to 100%) (GEREMIA et al., 2018). Young's Modulus was determined as the slope of the *stress-strain* curve between 50% and 100% of the maximal stress (GEREMIA et al., 2018). The slope of these curves between 50% and 100% were obtained by linear regressions.

Maximal Incremental Test

After MVIC, a familiarization was made with the athletes on the treadmill (super ATL, Inbrasport / Inbramed, Porto Alegre, Brazil). The test was started at a speed of 10 km.h^{-1}, and 1 km.h^{-1} was added every minute until exhaustion (BENTLEY et al., 2007). The $\dot{V}O_2$ was measured by the Breath by Breath method using an open-circuit indirect calorimetry system (Cosmed, Quark CPET, Rome, Italy). The $\dot{V}O_2$ analysis was performed by visual inspection using the software PFT ergo (Cosmed, Quark CPET, Rome, Italy). The VO_2 average values were calculated and plotted in the last minute of each speed, to exclude values with four standard deviations above or below the average of the moving windows of the entire curve, an average of three breaths each window (FERNANDES et al., 2012). $\dot{V}O_{2MAX}$ was determined as the highest value analyzed in the last test stage (BENTLEY et al., 2007). VT_1 and VT_2 were determined using the metabolic equivalent method. Therefore, there should be a non-linear increase in ventilation as a function of time and an increase in respiratory equivalents followed by a reduction in expired O_2 pressures. (BENTLEY et al., 2007).

Metabolic Cost

In the second visit, the VO_2 at rest was collected in the standing position for 6 minutes. Then, a 10 minutes warm-up was performed at 10 $km.h^{-1}$. Next, the submaximal tests were performed for 5 minutes at 12 and 16 $km.h^{-1}$ (approximately 60 and 80% of the maximal speed of the incremental test, respectively) and adopting 5 minutes between each test (SAUNDERS et al., 2004). The treadmill speeds were calibrated before tests (Mocap System).The VO_2 curves were analyzed using the software PFT ergo (Cosmed, Quark CPET, Rome, Italy), and the mean VO_2 values were calculated and plotted at the last minute of each speed, where all athletes reach the steady-state. The VO_2 obtained during the C_{MET} test followed a similar gas analysis as described above, using the Breath by Breath method. C_{MET} was calculated by dividing metabolic power (the difference between the exercise VO_2 and the rest VO_2, multiplied by the energy coefficient [20.9 $J.ml^{-1}$] and divided by the time in seconds [60 s]) by the respective speed in $m.s^{-1}$ (PRAMPERO et al., 1986).

3000 m Running Performance

Ten minutes after the RE tests, the athletes performed the 3000 m test on an official athletics track (400 m). Two digital stopwatches (Timex T5K 491Sr/Ti, USA) were used to measure the test time for each athlete. Two researchers were positioned separately at the start and finish line. Besides, the test time was defined by calculating the average between the two evaluators, and all athletes were verbally encouraged to run at their best time.

Statistical Analysis

The data are present as means, standard deviations and confidence intervals (95%). The data normality was performed using the Shapiro-Wilk test. Correlations between Achilles tendon properties, C_{MET} (12 and 16 km.h^{-1}) and 3000 m performance were obtained through Pearson's test ($p<0.05$). Correlation coefficient (r) was classified as null (0), low (0 - 0.3), moderate (0.3 - 0.6), high (0.6 - 0.9), very high (0.9 - 1) and perfect (1) (HOPKINS, 2000). All tests were performed using the software SPSS 20.0 for Windows (IBM, Chicago, EUA), using a significance level of $\alpha < 0.05$.

RESULTS

Table 1 shows the 3000 m running performance and the long-distance runners' physiological parameters of this study.

Table 1. Physiological parameters in mean, standard deviation and confidence interval.

Parameters	Mean	SD (±)	CI (95%)
3000 m performance (s)	646	56.8	603.6 to 687.8
VO_{2MAX} (ml.kg^{-1}.min^{-1})	65	7.2	59.7 to 70.3
vVO_{2MAX} (km.h^{-1})	20	1.9	18.6 to 21.4
VT_1 (ml.kg^{-1}.min^{-1})	37.9	8.1	31.9 to 43.9
VT_2 (ml.kg^{-1}.min^{-1})	54.9	5.7	50.7 to 59.2
C_{MET} 12 (J.kg^{-1}.m^{-1})	3.71	0.32	3.47 to 3.94
C_{MET} 16 (J.kg^{-1}.m^{-1})	3.69	0.15	3.58 to 3.81

VO_{2MAX} – maximal oxygen uptake; vVO_{2MAX} – speed associated with maximal oxygen uptake; VT_1 – first ventilator threshold; VT_2 – second ventilator threshold; C_{MET} 12 – metabolic cost at 12 km.h^{-1}; C_{MET} 16 – metabolic cost at 16 km.h^{-1}. CI – confidence interval.

Table 2 presents Achilles tendon morphological, material and mechanical properties of the long-distance runners of this study.

Table 2. Achilles tendon morphological, material and mechanical properties in mean, standard deviation and confidence interval.

Properties	Mean	SD (±)	CI (95%)
CSA (mm²)	48.7	10.3	41.1 to 56.4
TL-SL (%)	47.3	5.9	43 to 51.7
MA (m)	0.0466	0.0028	0.0445 to 0.0486
Force (N)	3286.5	476.4	2933.5 to 3639.5
Stress (N/mm²)	70.7	20	55.8 to 85.5
Strain (%)	10.7	2.7	8.6 to 12.7
Stiffness (N/mm)	164.3	40.1	134.6 to 194
Young's Modulus (MPa)	769.1	280	561.6 to 976.6

CSA – Cross-sectional area; TL-SL – tendon length normalized with shank length; MA – Achilles tendon moment arm. CI – confidence interval.

C_{MET} at 16 km.h^{-1} showed a high correlation with CSA (r=-0.834, p=0.02), *stress* (r=0.901, p=0.006) and Young's modulus (r=0.880, p=0.009) (Figure 2). Moreover, *stress* also showed a high correlation with CSA (r=-0.886, p=0.008), Young's modulus (r=0.878, p=0.009) (Figure 3).

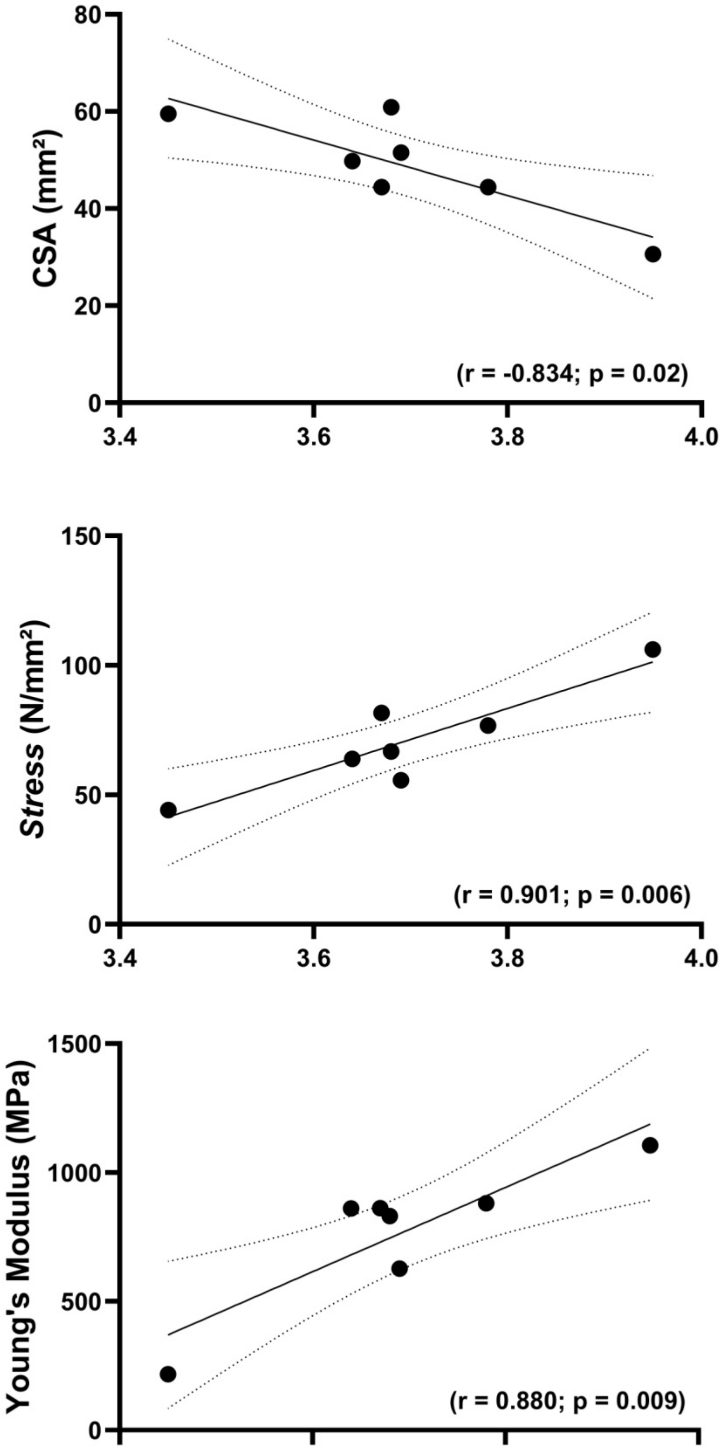

Figure 4. Person's correlation between C_{MET} (Metabolic cost) at 16 km.h^{-1}, CSA (Achilles tendon cross-sectional area), *stress* and Young's modulus. Dashed lines represent the confidence interval (95%).

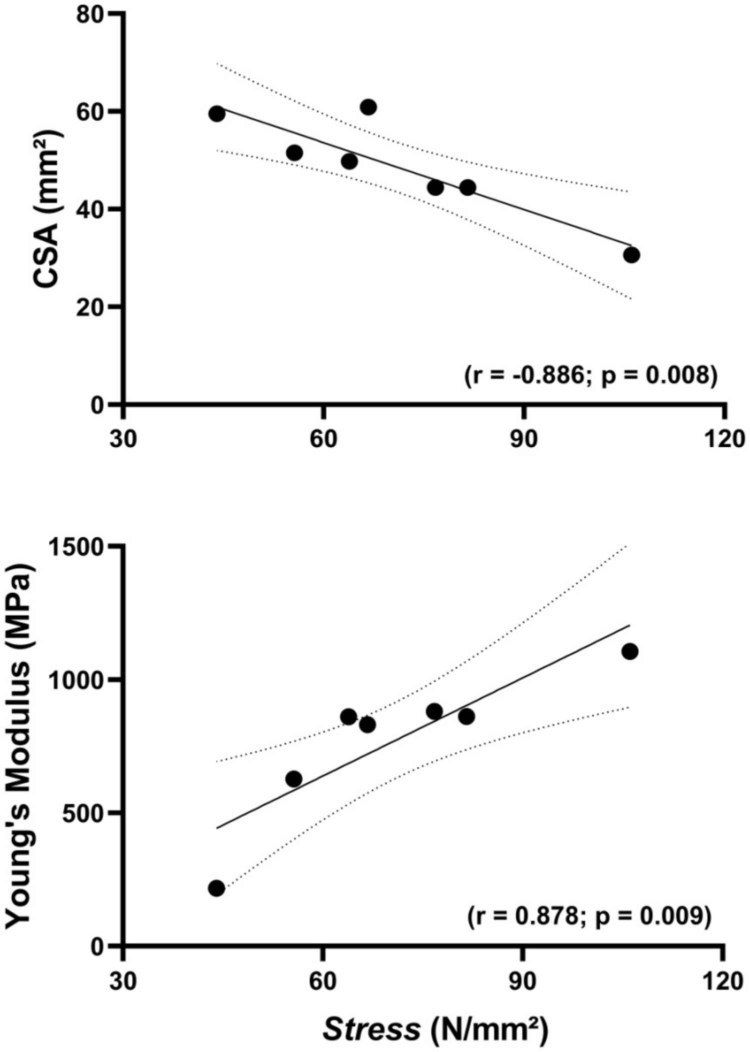

Figure 5. Person's correlation between *stress*, CSA (Achilles tendon cross-sectional area) and Young's modulus. Dashed lines represent the confidence interval (95%).

Finally, 3000 m running performance showed a high correlation with vVO_{2MAX} (r=-0.781, p=0.038).

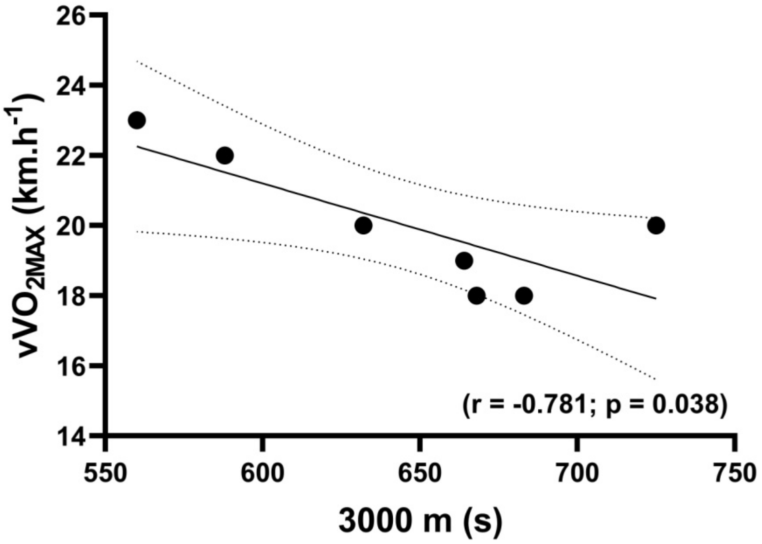

Figure 6. Person's correlation between 3000 m (3000 m running performance) and vVO_{2MAX} (speed at maximal oxygen uptake). Dashed lines represent the confidence interval (95%).

There was no correlation with TL or TL-SL, plantar flexor force, C_{MET} at 12 km.h^{-1}, VO_{2MAX}.

DISCUSSION

This study aimed to correlate the Achilles tendon properties, C_{MET} at 12 and 16 km.h^{-1} and 3000 m running performance in trained long-distance runners. The main findings of this study were that (i) RE at 16 km.h^{-1}, measured as C_{MET}, correlated with CSA, *stress* and Young's modulus; (ii) *stress* correlated with CSA and Young's modulus; (iii) 3000 m running performance correlated with vVO_{2MAX}. There was no correlation with TL or TL-SL, plantar flexor force, C_{MET} at 12 km.h^{-1}, VO_{2MAX}.

C_{MET} correlated with CSA, *stress* and Young's Modulus only at 16 km.h^{-1}. According to Cavagna (2006), above 14 km.h^{-1} of running speed the muscles acting almost isometrically allowing a greater activation. Thus, at high running speeds the muscle-tendon unit stretch and shortening in the lower extremities is determined by tendons (MONTE et al., 2020b; CAVAGNA, 2006). Therefore, long-distance runners at 16 km.h^{-1} probably demonstrated a greater tendon work explaining the correlations between C_{MET} and tendon material and morphological properties. This suggestion agrees with Monte et al. (2020b) that observed a negative correlation between tendon work and metabolic energy, affecting positively the apparent efficiency (AE = W_{TOT}/C_{MET}) of locomotion.

Moreover, Monte et al. (2020a) suggested that greater CSA could be associated with a possible improvement in the capability to store and release elastic *strain* energy, probably due to the higher mechanical resistance in greater CSA tendon, also explaining the increased metabolic economy. This result agrees with the negative correlation between CSA and C_{MET} at 16 km.h^{-1} found in the present study. However, greater CSA can be caused by greater extracellular matrix water content (e.g. edema after training), so we measured the CSA 4 days after the last training session to allow adequate recovery (GEREMIA et al, 2018; BOHM et al., 2014) during the first visit.

Therefore, a greater number of collagen fibers (tendon hypertrophy), main responsible to resist mechanical tension (e.g. stiffness), would decrease Achilles tendon *stress* during running, allowing the runner to decrease the applied force per unit area of the tendon (ROSAGER et al., 2002). Thus, the negative correlation between *stress* and CSA presented in this study agrees with this suggestion. Moreover, it can partly explain the positive correlation between *stress* and C_{MET} at 16 km.h^{-1}. The lower *stress* allows long-distance runners to performer greater distances with lower C_{MET} over a long period (ROSAGER et al., 2002).

In addition, several studies consider tendon *stress* determinant of Young's modulus (LACROIX et al., 2013; WAUGH et al., 2012). Our results are consistent with these previous findings, showing a positive correlation between *stress* and Young’s modulus. Tendon material properties can be altered by variations in collagen fibers amount, extracellular matrix protein, proteoglycan and collagen crimp (MALLIARAS et al., 2013), altering mainly the CSA dimension. Therefore, alterations in CSA dimension and *strain* possibly can explain the lower Young’s modulus in this study, explaining the lower C_{MET} according to our results.

Monte et al. (2020b) observed that tendon *strain* increased as a function of running speed. Thus, at high speeds, tendon elastic *strain* energy storage is most utilized than muscle fiber work (MONTE et al., 2020b; LICHTWARK et al., 2007). The capacity of the tendon to store elastic *strain* energy is related to the tendon stiffness (WIESINGER et al., 2015). Greater tendon stiffness is better to transmit forces to the bones efficiently (CARDINALE et al., 2010), being favorable to long-distance runners who produce submaximal forces for a long period. The present study found a negative correlation between tendon *strain* and stiffness. Analyzing these suggestions, runners with greater tendon stiffness probably present a lower *strain* due to the higher mechanical resistance (WIESINGER et al., 2015; ROSAGER et al., 2002). However, the movement efficiency is better due to the higher forces' transmission (CARDINALE et al., 2010) and, at high speeds, the elastic *strain* energy stored will be higher (MONTE et al., 2020b). Thus, the runners will present greater tendon stiffness and a lower C_{MET}, probably improving the running performance, disagreeing with Kubo et al. (2010) and Kubo et al. (2015). However, the optimal values for stiffness and *strain* at speeds most used by long-distance runners are still unknown.

Contrary to some studies (KUBO et al., 2015; ALBRACHT; ARAMPATZIS, 2013; FLETCHER et al., 2010; KUBO et al., 2010; ARAMPATZIS et al., 2006), our results showed no relationship between tendon stiffness and C_{MET} directly. However, there is an indirect relationship, runners with greater tendon stiffness will present lower C_{MET} due to the lower *strain* even at high speeds, consequently, decreasing Young's modulus.

Moreover, a high correlation between vVO_{2MAX} and 3000 m running performance was found. Lanferdini et al. (2020) showed that vVO_{2MAX} is highly associated with VO_{2MAX} and RE at 16 $km.h^{-1}$. Therefore, there is in the present study an indirect relationship between RE at 16 $km.h^{-1}$, measured as C_{MET}, and 3000 m running performance, disagreeing with Bragada et al. (2010).

CONCLUSION

According to the results presented in this study, RE at 16 $km.h^{-1}$, measured as C_{MET}, showed a high correlation with CSA, *stress* and Young's modulus. Moreover, *stress* correlated directly with CSA and Young's modulus. Tendon *strain* showed a high correlation with tendon stiffness and 3000 m running performance was correlated with vVO_{2MAX}. Therefore, runners with lower Young's modulus presented lower C_{MET} at 16 $km.h^{-1}$. Thus, we can infer that the lower C_{MET} can be achieved by modulations in Young's modulus, directly through CSA (related to the *stress*). In addition, runners with great 3000 m running performance are indirectly related to better RE at 16 $km.h^{-1}$. However, there was no correlation with TL or TL-SL, plantar flexor force, C_{MET} at 12 $km.h^{-1}$, VO_{2MAX}.

CHAPTER IV

GENERAL DISCUSSION

The main findings of this study were that (i) RE at 16 km.h^{-1} correlated with CSA, *stress* and Young's modulus; (ii) *stress* correlated with CSA and Young's modulus; and (iii) 3000 m running performance correlated with vVO_{2MAX}. There was no correlation with TL or TL-SL, plantar flexor force, C_{MET} at 12 km.h^{-1}, VO_{2MAX}.

According, the long-distance runners' profile that produces submaximal forces for a long period during running, greater tendon stiffness can be better to transmit forces to the bones, allowing more economical movements (CAVAGNA, 2017; CARDINALE et al., 2010). Achilles tendon can be made more stiffness mainly by an increase in the number of collagen fibers, increasing the tissue mechanical resistance (WIESINGER et al., 2015; ROSAGER et al., 2002) and probably the CSA. In the present study, we found a negative correlation between stiffness and *strain*, suggesting that runners with Achilles tendon more stiffness would present a lower *strain*. However, tendon *strain* increases as a function of speed (MONTE et al., 2020b). Then, at high speeds, runners with high stiffness and an optimal *strain* would present a greater capacity to store and release elastic energy (MONTE et la., 2020b). Besides, the greater CSA is associated with a possible improvement in the ability to store and release elastic energy (MONTE et al., 2020a), beyond correlating to the *stress* in this study. Thus, *stress* will decrease during the running stride, due to a better body weight distribution per unit area of the tendon (ROSAGER et al., 2020). These results are directly associated with the decrease in Young's modulus values, which consequently will allow runners to present a lower C_{MET} during running, according to the results of this study (Figure 5).

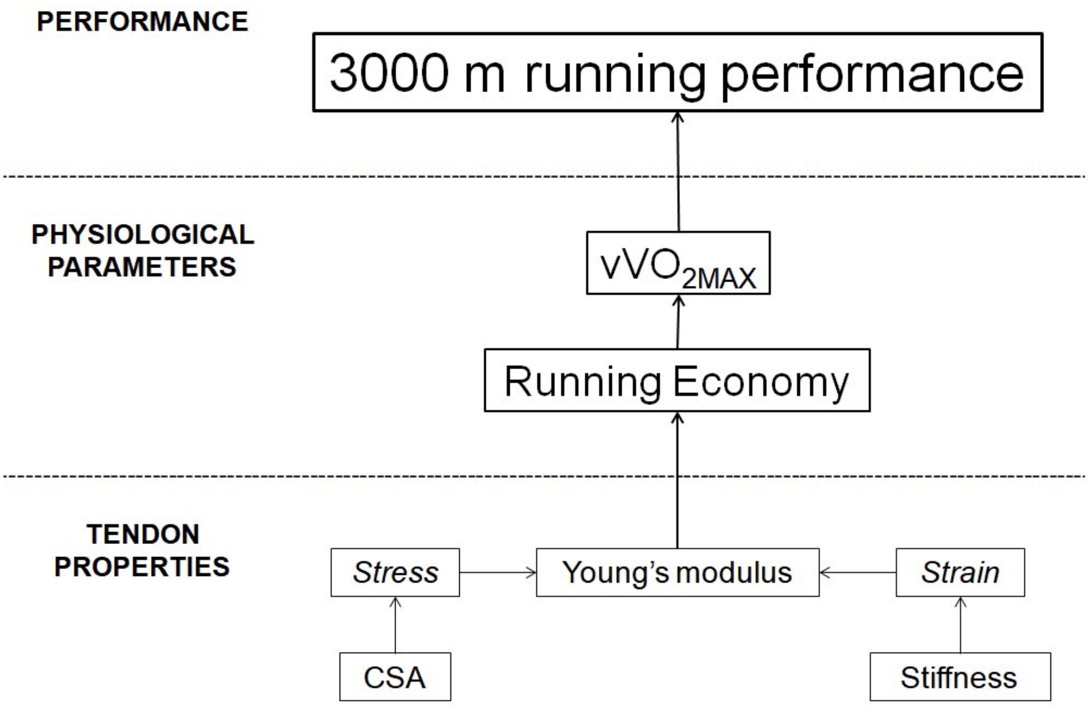

Figure 7. Physiological parameters and Achilles tendon properties affecting 3000 m running performance.

As a practical application, changes in the Achilles tendon mechanical, morphological and material properties through physical training can be an efficient strategy for technicians and trained long-distance runners that seek better running performance (Figure 5). Although Fletcher et al. (2010) did not find significant changes in the Achilles tendon mechanical properties after 8 weeks of isometric training, they observed that tendon stiffness and RE can change acutely and almost together possibly due to the high-intensity training they regularly performed in the days' previously. Geremia et al. (2018), in 12 weeks of high-intensity eccentric training, found an increase in the Achilles tendon stiffness and Young's modulus in the first 4 weeks, and an increase in stiffness, compared to week 4, and CSA after 8 weeks of training. Also, it was suggested that the increase in tendon stiffness was greater than the increase in CSA, therefore, the tendon material properties are mainly responsible for changes in tendon stiffness (GEREMIA et al., 2018; WIESINGER et al., 2015; BOHM et al., 2015). Thus, training will cause changes in the tendon properties and, consequently, in the C_{MET} of the long-distance runners according to our findings.

Moreover, 3000 m running performance presented a high correlation with vVO_{2MAX} in this study. According to Lanferdini et al. (2020), vVO_{2MAX} is determinate by VO_{2MAX} and RE at 16 $km.h^{-1}$. Therefore, RE at 16 $km.h^{-1}$, measured as C_{MET}, has an indirect relationship with 3000 m running performance in the present study (Figure 5). This suggestion agrees with several studies that consider RE the main determinant of the running performance (TARTARUGA et al., 2012; FOSTER; LUCIA, 2007; SAUNDERS et al., 2004; PRAMPERO et al., 1993; CONLEY; KRAHENBUHL, 1980; POLLOCK, 1977) and disagree with Bragada et al. (2010) that demonstrated RE as no related to the 3000 m running performance, due to the short event duration. Therefore, we can infer that changes in tendon properties can make changes in the RE, consequently, in the 3000 m running performance according to Figure 5.

Despite the importance of studying the relationship between tendon properties, RE and running performance in different genders and ages, the present study was carried out only with trained men, seeking to control a series of variables that may have direct implications on the tendon mechanical properties (e.g. tendon structure, hormonal characteristics). However, the number of subjects studied is still a limitation of this study. Therefore, all results presented are speculative. This study should be reproduced with a larger number of subjects, as well as analyzing these correlations at other running speeds on the treadmill and other race distances, such as 5000 m, 10000 m, half marathon and marathon.

GENERAL CONCLUSION

Due to the study limitation, all results obtained are only speculative. The literature review mainly presents conflicting data on the relationship between Achilles tendon mechanical properties and RE, as well as the relationship between RE and 3000 m running performance. Therefore, the results presented suggest that RE is better in long-distance runners with lower Young's modulus, lower *stress* and greater CSA. These results correlated only with C_{MET} at 16 km.h^{-1} possibly due to greater tendon work at this speed. Besides, long-distance runners with greater tendon stiffness had lower tendon *strain*. Runners with higher mechanical resistance (stiffness) perform movements more efficiently and the storage of elastic energy keeps high due to the high running speed, possibly explaining an increase in the metabolic economy. As the *strain* is directly linked to Young's modulus, which is mainly responsible for changes in tendon stiffness, we can infer that tendon stiffness can affect the RE indirectly, due to the relationship between Young's modulus and C_{MET}. Moreover, RE at 16 km.h^{-1} is indirectly related to the 3000 m running performance, due to the high correlation between vVO_{2MAX} and 3000 m running performance in this study. However, there was no correlation with TL or TL-SL, plantar flexor force, C_{MET} at 12 km.h^{-1}, VO_{2MAX}.

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APÊNDICE A - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Título do Projeto:

EFEITO DA TERAPIA DE FOTOBIOMODULAÇÃO SOBRE O DESEMPENHO DE CORREDORES EM DIFERENTES PROVAS DE ATLETISMO

Investigadores: Fábio Juner Lanferdini e Leonardo Alexandre Peyré Tartaruga.

Registro no comitê de ética em pesquisa com humanos da UFRGS (CEP-UFRGS)
Telefone CEP-UFRGS para contato 51- 3308 3738

JUSTIFICATIVA, OS OBJETIVOS E OS PROCEDIMENTOS:

Você está sendo convidado a participar desta pesquisa, que tem por objetivo investigar os efeitos terapia de fotobioestimulação (TFB), que é uma técnica de fototerapia (luz infravermelha) sobre o desempenho nos testes de 400, 800, 3000, 5000 e 10000m, recuperação muscular e economia de corrida (EC) em corredores ou triatletas. A TFB age no sentido de reduzir a fadiga ou cansaço muscular, e a compreensão dos efeitos de ação dessa terapia nos possibilitarão avaliar seu uso tanto na área do esporte de alto rendimento quanto em situações clínicas com o objetivo de reduzir a fadiga muscular.

DESCONFORTOS, RISCOS E BENEFÍCIOS: Toda e qualquer produção científica demanda alguns cuidados e oferta alguns desconfortos e possíveis riscos nesta incursão. Os benefícios oriundos da participação da pesquisa é a oportunidade de entender quais os benefícios da TFB sobre a possibilidade de melhora do desempenho e recuperação muscular. O desconforto que você poderá sentir, está relacionado a intensidade máxima dos exercícios a serem realizados (teste incremental máximo na esteira e testes de 400, 1500, 3000, 5000 e 10000m na pista de atletismo), bem como desconfortos musculares tardios decorrentes dos testes realizados anteriormente. Entre os possíveis desconfortos que você irá sentir durante o exercício, você terá aumento da frequência cardíaca, pressão arterial, maior sudorese, bem como poderá sentir dor muscular e tontura durante os testes. Além disso, após a realização dos testes, a dor muscular pode ser aumentada e com isso poderá sentir dificuldade de locomoção (movimentar os segmentos corporais), devido ao exercício ter sido máximo.

FORMA DE ACOMPANHAMENTO E ASSINTÊNCIA:

Esse termo de consentimento, cuja cópia lhe foi entregue, explica todo o processo que você voluntário passará neste projeto de pesquisa. No primeiro dia de teste, todos os sujeitos deverão fornecer informações pessoais como nome, idade, e serão avaliadas a estatura, a massa corporal, além da aplicação de três questionários. Um questionário será utilizado para avaliar qual o seu membro inferior dominante (perna). Outro questionário será utilizado para coletar dados sobre a idade, massa, estatura, descrição das atividades de vida diária, como tipos e tempo de prática de exercícios realizados durante a semana. Todos os sujeitos realizarão cinco dias de testes, sendo que no primeiro dia será realizado um teste progressivo na esteira ergométrica, ou seja, a esteira aumentará a velocidade a cada minuto até a sua exaustão (até você não conseguir mais suportar o aumento da velocidade correndo, ou seja, o teste será interrompido); após 30 minutos será realizado dois testes de 4 minutos de corrida na esteira a 12 e 16km/h; e finalmente será realizado os testes de 400, 800, 3000, 5000 e 10000m, na pista de atletismo. Após o intervalo mínimo de 72 horas, você realizara a segunda visita ao laboratório onde será explicado quais os testes a serem realizados e após isso será realizado a avaliação da dor muscular nas pernas, bem como da ecografia de músculos das pernas, e saltos verticais; após isso você realizará um aquecimento de 10 minutos na esteira em velocidade controlada (10km/h), em seguida, será realizada a aplicação de TFB ou Placebo com duração de aproximadamente 10 minutos. Imediatamente após, será posicionado sobre a pele na sua perna direita, o medidor de oxigenação muscular (equipamento de tamanho parecido com um celular de 3 polegadas, sem qualquer fio); termografia dos membros inferiores (temperatura da pele com uma câmera com fotografia térmica); Finalmente você será convidado a se deslocar até a pista de atletismo, onde realizará os testes de 400, 800, 3000, 5000 e 10000m, imediatamente após os testes de 400, 800, 3000, 5000 e 10000m, você realizará novamente todos os testes realizados anteriormente no laboratório. No dia seguinte, 24h após os testes de 400, 800, 3000, 5000 e 10000m, você deverá voltar ao laboratório para realizar novamente a avaliação de dor muscular e ecografia de músculos das pernas. A sua quarta visita ao laboratório, será realizada com intervalo mínimo 72h após a segunda e consistira nas mesmas avaliações e testes. Além disso, 24h após os testes de 400, 800, 3000, 5000 e 10000m, da quarta visita, você retornará ao laboratório para uma nova avaliação de dor muscular e ecografia de músculos das pernas. Além do mais, durante as coletas de dados, os pesquisadores serão responsáveis pela prestação do seu socorro em caso de algum incidente por trauma, ou mesmo algum descomforto acontecer durante as avaliações. Desta forma, se necessário os pesquisadores terão telefone em mãos para procurar socorro médico.

Obs. Para a realização dos testes, você não poderá ter qualquer doença cardíaca, pulmonar ou qualquer histórico de lesão musculoesqueléticas a pelo menos dois anos do início do projeto, bem como durante o projeto.

Se você quiser mais detalhes sobre algo mencionado aqui, ou outra informação, sinta-se livre para solicitar. Leia atentamente esse termo, a fim de que você tenha entendido plenamente o objetivo desse projeto, e o seu envolvimento nesse estudo como sujeito participante.

O investigador tem o direito de encerrar o seu envolvimento nesse estudo, caso isso se faça necessário, o que poderá ocorrer se você apresentar qualquer proibição médica à execução de testes máximos ou se você apresentar limitações físicas, cognitivas e/ou psicológicas a execução e/ou compreensão dos testes. Além disso, caso você não siga as recomendações nutricionais, sinta fortes dores musculares ou articulares durante os testes, ou ainda altere suas atividades de vida diária no período das avaliações, o pesquisador pode optar por sua exclusão do estudo. Da mesma forma, você pode retirar o seu consentimento em participar do estudo a qualquer momento se assim o desejar.

Por fim, o Registro alimentar fornecerá dados referentes à sua alimentação nas 48 horas anteriores a realização de cada um dos testes descritos a seguir.

GARANTIA DE ESCLARECIMENTO, LIBERDADE DE RECUSA E GARANTIA DE SIGILO:

Você será esclarecido sobre a pesquisa em qualquer aspecto que desejar. Você é livre para recusar-se a participar, retirar seu consentimento ou interromper a participação a qualquer momento. A sua participação é voluntária e a recusa em participar não irá acarretar qualquer penalidade ou perda de benefícios. Os pesquisadores irão tratar da sua identidade com padrões profissionais de sigilo. Os resultados da pesquisa serão enviados para você e permanecerão confidenciais. Seu nome ou o material que indique a sua participação não serão liberados sem a sua permissão. Durante a realização da pesquisa serão tiradas fotos e gravados vídeos dos procedimentos de avaliação, desta forma, este termo também refere-se ao seu consentimento quanto a liberação e divulgação das imagens e vídeos destas avaliações. Uma cópia deste consentimento informado será arquivada no Laboratório de Pesquisa do Exercício – LAPEX, da Universidade Federal do Rio Grande do Sul - UFRGS e outra será fornecida a você.

Esta pesquisa corresponde e atende as exigências éticas e científicas indicadas na Resolução CNS 466/12 que contém as diretrizes e normas regulamentadoras de pesquisas envolvendo seres humanos. Além disso, os dados coletados nesta pesquisa serão armazenados por no mínimo cinco anos, os quais estarão disponíveis para possíveis esclarecimentos após a realização da pesquisa.

CUSTOS DA PARTICIPAÇÃO, RESSARCIMENTO E INDENIZAÇÃO POR EVENTUAIS DANOS: A participação no estudo não acarretará qualquer custo para você e também não será disponibilizada qualquer compensação financeira adicional. No caso de você sofrer algum dano decorrente dos testes realizados por esta pesquisa, os pesquisadores assumirão as suas responsabilidades e farão o necessário para melhor lhe atender e resolver o dano ocasionado.

A sua assinatura nesse formulário indica que você entendeu satisfatoriamente a informação relativa à sua participação nesse projeto e você concorda em participar como sujeito desta pesquisa, o qual foi informado a você de forma clara e detalhada de qualquer forma de constrangimento e coerção, dos objetivos, da justificativa, dos procedimentos que serei submetido, dos riscos, desconfortos e benefícios. De forma alguma, esse consentimento lhe faz renunciar aos seus direitos legais, e nem libera os investigadores, patrocinadores, ou instituições envolvidas de suas responsabilidades pessoais ou profissionais. A sua participação continuada deve ser tão bem informada quanto o seu consentimento inicial, de modo que você deve se sentir à vontade para solicitar esclarecimentos ou novas informações durante a sua participação. Se tiver qualquer dúvida referente a assuntos relacionados com esta pesquisa, favor entrar em contato com o: Dr. Fábio Juner Lanferdini (Fone: (51) 999883262; email: fabiolanferdini@gmail.com), ou Prof. Dr. Leonardo Alexandre Peyré Tartaruga (Fone: (51) 3308.5817; email: leonardo.tartaruga@ufrgs.br) ou ainda com o Comitê de Ética em Pesquisa da UFRGS [Fone: (51) 3308.3738].

______________________	____/____/____	_______________________
Assinatura do Investigador	Data	Assinatura do Participante