UNIVERSITY OF UDINE

DEPARTMENT OF MEDICAL AND BIOLOGICAL SCIENCES

PhD COURSE IN BIOMEDICAL AND BIOTECHNOLOGICAL SCIENCES

XVIII CYCLE

ENERGETICS AND MECHANICS OF RUNNING:

Metabolic and mechanical changes in ultra-endurance running races and the effects of a specific training on energy cost of running

Supervisors

Prof. Stefano Lazzer

Prof. Alessandro Gasparetto

PhD Student

Nicola Giovanelli

ACADEMIC YEAR 2014/2015

INDEX

CHAPTER I: GENERAL INTRODUCTION

CHAPTER II

PART I: MECHANICAL CHANGES DURING AN ULTRA-ENDURANCE EVENT AND THE EFFECTS OF A SPECIFIC TRAINING ON THE COST OF RUNNING

PART II: DEVELOPMENT OF INSOLE SHOE SENSOR FOR GAIT ANALYSIS: A PILOT STUDY

ABSTRACT

The present thesis is divided into two parts.

Part I: The objectives of the first part were to examine the factors affecting the ultra-endurance performance and in particular which aspects influence the cost of running (Cr). Consequently, we defined how the Cr and running mechanics changed during different types (i.e. level and uphill) of ultra-endurance races. Finally, we proposed a specific training protocol for improving the Cr in high-level ultra-marathoners.

We assessed the Cr by measuring the oxygen consumption at one (or more) fixed speeds using a metabolic unit. Further, for the running mechanics measurement and the spring-mass model parameters computation we used video analysis. Other parameters such as maximal muscle power of the lower limbs (MMP), morphological properties of the gastrocnemius medialis and Achilles tendon stiffness were also measured.

Our studies showed that the maximal oxygen uptake, the fraction of it maintained throughout the race and the Cr are the main physiological parameters affecting the ultra-endurance performance, both in level and uphill competitions. Moreover, low Cr values were related to high MMP, vertical stiffness (k_{vert}), low foot print index (FPI), Achilles tendon stiffness and external work. These results indicate that MMP, k_{vert} and FPI are important factors in determining ultra-endurance performance. Also, our studies reported that during ultra-endurance competitions athletes tend to change their running mechanics after a certain time (~4 hours) rather than after a certain distance covered. Then, by adding strength, explosive and power training to the usual endurance training it is possible to lower the cost of running leading to a better performance.

From these conclusions we suggest new training protocol for the ultra-marathoners including strength, explosive and power training which maintain a correct and less expensive running technique during ultra-endurance events.

Part II: The aim of the second part was to develop and validate a customized thermoplastic polyurethane insole shoe sensor for collecting data about the ground reaction forces (GRF), contact and aerial times. This prototype allowed us to collect vertical GRF and contact time by using piezoresistive force sensors (RFS). Our final model was composed by a rubber insole, five RFSs, an s-beam load cell, an acquisition device (NI myRIO) and a battery case. By using this device we can collect data on field, avoiding the restrictions imposed by the laboratory environment.

LIST OF PUBLICATIONS

I. Lazzer S, Taboga P, Salvadego D, Rejc E, Simunic B, Narici MV, Buglione A, **Giovanelli N**, Antonutto G, Grassi B, Pisot R, di Prampero PE. Factors affecting metabolic cost of transport during a multi-stage running race. J Exp Biol. 2014;217(Pt 5):787-95. doi: 10.1242/jeb.091645. PubMed PMID: 24265425.

II. Lazzer S, Salvadego D, Taboga P, Rejc E, **Giovanelli N**, di Prampero PE. Effects of the Etna uphill ultramarathon on energy cost and mechanics of running. Int J Sports Physiol Perform. 2015;10(2):238-47. doi: 10.1123/ijspp.2014-0057. PubMed PMID: 25117400.

III. **Giovanelli N**, Taboga P, Rejc E, Simunic B, Antonutto G, Lazzer S. Effects of an Uphill Marathon on Running Mechanics and Lower Limb Muscles Fatigue. Int J Sports Physiol Perform. 2015. doi: 10.1123/ijspp.2014-0602. PubMed PMID: 26390075.

IV. **Giovanelli N**, Ortiz ALR, Henninger K, Kram R. Energetics of vertical kilometer foot races; is steeper cheaper? J Appl Physiol (1985). 2016 Feb 1;120(3):370-5. doi: 10.1152/japplphysiol.00546.2015. Epub 2015 Nov 25.

LIST OF UNDER REVISION PAPERS

V. **Giovanelli N**, Taboga P, Rejc E, Lazzer S. Effects of a strength, explosive and plyometric training protocol on ultra-endurance athltes' cost of running. Under revision Eur J Appl Physiol

VI. **Giovanelli N**, Taboga P, Lazzer S. Changes in running mechanics during a six hours running race. Under revision Int J Sports Physiol Perform

OTHER RELATED PUBLICATIONS AND UNPUBLISHED PAPERS

VII. Da Ponte A, **Giovanelli N**, Nigris D, Lazzer S. Effects of hydrogen rich alkaline water on prolonged intermittent exercise. Under revision J Int Soc Sport Nutr.

VIII. Da Ponte A, Antonutto G, **Giovanelli N**, Curcio F, Cortese P, Lazzer S. Effects of an uphill only running marathon on biomarkers of cardiac and muscle damage . In Prep.

CONFERENCE PROCEEDINGS

I. **Giovanelli N**, Taboga P, Rejc E, Simunic B, Antonutto G, Lazzer S. Changes in spring.-mass model parameters and lower limb muscle fatigue during an uphill ultra-marathon. Presentation at the 2015 Rocky Mountain Regional American Society of Biomechanics Meeting. April 17th-18th, 2015. Estes Park, USA.

II. Giovanelli D, **Giovanelli N**, Taboga P, Shojaei Bariuei E, Boscariol P, Vidoni R, Gasparetto A, Lazzer S. A mechatronic system mounted on insole for analyzing human gait. International Conference on Robotiscs and Mechatronics. October 15th-17th, 2014. Tehran, Iran.

III. Lazzer S, Taboga P, Salvadego D, Rejc E, Simunic B, Narici MV, Buglione A, **Giovanelli N**, Antonutto G, Grassi B, Pisot R, di Prampero PE. Factors affecting energy cost of running during an ultra-endurance race. Med Sci Sports Exerc. 2014; 46(5):S733

AWARDS

I. Boscariol P, Gasparetto A, **Giovanelli N**, Lazzer S, Taboga P, Shojaei Barjuei E. Best application “Advanced research” – Forum Tecnologico NIDays 2015. March 12th Milan, Italy

LIST OF ABBREVIATIONS

ΔL	Leg length variation during the stance phase
Δz	Vertical displacement of the center of mass during the stance phase
BW	Body weight
CG	Control group
CoM	Centre of mass
CoT	Cost of transport
Cr	Cost of running
Cr_{vert}	Vertical cost of running
Cw	Cost of walking
Cw_{vert}	Vertical cost of walking
E_{es}	Elastic energy
EG	Experimental group
E_{kf}	Forward kinetic energy
E_p	Potential energy
FPI	Foot print index
GM	Gastrocnemius medialis
GRF	Ground reaction force
HS	Heel strike
Kleg	Leg stiffness
K_{tendon}	Stiffness of the Achilles' tendon
K_{vert}	Vertical stiffness
LT	Lactate threshold
MMP	Maximal muscle power
MS	Mid-stance
MTS	Muscle tendon stiffness
MUM	Mountain ultra-marathon
RE	Running Economy
RFD	Rate of force development
SEP	Strength, endurance and plyometric
Sm	Optimal metabolic transition speed
SMM	Spring-mass model
Ss	Spontaneous transition speed
Ta	Aerial time
Tc	Contact time
TMG	Tensyomyography
TO	Toe off

$\dot{V}CO_2$	Carbon dioxide production
VK	Vertical kilometer
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_2max$	Maximal oxygen uptake
Wext	External work
Wint	Internal work

CHAPTER I

GENERAL INTRODUCTION

Ultra-endurance: from the origins to the present days

The term *ultra-endurance* identifies efforts greater than four (Hawley and Hopkins 1995; Laursen and Rhodes 2001) or six hours (Zaryski and Smith 2005). Several multi-stage cycling events (Tour de France, Giro d'Italia) that took place at the beginning of last century presented stages longer than 300-400 km. However, one of the oldest "official" ultra-endurance competition is the Comrades Marathon (www.comrades.com), which take place in South Africa. It is a point-to-point 90-km running race and the first edition took place in 1921. The racecourse direction is alternated each year between the cities of Durban and Pietermaritzburg. The course record was set in the 2007 edition by the Russian Leonid Shvetsov in 5 hours 20 minutes and 49 seconds.

Ultra-endurance performance is usually achieved by swimming, cycling or running (or the combination of these three specialty, e.g. the Ironman). Our field of interest is the running competition (i.e. ultra-marathons) and we will refer to this type of race throughout the text.

Endurance running is defined as running many kilometers over extended time periods using aerobic metabolism (Bramble and Lieberman 2004). It is a type of locomotion typical of the humans that influenced human evolution. Indeed, humans are the only primates capable of endurance running. Apes and other primates can sprint rapidly, but they do so only for short distances (Bramble and Lieberman 2004). Millions people all over the world regularly jog or run several kilometers a day. Amateur runners can regularly run up to 10 km or even longer distances such as marathons. Recent trends show how races longer than a marathon have become more popular among the running community lately.

Ultra-marathons are footraces longer than the traditional marathon distance (>42.195 km) and they can be divided into two types: those that cover a specified distance (i.e. 50-100 km, in which the ranking depends on the time spent to cover that distance) and those that take place during specified time (i.e. 6-12-24 h, in which the ranking depends on the distance covered). Further, some races are performed on a 200-m (indoor) or 400-m (outdoor) track loop, others are on pavement road and others are on trail or technical terrain (e.g. mountain path). The number of participants in these types of race is rapidly increasing and many "classical" marathoners are eager to race in longer distances. For these reasons, over the last few years the interest of different research groups has been directed to better understand the factors that affect the ultra-endurance performance (Gimenez et al. 2013; Hoffman 2014; Lazzer et al. 2012; Martin et al. 2010; Millet 2011; Millet et al. 2011a; Morin et al. 2011a; Rejc et al. 2010; Schena et al. 2014; Vernillo et al. 2015a).

Factors affecting ultra-endurance performance

Lazzer et al. (2012) reported that the main physiological factors determining the ultra-endurance performance during a multi-stage running race were the maximal oxygen uptake ($\dot{V}O_2max$), the fraction of it sustained throughout the race (F) and the cost of running (Cr) (Fig. 1.1).

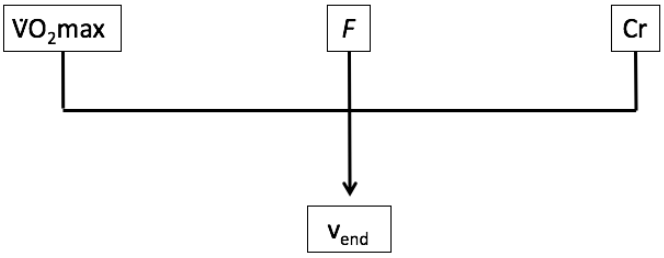


Fig. 1.1 – Factors related to distance running performance. $\dot{V}O_2max$: maximal oxygen uptake; F: % of $\dot{V}O_2max$ maintained during the race; Cr: cost of running; v_{end}: speed in endurance running.

Specifically, the combination of these three components explains 87% of the total competition time variance. As described previously (di Prampero et al. 1986) the maximal theoretical speed in endurance running (v_{end}) can be predicted by the equation

$$v_{end} = \dot{V}O_2max \cdot F \cdot Cr^{-1} \qquad (1)$$

Consequently, if athletes want to improve their performance they should focus the training on improving one or more of these parameters. $\dot{V}O_2max$ and F can be enhanced by specific running or cycling training (Laursen et al. 2002; Midgley et al. 2006), whereas the Cr can be improved by including various forms of training (Billat et al. 1999; Enoksen et al. 2011; Hoff et al. 2002; Hoff et al. 1999; Ronnestad et al. 2012; Ronnestad and Mujika 2014; Spurrs et al. 2003; Storen et al. 2008; Turner et al. 2003).

Maximal oxygen uptake

Maximal oxygen uptake is defined as the highest rate at which oxygen can be taken up and utilized by the body during severe exercise (Bassett and Howley 2000). Potential factors limiting $\dot{V}O_2max$ operate along the route from the air to the mitochondria, where the oxygen is used. They include the pulmonary diffusion capacity, the maximal cardiac output, the blood capacity to transport oxygen and/or the muscle's capacity to consume oxygen (Bassett and Howley 2000) (Fig. 1.2).

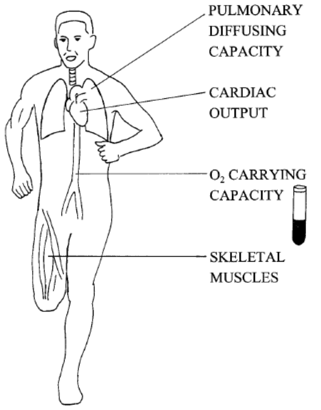


Fig. 1.2 – Physiological factors limiting $\dot{V}O_2max$ during exercise in human. From Bassett and Howley (2000)

The *maximal cardiac output* is considered the main limiting factor, affecting the $\dot{V}O_2max$ by 70-85%. Since during maximum exercise almost all the available O_2 is extracted from the red blood cells as they perfuse the active muscles, it follows that the mechanism for the increase in $\dot{V}O_2max$ is by increasing the blood flow (and O_2 delivery) (Bassett and Howley 2000).

The *oxygen carrying capacity* can be enhanced by increasing the hemoglobin content of the blood (Ekblom et al. 1976). It can be induced by specific training (i.e. training in altitude) or as a consequence of unethical and illegal doping activity. Indeed, reinfusion of 900-1,350 mL blood leads to an increase in $\dot{V}O_2max$ by 4-9% (Gledhill 1982; Gledhill 1985).

The *pulmonary system* may limit $\dot{V}O_2max$ under certain circumstances, but in "normal" conditions (e.g. sea level, healthy subjects…) it is not the most important factor in determining the $\dot{V}O_2max$. Authors (Dempsey et al. 1984) demonstrated that elite athletes with high maximal cardiac output (~40 L/min) can be penalized by the lower transit time of the red blood cell in the pulmonary capillary. This leads to a lower time to saturate the blood with O_2 before it exits the pulmonary capillary net.

Other limitations can be detected in the *skeletal muscles*. Peripheral diffusion gradients, mitochondrial enzyme levels and capillary density have a role in limiting the $\dot{V}O_2$max. However, when limiting factors for $\dot{V}O_2$max are discussed, there is agreement in saying that it is mainly the ability of the cardiorespiratory system (i.e. heart, lungs and blood) to transport O_2 to the muscles, not the ability of muscle mitochondria to consume O_2 that limits $\dot{V}O_2$max (Bassett and Howley 2000).

The fraction of maximum oxygen uptake

Another important factor in determining the performance in ultra-endurance race is the fraction of $\dot{V}O_2$max maintained during the competition. It decreases with increasing the exercise duration and it is strongly dependent from the training status of the athlete. Subjects are able to sustain the 100% of $\dot{V}O_2$max for ~6 minutes (Billat et al. 1994; Renoux et al. 2000) while it is much lower when they perform a longer exercise. Indeed, ultra-marathoners may be able to sustain a running intensity of 88% of $\dot{V}O_2$max for 1 hour, 66% $\dot{V}O_2$max for 8 hours and 39 – 47% for 24 hours (Davies and Thompson 1979; Gimenez et al. 2013; Millet et al. 2011a). Conversely to $\dot{V}O_2$max, which increases during the first 2 months of training and then it stabilizes, *F* continues to change over time (Bassett and Howley 2000).

The energy cost of walking and running

The cost of transport (CoT) is determined by measuring the steady-state oxygen consumption ($\dot{V}O_2$, in ml/kg m^{-1}) and the respiratory exchange ratio (RER) at a fixed speed or power. CoT can be expressed as joule or kilojoule per distance covered, or in mlO_2 used per unit distance and it is lower in trained athletes. Moreover, many studies used the term running economy (RE) as synonymous with oxygen cost of running (Lacour and Bourdin 2015). It can be applied to different actions (cycling, swimming, rowing, walking, running, etc.). However, we will focus on the cost of walking (Cw) and running (Cr).

It has been reported that the importance of Cr is higher in determining the performance when athletes with similar $\dot{V}O_2$max are compared and its relevance increases in longer races (Horowitz et al. 1994). The Cr is affected by several factors (Fig. 1.3), nonetheless in the present thesis we will discuss only few of them.

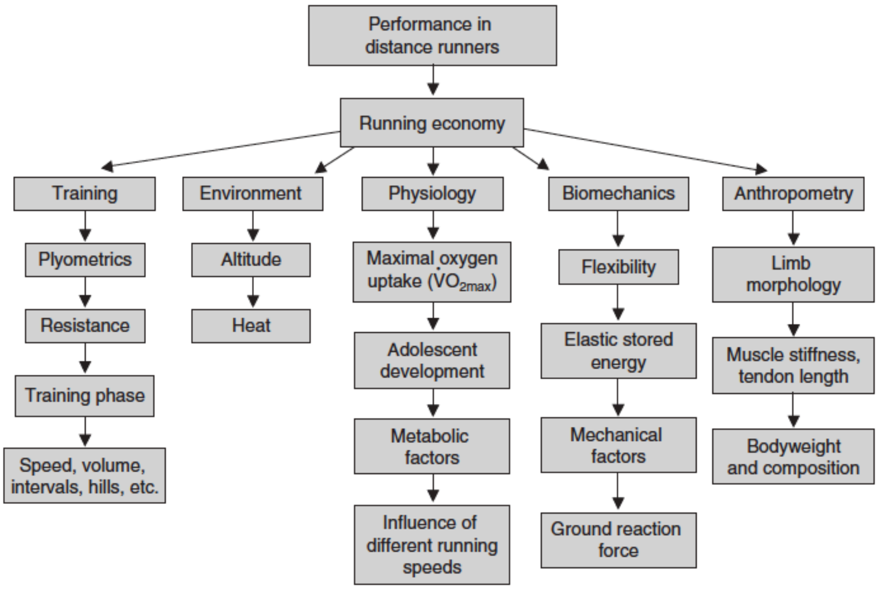


Fig. 1.3 – Factors affecting running economy. From Saunders et al. (2004)

The metabolic cost of walking for humans, like that of other mammals, is a “U”-shaped curve, in which optimal speed is ~1.3 m·s^{-1} (Abe et al. 2015; Minetti et al. 1994b). On level ground or treadmills, at speeds slower than ~2 m·s^{-1}, walking requires less metabolic energy than running (di Prampero 1986; Minetti et al. 1994b). From ~2 m·s^{-1} running is the preferred gait. However, the speed at which subjects decide to switch from walking to running (preferred transition speed) does not always correspond with the optimal metabolic transition speed (Mercier et al. 1994) (Fig. 1.4).

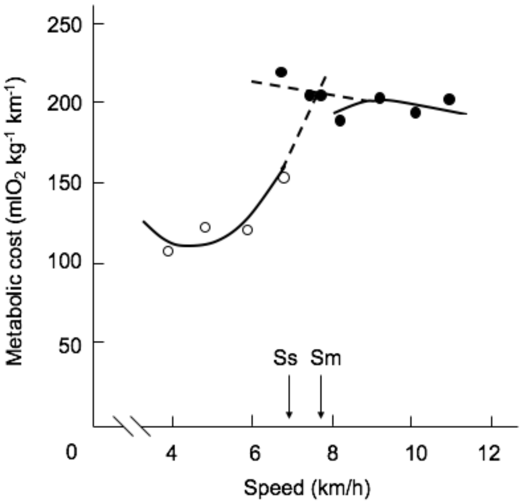


Fig. 1.4 – The optimal metabolic transition speeds (Sm) and spontaneous transition speed (Ss) do not always overlap. Adapted from Minetti et al. (1994b).

The difference between cost of walking and running is generally attributed to the more effective inverted pendulum-like exchange of mechanical energy at slower walking speeds and the superior elastic energy storage and recovery of running at faster speeds (see the “Walking and running mechanics” paragraph) (Farley and Ferris 1998) (Fig. 1.5). Indeed, at higher speeds running is less costly than walking because the exchange of kinetics and potential energy is in phase and a spring-mass mechanism is involved. Tendons and ligaments in the leg store elastic strain energy during the initial, braking part of the support phase, and then release the energy during the subsequent propulsive phase (Cavagna et al. 1976).

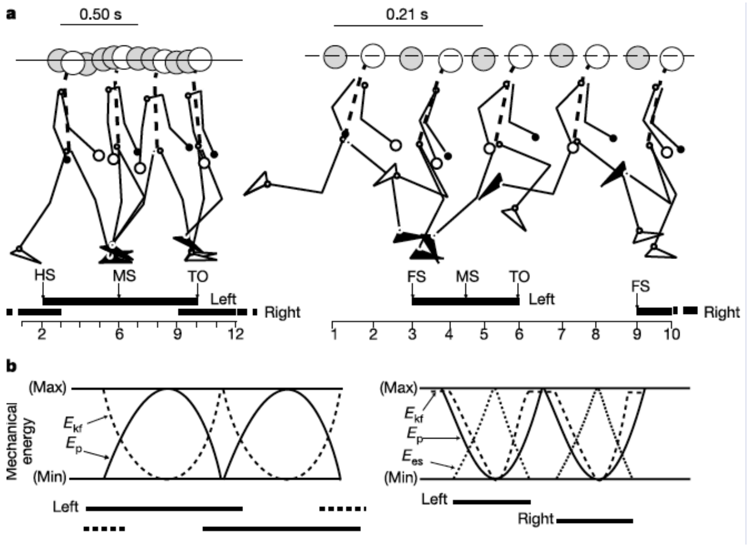


Fig. 1.5 – Comparison of walking and running. **a**, kinematics of walking (left) and running (right). **b,** biomechanical contrasts between human gaits. HS: heel strike; TO: toe off; MS: mid-stance; E_{kf}: forward kinetic energy; E_p: potential energy; E_{es}: elastic energy. Adapted from Bramble and Lieberman (2004).

Conversely to walking, it is widely accepted that the metabolic rate during running increases linearly as a function of the speed. Thus, it has been proposed that Cr is independent from the running speed (di Prampero et al. 1986; di Prampero et al. 2009; Margaria et al. 1963) (Fig. 1.6).

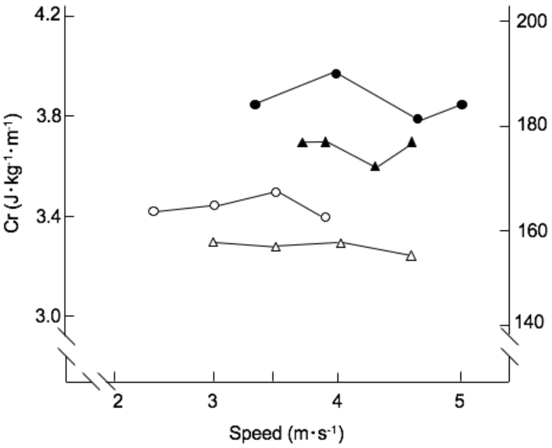


Fig. 1.6 - Energy cost of running as a function of treadmill speed in four subjects. Adapted from di Prampero et al. (1986).

Indeed, researchers have suggested that the amount of energy used to run a given distance is (nearly) the same, independently from the speed (Kram and Taylor 1990; Margaria et al. 1963). Conversely, more recently some authors have sustained that there is an optimal running speed (Steudel-Numbers and Wall-Scheffler 2009) which can derive from an evolutionary selection (Fig. 1.7).

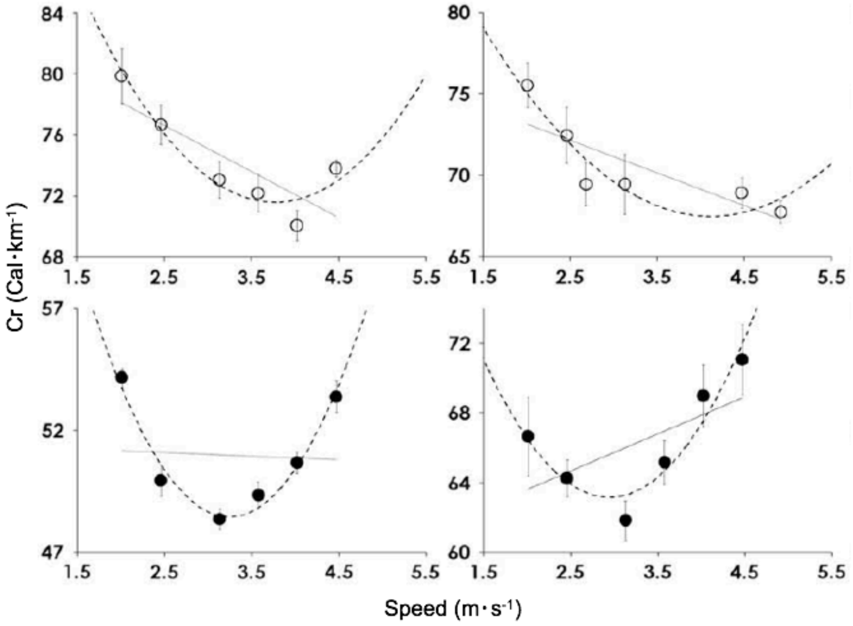


Fig. 1.7 - Individual's Cr with both a linear and curvilinear line fit. Open circles are males, closed circles are females. Error bars are the standard error of mean values averaged over the trials at each speed. Adapted from Steudel-Numbers and Wall-Scheffler (2009).

Cost of running and ultra-endurance

It is reported that during a marathon race the Cr at a fixed speed increases by ~5% (Brueckner et al. 1991). During ultra-marathons different results are reported. Most of authors showed that Cr increases with the distance covered in ultra-endurance performance (Gimenez et al. 2013; Lazzer et al. 2012; Millet et al. 2009). Gimenez et al. (2013) reported that Cr increases after 8 hours of treadmill run compared with the PRE and this increment may be explained by peripheral muscular alterations. In particular, authors (Fernstrom et al. 2007) demonstrated that the reduction of mitochondrial efficiency might explain the increased oxygen cost. Also, changes in muscle activation, or changes in running mechanics pattern could affect the Cr (Morin et al. 2011b). Moreover, Lazzer et al. (2012) showed increased Cr during a multi-day competition by ~18% after the third day of race suggesting that Cr, along with $\dot{V}O_2$max and *F,* explains 87% of the variance in the total race time. Conversely to previous works, Vernillo et al. (Vernillo et al. 2015b; Vernillo et al. 2014) showed lower Cr after mountain ultra-marathons. These authors reported unchanged or lower uphill Cr after 65-km and 330-km trail running competitions. Also, Cr on level did not change in both races. Authors explain the unchanged Cr by a greater eccentric contribution during level as compared with uphill running. They hypothesize that the return of elastic energy in the concentric phase of the stretch-shortening cycle may have compensated for any deficit in the force-generating capacity after the race and resulted in unchanged level-running steps mechanics that probably did not affect the level Cr (Vernillo et al. 2015b).

How can the cost of running be improved?

Several authors reported that different types of training can positively affect the Cr (Barnes and Kilding 2015) (Fig. 1.8) but most studies deal with different approaches to resistance training.

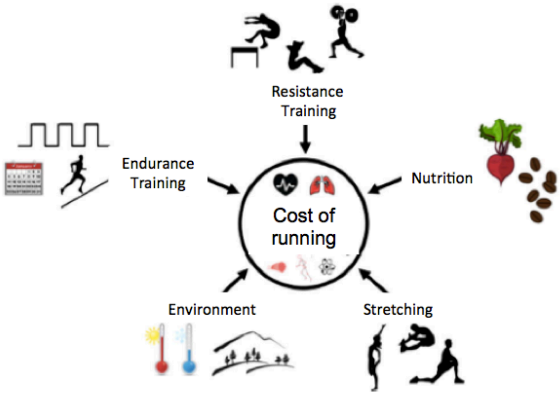


Fig. 1.8 – Schematic of strategies to improve running economy. Adapted from Barnes and Kilding (2015).

Indeed, strength, explosive and plyometric (SEP) training positively affect short (<15 minutes) and long (>15 minutes) endurance performance in different types of subjects and sport situations (Aagaard et al. 2011; Hakkinen et al. 2003; Hoff et al. 2002; Hoff et al. 1999; Millet et al. 2002; Paavolainen et al. 1999; Ronnestad et al. 2012; Ronnestad and Mujika 2014; Spurrs et al. 2003; Storen et al. 2008; Sunde et al. 2010; Turner et al. 2003). Moreover, lower values of Cr in trained runners were related with higher values of MMP and vertical stiffness supporting previous studies that underlined the role of muscle-tendon complex stiffness (MTS) in storing and releasing elastic energy and the importance of a good ankle stability (Spurrs et al. 2003). SEP training affects the performance without affecting $\dot{V}O_2$max, lactate threshold and body weight (Hoff et al. 1999; Millet et al. 2002; Spurrs et al. 2003; Storen et al. 2008; Turner et al. 2003). Indeed, adding strength training to an athlete's usual endurance training may improve endurance performance by acting on exercise economy, anaerobic capacity, maximal speed and reducing the fatigue effects (Ronnestad and Mujika 2014). Mechanisms involved in improving performance consequently of concurrent strength and endurance training include an increased proportion of more fatigue-resistant type IIA fibers and reduced proportion of type IIX fibers (Aagaard et al. 2011). This adaptation results in an increased maximal muscle strength and RFD without any change in body mass nor in muscle fiber size (Aagaard et al. 2011). Also, in activity where the stretch-shortening cycle is involved and the storage and return of elastic energy plays an important role such as running, explosive strength training (Spurrs et al. 2003) and heavy strength training (Millet et al. 2002) added to normal endurance training can increase the MTS, which may improve the storage and release of elastic energy (Spurrs et al. 2003).

Walking and running mechanics

The gait cycle

The gait cycle begins when one foot touch the ground and ends when the same foot contacts the ground again. These moments are referred to as initial contact (or heel strike, HS). Stance ends when the foot is no longer in contact with the ground. Toe off (TO) marks the beginning of the swing phase of the gait cycle. The stance phase in walking is longer than 50%. This part of the gait cycle is referred as duty factor (i.e. the percent of the total cycle which a given foot is on the ground). In walking there are two periods of double support whilst in running *usually* there are no periods when both feet are in contact with the ground (Novacheck 1998) (Fig. 1.5). However, distinguish walking and running just from the presence or not of the aerial phase is not always correct. Indeed, more appropriate is to differentiate running from walking by analyzing the center of mass (CoM) trajectory.

The CoM trajectory and the spring-mass model

In walking, the CoM reaches its lowest point near toe-off and highest at mid-stance (MS) where the leg is relatively straight. During running, the CoM reaches its highest point during the aerial phase and lowest at MS, when the hip, knee and ankle are flexed (Bramble and Lieberman 2004). This latest definition is true also when humans run without an aerial phase. Indeed, McMahon first defined this running pattern as “Groucho running” (McMahon et al. 1987) whereas Rubenson et al. (2004) named it “grounded running”. Groucho (or grounded) running requires increased knee flexion. In this posture, contact time increases until the aerial phase disappears entirely. However, with this running pattern, CoM continues to reach its lowest height near MS and the bouncing gait is present, as is the case with “normal” running.

Video analysis and force plate can be used to investigate walking and running mechanics. Indeed, with these devices is possible to compute the total work done during gait. In pioneering age (Fenn 1930) studied first a model in which the total work (W_{tot}) done to move the body during walking and running is the sum of the external work, done to accelerate and lift the CoM (W_{ext}), and the internal work, done to accelerate the limbs relatively to the CoM (W_{int}). The positive work done by the muscles derives from the chemical energy transformed by their contractile machinery and the mechanical energy stored in the muscles and tendons complex. The ratio of the positive work done by the muscles to the chemical energy used (e.g. efficiency) gives an indication of the role of the elastic energy returned during locomotion. Indeed, values greater than 0.25 (which is the efficiency of the concentric contraction) indicate that part of the positive work is “free of cost” and it is permitted by elastic elements stretched by some external force during a preceding phase of negative work. Cavagna and Kaneko (1977) reported that the efficiency during walking is always lower than during running and it reaches a maximum (0.35-0.40) at intermediate speeds. Conversely, during running it increases linearly with speed, attaining values of ~0.50.

The different efficiency between walking and running can be explained by the different mechanism involved in the two gaits. The difference in phase of potential and kinetic energy in walking (Fig. 1.5) suggests that the mechanism of walking is similar to that of a “rolling egg” or a pendulum (Cavagna et al. 1976). Indeed, during walking an inverted pendulum mechanism exchanges forward kinetic energy (E_{kf}) for gravitational potential energy (E_p) between heelstrike (HS) and MS; the exchange is reversed between MS and TO. Conversely, in running, the potential and kinetic energy are in phase, as in a “bouncing ball”. Leg tendons and ligaments partially store the elastic strain energy (E_{es}) derived from the decrease of E_p and E_{kf} during the first half of the stance, which is subsequently returned between MS and TO (Bramble and Lieberman 2004). The bouncing ball model was proposed by Cavagna et al. (1964) and more recently it was developed by McMahon and Cheng (1990) into the spring-mass model. In this model the legs can be described as springs loaded by the runner’s body mass (Blickhan 1989). The main mechanical parameter studied when using the spring-mass model is the stiffness of the leg spring (k_{leg}), which is defined as the ratio of the maximal force and the leg deformation (ΔL) at the middle of the stance phase. Also, the vertical stiffness (k_{vert}) is calculated by the ratio of the maximal force and the displacement of CoM (Δz) at the middle of the stance phase (Farley and Gonzalez 1996; McMahon and Cheng 1990). Measurement of the maximal ground reaction forces (Fmax) and Δz during running required expensive equipment (i.e. force plate, video-motion analysis). However, Morin et al. (2005) validated a method for assessing leg and vertical stiffness during running. Given athlete’s leg length (L, in m), body mass (BM, in kg), contact and aerial time (t_c and t_a, in s) and running velocity (v, $m \cdot s^{-1}$) k_{vert} (in $kN \cdot m^{-1}$) can be calculated as follows:

$$k_{vert} = \frac{F_{max}}{\Delta z} \tag{2}$$

with

$$F_{max} = BMg\ \frac{\pi}{2}(\frac{t_a}{t_c} + 1) \tag{3}$$

and

$$\Delta \mathrm{z} = -\frac{F_{max}}{m}\frac{t_c^2}{\pi^2} + g\,\frac{t_c^2}{8} \tag{4}$$

then, k_{leg} (in kN·m^{-1}) can be calculated as:

$$k_{leg} = \frac{F_{max}}{\Delta L} \tag{5}$$

with

$$\Delta L = L - \sqrt{L^2 - \left(\frac{vt_c}{2}\right)^2} + \Delta z \tag{6}$$

Ground reaction forces

Ground reaction forces (GRF) are used to quantify impacts, understand propulsion and braking, compute muscle forces, and calculate mechanical energy fluctuations (Gottschall and Kram 2005). These forces are dependent from the running speed, running pattern, characteristics and inclination of the terrain. For instance, during uphill and downhill running GRF behaves in different way compared to level running (see "*Uphill and downhill running*" paragraph).

GRF have three components: vertical, horizontal and mediolateral. The vertical component presents two peaks in rear-foot strikers while in mid-foot and fore-foot strikers there is only an active peak. At a moderate pace of 3 m·s^{-1}, for runners who land on their rear-foot, the vertical component of the GRF quickly rises and falls, forming the impact peak (~1.6 body weight). The vertical component then more slowly increases to a second peak at mid-stance, termed the active peak (~2.5 BW), before decreasing prior to toe-off. The horizontal component is negative at foot strike, since a braking force is applied reaching a minimum (~0.3 BW) at about one-quarter of stance time before decreasing in magnitude and becoming positive during the propulsion phase prior to toe-off (Fig. 1.9)

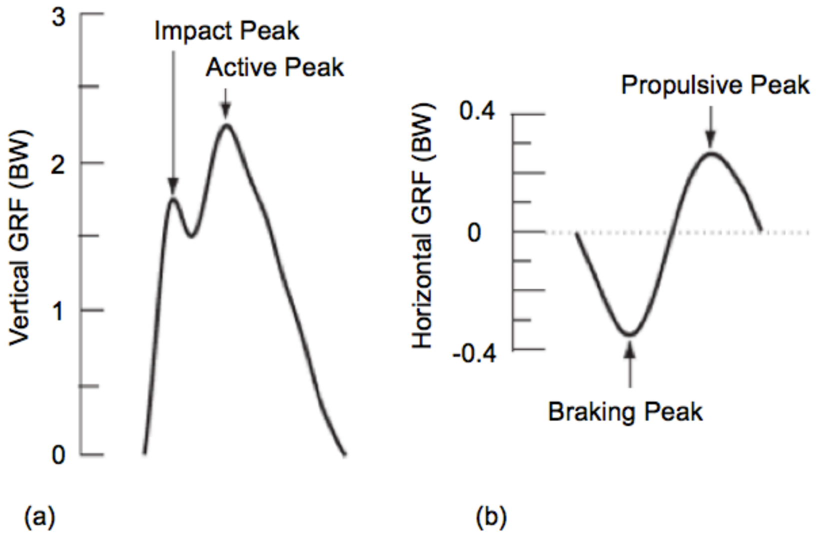


Fig. 1.9 - Typical traces of the vertical (a) and horizontal (b) GRF during level running at 3 m·s^{-1}. Adapted from Gottschall and Kram (2005).

The mediolateral component is usually small compared to the vertical and anteroposterior components. Indeed, it equals to 9% of the peak vertical component and 26% of the horizontal component (Cavanagh and Lafortune 1980).

Running mechanics in ultra-endurance events: does it change?

In the latest years many researchers attempted to clarify how the running biomechanics change during long-lasting events. Few years ago authors (Millet et al. 2009) examined the physiological and biomechanical changes occurring in a subject after running 8,500 km in 161 days. They reported that contact time (t_c) did not change but there was a tendency toward a "smoother" running pattern with higher step frequency (SF) and duty factor (DF) with lower aerial time (t_a), maximal vertical GRF (Fmax) and loading rate at impact. In the following years different groups have studied the ultra-endurance performance in different situations. All studies agree that Fmax decreases with the distance covered but there is not agreement about other biomechanical parameters' behavior. Indeed, some authors (Degache et al. 2013; Morin et al. 2011a) reported similar changes both after a 5-hour hilly running and 24-hour treadmill running. Indeed, they showed an increase in SF caused by a decrease in t_c with constant ta. Further, spring-mass parameters (Δz and ΔL) decreased significantly. Consequently, vertical and leg stiffness increased. After a 160 km mountain ultra marathon (MUM) authors reported a similar trend but in this event ta decreased (Morin et al. 2011b). Also, Vernillo et al. (2014) studied the world's most challenging MUM (Tor des Geants: 330

km, 24,000 m elevation gain) on energetics and mechanics of running in two conditions (level running at 9 km/h and uphill running at 6 km/h with an inclination of 15%). No differences were reported during the level-running condition while in uphill running t_c and DF increased. Similar results were reported after a 65 km MUM with higher tc, DF and SF and shorter stride length (SL) (Vernillo et al. 2015b).

Differences among these studies may be in part explained by the different experimental design (treadmill vs. overground running) and different racecourses (distance, flat or with elevation gain/loss).

Relationships between Cr and mechanical parameters

Stride frequency and stride length

As speed is the product of SF and SL, a wide range combinations is possible for a given speed. Usually runners adopt a SF which is very close to the energetically optimal SF (i.e. less expensive) (Cavanagh and Williams 1982), which is between 85 and 90 strides $\cdot \text{min}^{-1}$ for a range of speeds below 6 $\text{m} \cdot \text{s}^{-1}$ (Cavanagh and Kram 1989; Cavanagh and Williams 1982). Cavanagh and Kram (1989) reported that as speed increased in the range of 3.15-4.12 $\text{m} \cdot \text{s}^{-1}$, SF remained nearly constant (+4%) while SL increased by 28%. Runners adjust their SL in order to minimize the metabolic Cr when running speed changes.

Vertical stiffness

Authors (Heise and Martin 1998) found inverse relationships between vertical stiffness (k_{vert}) and the Cr, suggesting that higher stiffness led to lower Cr. Thus, changes in stiffness with fatigue may help to explain increased metabolic cost in endurance and ultra-endurance running. Stiffness coefficients play a role in determining the final performance in running. Indeed, it is reported that higher k_{vert} corresponds to lower Cr (Dalleau et al. 1998). Thus, greater plantar flexor muscle strength and greater tendon-aponeurosis stiffness in the triceps surae lead to lower Cr (Arampatzis et al. 2006).

Muscle tendon stiffness

Also, authors underlined the role of muscle-tendon complex stiffness (MTS) in storing and releasing elastic energy and the importance of a good ankle stability (Spurrs et al. 2003). Indeed, in activities where the stretch-shortening cycle is involved and the storage and return of elastic energy plays an important role such as running, a high MTS may improve the storage and release of elastic energy. Higher MTS can be attained by adding explosive strength training (Spurrs et al. 2003) and heavy strength training (Millet et al. 2002) to normal endurance training.

Uphill and downhill running

Uphill running was studied first by Margaria (1938) and in more recent years authors studied different gradients/gaits combinations (Minetti et al. 1994a; Minetti et al. 2002). The increase number of uphill competitions stimulated this exploration. Indeed, there are uphill running races of various distance and inclination. Uphill running requires higher cost of running (Margaria 1938; Minetti et al. 1994a; Minetti et al. 2002), and lower vertical GRF (Gottschall and Kram 2005). During uphill running the energy demand increases linearly up to +0.45. At this steep gradient, it is ~5 fold higher than on level ground. Conversely, the energy cost of running decreases in downhill and reaches a minimum at approximately -20% (Minetti et al. 2002) (Fig. 1.10).

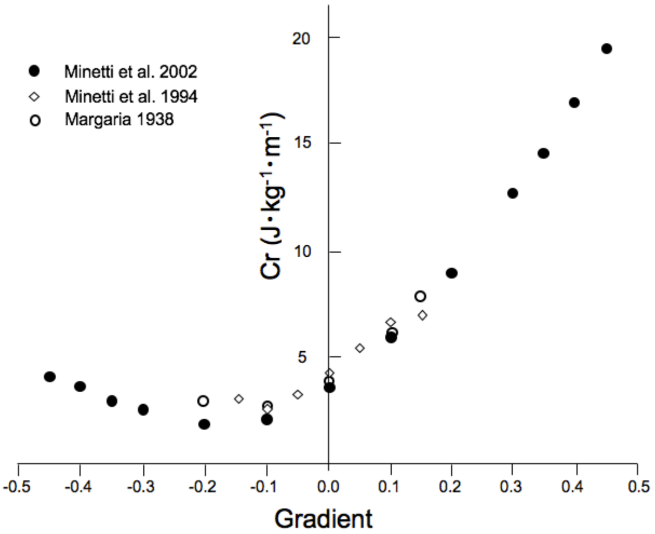


Fig. 1.10 - Metabolic energy cost of running as a function of the gradient from the works of Margaria (1938), Minetti et al. (1994a) and Minetti et al. (2002). Adapted from Minetti et al. (2002) .

Also the GRF are dependent on the gradient. Indeed, Gottschall and Kram (2005) reported that in downhill running the impact peaks were dramatically larger compared to level running and they were smaller in uphill running (Fig. 1.11). Particularly, compared to level running, at -9° the normal impact peak of the vertical GRF increased by 54%. The horizontal braking force peaks were larger for downhill running and smaller for uphill running. Running at -9° the parallel braking force peaks increased by 73% and during uphill running at 9° it decreased by 54%.

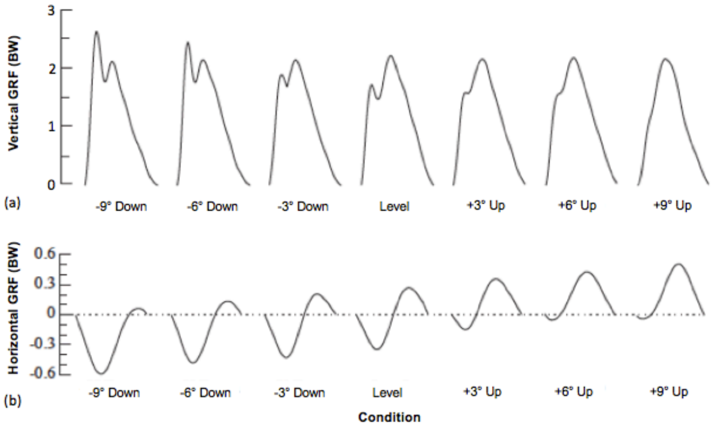


Fig. 1.11 - Vertical (a) and horizontal (b) ground reaction forces versus time traces for a 73-kg subject running at 3 m·s^{-1}. Adapted from Gottschall and Kram (2005).

In level running, gravitational potential energy and kinetic energy of the CoM fluctuations are symmetrical and in-phase (Fig. 1.5). Part of this energy is stored elastically in the tendons and subsequently recovered (Cavagna et al. 1977). Conversely, in downhill and uphill running, some net mechanical energy dissipation and generation is required. Minetti et al. (1994a) reported that positive external work per unit distance decreased linearly with slope during downhill running and increased linearly with slope during uphill running. At angles steeper than ±17%, exclusively negative and positive work is performed. Further, other authors (Snyder et al. 2012) reported that at -9° mechanical energy must no longer be generated while when running uphill additional energy must be generated to offset reduced elastic energy storage and return.

Conclusions of the general introduction

In this introduction we presented the physiological and mechanical determinants of endurance and ultra-endurance running. $\dot{V}O_2max$, *F* and Cr are all factors that can be improved by specific training. Consequently, endurance and ultra-endurance performance can be enhanced. Further, in the latest years the interest for studying the running mechanics changes during ultra-endurance event has grown up.

Ultra-endurance running is a relatively new field of study that can provide new insight of the human behavior in extreme fatigue conditions. For this reason we designed a study to investigate the human reaction from a physiological and mechanical point of view. In the next part I will present the aims of this thesis and the works generated throughout the last three years.

AIMS OF THE THESIS

This thesis is divided into two parts. The first part elucidates the energetics and mechanics of ultra-endurance running whereas the second part describes the development of a device for the gait mechanical analysis.

Part I: The objectives of the first part were to examine the factors affecting the ultra-endurance performance and in particular which aspects influence the cost of running (Cr). Consequently, we defined how the Cr and running mechanics changed during different types (i.e. level and uphill) of ultra-endurance races. Finally, we proposed a specific training protocol for improving the Cr in high-level ultra-marathoners.

Part II: The aim of the second part was to develop and validate an insole shoe sensor to collect data about the ground reaction forces, contact and aerial times. I will present the different prototypes we developed and the one we used for the validation. This device should be flexible, comfortable and low cost. Moreover it should allow to collect data in the field without the use of force plate nor video analysis.

CHAPTER 2

PART I

MECHANICAL CHANGES DURING ULTRA-ENDURANCE EVENTS AND THE EFFECTS OF A SPECIFIC TRAINING ON THE COST OF RUNNING

Introduction

As described in the previous chapters several factors affect the endurance and ultra-endurance performance. Physiological, mechanical, environmental, psychological and nutritional aspects influence the final results.

We confirmed that in ultra-endurance events the most important physiological factors determining the final performance are $\dot{V}O_2max$, *F* and Cr (Lazzer et al. 2014). Also, we reported that the mean velocity in this type of race can be predicted by the equation 1) previously proposed by other authors (di Prampero 2003; di Prampero et al. 1986).

In particular, we focused our efforts in studying the Cr under different conditions. In the first project (Lazzer et al. 2014) we analyzed the energetics and mechanics of running before and after a trail-running *flat* race (www.magraid.it). We reported that low Cr values before the race were related to high MMP and k_{vert}, and low foot print index (i.e. the mediolateral displacement of the foot during the stance phase, FPI) and W_{ext} (Lazzer et al. 2014). These results are important in suggesting that MMP, k_{vert} and FPI are relevant for the final performance. Thus, athletes may improve their results by acting on these parameters with specific training programs.

Since more recently uphill running races have become more popular among the athletic population, in another work (Lazzer et al. 2015) we studied the effects of an uphill-only race on the running mechanics and the Cr. We analyzed the changes occurred after the "Supermaratona dell'Etna", (www.supermaratonadelletna.it), an uphill-only marathon from sea level to the top of Monte Etna, Sicily, Italy (43 km with 3000 m of elevation gain). In this study we compared pre- and post-race MMP, Cr and running mechanics. We concluded that there is a direct relationship between Cr measured before the race and performance time. Further, in agreement with other studies (Brueckner et al. 1991; Lazzer et al. 2014) the Cr increased with the distance covered by ~9%. This increase was associated with changes in running mechanics, particularly with a decrease in k_{vert} and k_{leg}. We hypothesized that the decrease of k_{vert} and k_{leg} due to fatigue, induced the runner to lower the CoM during contact, increasing t_c and Δz. Furthermore, the decreased stride frequency likely led runners to a less efficient elastic energy utilization (Snyder and Farley 2011). In this study an important role in determining the final performance was played by the MMP, which was related with ΔCr (i.e. the increase of the Cr during the race). Indeed, athletes with higher MMP showed a lower ΔCr, suggesting that MMP may limit the variation of the Cr.

In previous works (Lazzer et al. 2015; Lazzer et al. 2014) our group reported that ultra-endurance races induce changes both in running mechanics and energetics. However, in these studies we were not able to define at which point of the race such changes happen.

Thus, in another work (Giovanelli et al. 2015b) we investigated when (and at which point of the race) the running mechanics change during the Supermaratona dell'Etna. Studying the running mechanics at four different points of the race (at km 3, 14, 30 and immediately after the finish line) we could define when athletes changed their running pattern. Further, we evaluated the effect of race-induced fatigue on muscle contractile properties by using tensiomyography (TMG). Finally, we compared the fastest runners with the slowest ones to determine if the running mechanics changed in a different way between the two groups. The results of this work underlined once again the role of the MMP and k_{vert} in defining the performance. Moreover, we reported that the fastest athletes did not change their running mechanics throughout the race while it changed from the 30th km onward in the slowest runners suggesting that the spring-mass parameters change after a certain time of exercise performed (~4 hours) rather than after a certain amount of distance covered. Further, in this study we analyzed the muscle response to an electrical stimulus by using TMG. Our results showed a decrement in muscle stiffness and higher sensibility of the muscle to the electrical stimulus, suggesting that the potentiation of fast twitch fibers and the fatigue of the slow twitch fibers are two parallel mechanism involved in this type of race.

When considering uphill running performance, particular attention deserves the vertical kilometer (VK) foot races in which athletes have to cover 1000 m of elevation gain in less than 5 km. The mean slope in this competition can exceed 30° and athletes can choose running, walking and the combination of the two gaits. To the best of our knowledge, there were no prior scientific studies of human walking or running at the steep angles that are encountered in the fastest VK races. To date, the only study in which authors analyzed steep slopes was by Minetti et al. (2002). Authors measured the metabolic cost of walking (Cw) and running (Cr) on a range of slopes up to 24.2°.

A new experimental design (Giovanelli et al. 2015a) explored steeper slopes than Minetti et al. by quantifying the metabolic costs of walking and running across a wide range of inclines up to and beyond those used in VK races. We also analyzed the mechanics of walking vs. running on steep inclines. We found that there is a range of angles for which energy expenditure is minimized (between 20° and 35°). Further, at the vertical velocity tested (0.35 $m \cdot s^{-1}$), on inclines steeper than 15.8°, athletes can reduce their energy expenditure by walking rather than running.

We reported (Giovanelli et al. 2015b; Lazzer et al. 2015; Lazzer et al. 2014) that in uphill-only race the running mechanics change after a certain time of exercise performed rather than a certain distance covered. Our next goal was to define if this happens also in *flat* races. In the race "6 ore città di Buttrio" athletes were required to run as many 874 m loops as possible in six hours. In this context we analyzed the running mechanics evolution every ~30 minutes of race to define whether and when any change occurs. The measurements performed and results obtained are explained in the following manuscript, which is currently in revision in the Int J Sports Phiol Perf.

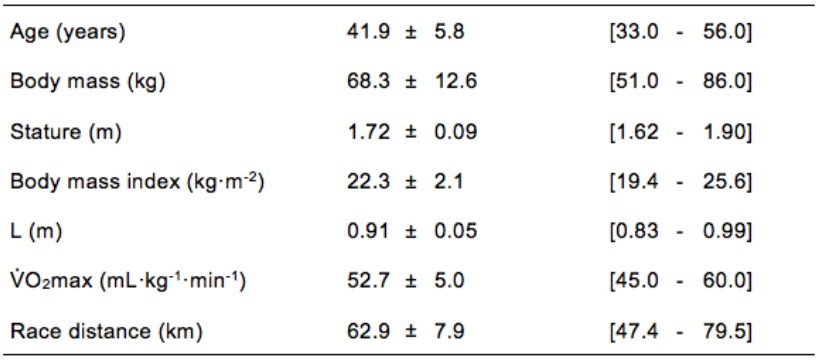
Changes in running mechanics during a six hours running race

To date, different types of ultra-endurance race have been analyzed and from previous work it seems that the running mechanics change after a certain time of exercise performed rather than a certain distance covered (Giovanelli et al. 2015b). The aim of this study was to analyze continuous changes in running mechanics during a six hours running competition on an 874 m flat loop. We hypothesized an increase in t_c and a decrease in t_a and k_{vert} between the third and fourth hour of the race. Also, we hypothesized that during a flat running race the changes in biomechanical parameters were smaller when comparing with an uphill-only race of similar duration.

Materials and Methods

Participants

Nineteen healthy Italian male runners (age: 41.9±5.8 years; body mass index: 22.3±2.1 $kg \cdot m^{-2}$, Table 2.1) were enrolled in this study as participants in the “6 ore Città di Buttrio”. The experimental protocol was conducted according to the Declaration of Helsinki and it was approved by the Ethics Committee of the University of Udine. Before the study began, the purpose and objectives were carefully explained to each participant and written informed consent was obtained from all of them. The participants were recruited among experienced ultra-endurance runners (12.4±8.5 years of training history in running and 6.5±3.5 years of ultra-endurance running race experience; they reported to run on average 73.3±19.5 km every week). Athletes were asked to fill out a questionnaire on physical exercise activity, demographics, medical history and lifestyle (Craig et al. 2003). All the nineteen athletes who were eligible for the study began the race but only 12 completed the entire competition. The athletes dropped out because of gastrointestinal problems (n=4) and muscular cramps (n=3). Therefore, only the runners who concluded the race (n=12) were taken into account for the data analysis.



Age (years)	41.9 ± 5.8	[33.0 - 56.0]
Body mass (kg)	68.3 ± 12.6	[51.0 - 86.0]
Stature (m)	1.72 ± 0.09	[1.62 - 1.90]
Body mass index ($kg \cdot m^{-2}$)	22.3 ± 2.1	[19.4 - 25.6]
L (m)	0.91 ± 0.05	[0.83 - 0.99]
$\dot{V}O_2max$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	52.7 ± 5.0	[45.0 - 60.0]
Race distance (km)	62.9 ± 7.9	[47.4 - 79.5]

TABLE 2.1. Physical characteristics of participants measured before the race in the athletes who concluded the race (n: 12). All values are mean ± standard deviation (SD). Range in square brackets. L: lower limb length; $\dot{V}O_2max$: maximal oxygen uptake.

Experimental design

The athletes were required to run as many 874 m flat loops as possible in six hours. In the week preceding the race, the participants were asked to come to the laboratory to perform a graded exercise test on a treadmill to evaluate their maximal oxygen uptake ($\dot{V}O_2max$). Athletes were also asked to refrain from performing any vigorous physical activity during the day preceding the test and during the preliminary testing session they performed to familiarize with the equipment. During the race, running mechanics in the first lap and every 30 minutes thereafter (±2 minutes, depending on the athlete's position along the circuit) were evaluated. Athletes were free to choose their own running velocity during the race to achieve their best performance (i.e. the highest distance covered).

Physiological measurements before the race

Body mass (BM) was measured by a manual weighing scale (Seca 709, Hamburg, Germany) and stature by standardized wall-mounted height board. Then, body mass index (BMI) was calculated as BM (kg)·stature^{-2} (m). $\dot{V}O_2max$ (mL·kg^{-1}·min^{-1}) was measured by means of a graded exercise test on a treadmill (Saturn, HP Cosmos, Germany) as previously described elsewhere (Lazzer et al. 2015; Lazzer et al. 2014).

Mechanical measurements during the race

A digital camera with a sample frequency of 400 Hz (Nikon J1, Japan) has been used to record participants during the race. The camera was placed perpendicular to the running direction of the athletes in a flat section of the loop. Running velocity was measured by means of two photocells placed 20 m apart (Fig. 2.1). Five subsequent steps were analyzed in order to measure t_c (s) and t_a (s) (Giovanelli et al. 2015b; Morin et al. 2011b). Afterwards, step frequency (f, step·s^{-1}) was calculated as $f=1/(t_c+t_a)$. Finally, the spring-mass model parameters (F_{max}, Δz, ΔL, k_{vert}, k_{leg}) were calculated using the method proposed by Morin et al. (2005).

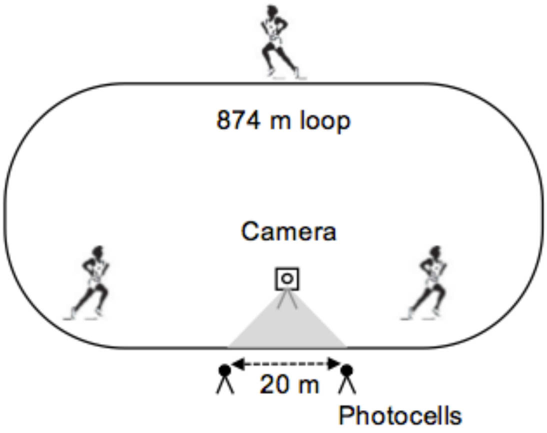


Fig. 2.1 – The experimental setup

Statistical analyses

Statistical analyses were performed using PASW Statistic 18 (SPSS Inc., IL, USA) with significance set at $p<0.05$. All results are expressed as means and standard deviation (SD).

Normal distribution of the data was tested using the Kolmogorov-Smirnov test. Changes of speed and mechanical parameters during the race were studied with the General Linear Model repeated measures. When significant differences were found, a Bonferroni post-hoc test was run to determine the exact location of the difference. The magnitude of the changes was assessed using effect size (ES) statistic and percentage change. The interpretation of effect size was as follows: <0.2 = trivial, 0.2–0.49 = small, 0.5–0.79 = medium, >0.80 = large (Cohen 1992).

Results

The physical characteristics of the participants measured before the race are reported in Table 1, together with the distance covered. The average running velocity during the race was 2.91±0.37 $m \cdot s^{-1}$.

Running velocity decreased significantly starting from 4h30' onward, compared to the running velocity measured at the first check point (first lap) (mean: -5.6±0.3%; $p<0.05$, ES=0.64, medium).

Figure 2.2 shows the trends of mechanical parameters during the race. All the changes are related to the first check point. Contact time (Fig. 2.2(a)) increased significantly from

4h30’ onward, reaching the maximum difference at 5h30, (+6.1%, $p=0.015$, ES=0.97, large). Aerial time (Fig. 2.2(b)) and F_{max} (Fig. 2.2(c)) decreased significantly from 4h30’ throughout the end of the race (mean: -29.2% and -5.1%, $p<0.05$ and $p<0.05$; ES= 0.55 and ES=0.72, medium; respectively). Also, Δz (Fig. 2.2(d)) decreased only in the last check point (-6.5%, $p= 0.02$; ES=0.64, medium). Consequently, k_{vert} (Fig. 2.2(e)) decreased significantly after 4h00’ reaching the lowest value after 5h30’ (-6.5%, $p=0.008$; ES=0.33, small). Finally, SL (Fig. 2.2(f)) decreased significantly from 5h00’ throughout the end of the race (mean: -5.1%, $p=0.010$; ES=0.41, small).

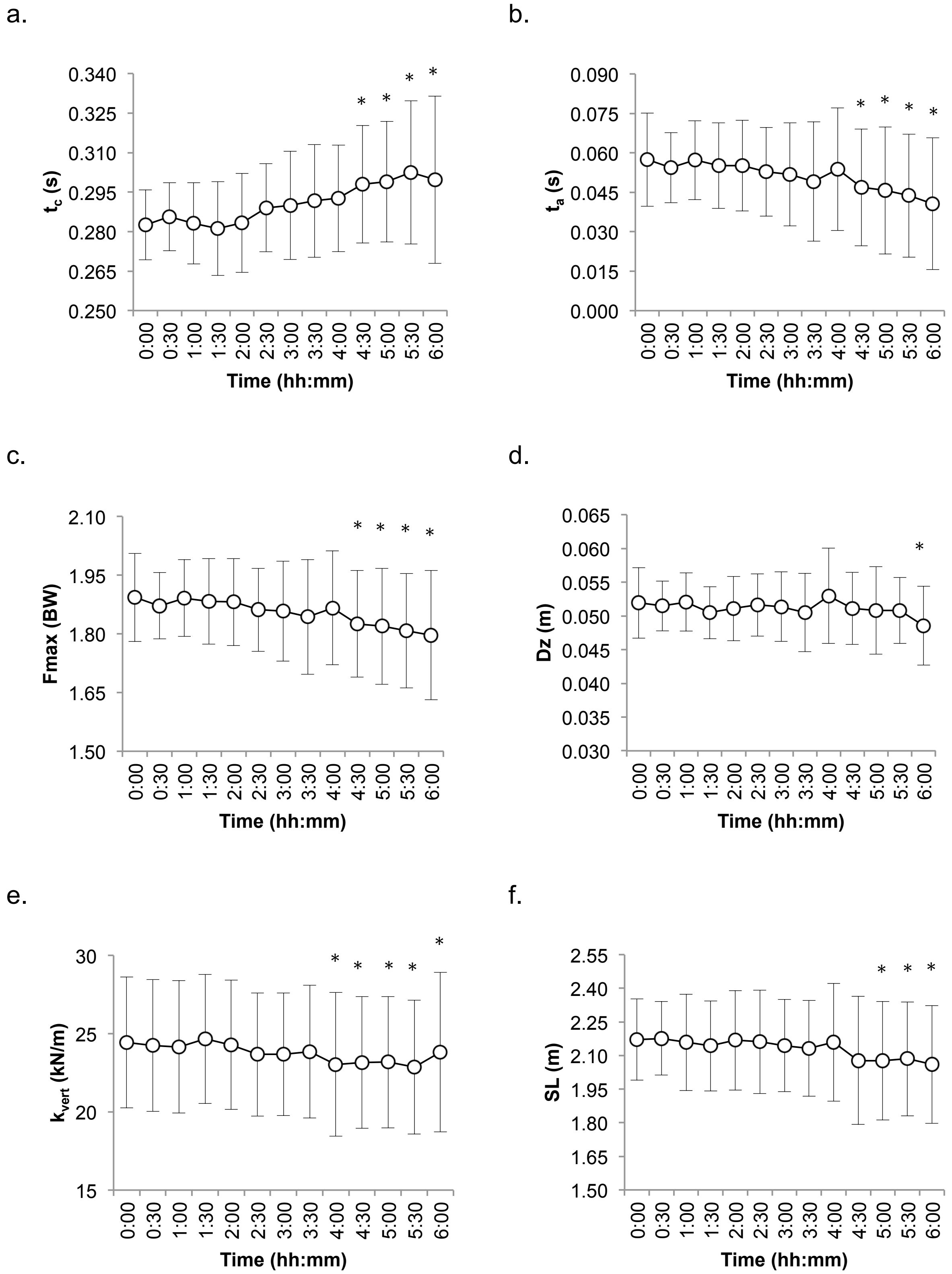


FIG. 2.2 – mechanical parameters measured every 30 minutes (a: contact time, t_c; b: aerial time, t_a; c: vertical ground reaction force, F_{max}; d: vertical displacement of the center of mass, (Δz); e: vertical stiffness, (k_{vert}); f: stride length, SL. *: $p<0.05$, significantly different from the first point.

Discussion

The main results of the present study show that: 1) running mechanics change significantly after the fourth hour of exercise, regardless of absolute running velocity; and 2) athletes chose their preferred pace throughout all the race.

Running mechanics did not change in the first part of the race, rejecting our hypothesis that changes would be seen between the third and fourth hour of the race. Indeed, before the fourth hour we did not observe changes in the spring-mass model parameters, while at the fourth hour only k_{vert} changed. After $4^h30'$ of running, also F_{max}, t_a, t_c and SL changed significantly, suggesting that low-frequency fatigue affects the gait (Fourchet et al. 2012). Indeed, a lower force production capacity and/or a reduced stretch-shortening-cycle efficiency of the lower limb extensor muscles may affect the mechanical behavior after this time-threshold (Dierks et al. 2010; Fourchet et al. 2015). The decrease observed in F_{max} was in agreement with all previous studies that analysed both shorter and longer events than six hours (Degache et al. 2013; Giovanelli et al. 2015b; Girard et al. 2013; Lazzer et al. 2015; Lazzer et al. 2014; Morin et al. 2011a; Morin et al. 2011b; Rabita et al. 2011). In our study athletes decreased their F_{max} by -5.1% and this value is similar to the values reported by the abovementioned studies: -4.4% showed after a 24h treadmill running (Morin et al. 2011a), -6.3% after a mountain ultra-marathon (Morin et al. 2011b) and -2.4% after 5h hilly running (Degache et al. 2013). A greater difference is registered when our results are compared to the uphill-only marathon in which Lazzer et al. (2015) showed a decrease of -17.6% in F_{max}. This difference could be due to the peculiar elevation profile of the race analyzed in this study. As a matter of fact our results are comparable to those reported for longer (≥24 hours) races. An explanation could be that F_{max} decreases in the first few hours of exercise reaching a plateau after a certain time (~4 hours) of running as previously pointed out (Morin et al. 2011a). The decrease in F_{max} could be due to functional and structural alterations of muscle fibers as well as to an increase in the inflammatory status occurring during this kind of races, in particular in the downhill sections (Millet et al. 2011b; Saugy et al. 2013).

However, our results are not consistent with the findings of other studies (Degache et al. 2013; Morin et al. 2011a; Morin et al. 2011b) which demonstrate different trends for Δz, k_{vert}, k_{leg}, f, t_c and t_a. These authors showed an increase in k_{vert} due to a higher decrease in Δz than in F_{max} explaining these changes as the result of a research of a smoother and less traumatic way to run. However, in our study, k_{vert} decrease was due to a decrease in F_{max} without changes in Δz, suggesting the inability of the system to maintain an optimal stiffness when the subject is running at constant speed in a fatigued state (Dutto and Smith 2002).

As we hypothesized, our results are in agreement with Lazzer et al. (2015) who analyzed running mechanics during an uphill-only race. Despite the fact that in uphill race the running pattern is different (Gottschall and Kram 2005; Padulo et al. 2012), we hypothesized that similar results could be achieved because of the similar duration of the race (~ six hours). Indeed, if we compare the present work with the work of Lazzer et al. (2015), changes in all parameters are smaller in a flat race compared to an uphill-only running race of similar duration (F_{max}: -5.1 *vs* -17.6%; Δz: -6.5 *vs* +52.9%; ΔL: NS *vs* +44.5%; k_{vert}: -6.4 *vs* -45.6%; k_{leg}: -7.2 *vs* -42.3%; t_c: +6.1 *vs* +28.6%; t_a: -29.2 *vs* -58.6%). These differences could be due to the work done to elevate the center of mass during uphill running, which involves higher fatigue and bigger changes in running mechanics. Further, the different trend of Δz can be explained as the different effort sustained by the athletes (flat vs. uphill running). Also, Vernillo et al. (2015b) explained that changes in stiffness due to fatigue could induce the runners to generate force less rapidly, thus having longer t_c, according to the cost of generating force hypothesis proposed by Kram and Taylor (1990).

In sprint and middle-distance running events athletes start fast, then slow down (Hanon et al. 2010) and then increase their speed again in the last part of the race (Girard et al. 2013). Conversely, in endurance races, athletes try to keep a regular and comfortable pace for the whole duration of the event (Hoffman 2014). Considering the mean speed over the six hours, the average velocity was 2.91±0.37 $m \cdot s^{-1}$, which corresponds to 67.4 ± 6.9 % of the running velocity at $\dot{V}O_2max$, as reported by previous studies (Davies and Thompson 1979). This speed was slightly slower than the running velocity at which the mechanical analysis was carried out because it actually includes also the rest time to enable athletes to feed during the six hours. Unlike other protocols (Martin et al. 2010; Millet et al. 2011a; Morin et al. 2011a), athletes organized their own feeding strategy, so that we could not control when and how long for they rested during the race. However, we supposed that these breaks did not affect running mechanics. Furthermore, we allow that the method we used to calculate the spring-mass parameters (Morin et al. 2005) is dependent upon ground contact times. Nevertheless, previous studies considered this model for measuring the changes in running mechanics during both short (Hobara et al. 2010) and long performance (Giovanelli et al. 2015b; Lazzer et al. 2015; Morin et al. 2011a; Morin et al. 2011b). In addition, the changes in running velocity (coefficient of variation 3.7±2.6%) observed in this study were not great enough to affect the computation of the F_{max} and k_{vert}. In effect, in agreement with previous study (Lazzer et al. 2015), a decrease of -5.6±0.3% in the running velocity had only a partial effect on the changes in the mechanical parameters. As well, the range of speed accepted by Morin et al. (2011b) for calculation of the mechanical parameters during running was ±5% the reference speed (3.33 $m \cdot s^{-1}$). In particular, Arampatzis et al. (1999) showed that k_{vert} did not change significantly for speeds ranging from 2.5 to 3.5 $m \cdot s^{-1}$, which are very similar to the range of speeds that our athletes maintained throughout the whole race (min= 2.4 $m \cdot s^{-1}$; max= 3.9 $m \cdot s^{-1}$).

Conclusions

In conclusion, we observed that, most mechanical changes happen after four hours of continuous running. This suggests the existence of a “time threshold” that could affect performance regardless of absolute running speed. Future studies should focus on the reason why spring-mass model changes after this “threshold”, proposing some specific training to preserve the correct running mechanics for more hours.

Effects of strength, explosive and plyometric training protocol on energy cost of running in high-level ultra-endurance athletes

As extensively reported throughout this thesis, Cr plays an important role in determining the performance and it is affected by several factors. We suggested to add strength and explosive training in the ultra-marathoners training program because an increase in MMP might lead to a decrease in Cr (Lazzer et al. 2015; Lazzer et al. 2014). Thus, our next objective was to evaluate the effects of a 12-week of strength, explosive and plyometric (SEP) training protocol on the Cr in ultra-marathoners and to define which biomechanical parameters and anthropometric characteristics of the gastrocnemius medialis and Achilles tendon affected the Cr. The experiments performed and the results of this study are exposed in the below manuscript, which is actually in revision of the Eur J Appl Physiol.

Effects of strength, explosive and plyometric (SEP) training on short (<15 minutes) and long (>15 minutes) endurance performance have been extensively studied in different populations and sport situations (Aagaard et al. 2011; Hakkinen et al. 2003; Hoff et al. 2002; Hoff et al. 1999; Millet et al. 2002; Paavolainen et al. 1999; Ronnestad et al. 2012; Ronnestad and Mujika 2014; Spurrs et al. 2003; Storen et al. 2008; Sunde et al. 2010; Turner et al. 2003). Cyclists, rowers, triathletes and runners underwent different types of training protocols to improve the endurance performance, but literature misses data about ultra-marathoners. Several sports are analyzed in the above-mentioned studies. Although different points of view are present between authors, it appears that the optimal training regime includes concurrent strength, explosive and plyometric exercises added to endurance training. Indeed, concurrent endurance and strength training may improve endurance performance more than endurance training alone (Aagaard et al. 2011; Hoff et al. 1999; Spurrs et al. 2003; Storen et al. 2008).

The first objective of the present study was to evaluate the effects of a 12-week SEP training protocol on the Cr in high-level ultra-marathoners. We hypothesized I) a decrease in Cr and we expected II) higher MMP of the lower limbs. Secondly, if the training protocol led to a decrease in cost of running, we aimed to define which factors affect this change analysing some running mechanical parameters and some characteristics of the Achilles tendon and gastrocnemius medialis (GM). We also hypothesized that SEP training led III) higher tendon stiffness.

Materials and methods

Participants

Twenty-five male runners (38.2±7.1 years; BMI: 23.0±1.1 kg·m^{-2}; $\dot{V}O_2$max: 55.4±4.0 mlO$_2$·kg^{-1}·min^{-1}, Table 2.2) participated in this study and provided informed consent. The experimental protocol was approved by the Ethics Committee of the University of Udine, Italy. The participants were recruited among Italian high-level ultra-endurance runners (both road- and trail-runners); some of them joined the national team in the latest two years (Italian Ultra-Marathon and Trail Association, IUTA). The inclusion criteria were: the athletes had run at least one race longer than 50 km and their training volume in the latest 3 months was more than 60 km·week^{-1}. Further, they did not perform strength training in the last six months and none of the athletes had a history of neuromuscular or musculoskeletal impairments at the time of the study that could affect the results.

On average their training experience amounted to (mean ± s.d.) 11.7±8.6 years, of which 4.7±3.4 years involved in ultra-endurance running. They reported to run on average 88.0±33.1 km·week^{-1} and their personal best on marathon race and 100 km race were (hours:minutes) 3:00±0:17 and 9:00±1:56, respectively.

	All runners (n: 25)		Control group (n:12)	Exercise group (n:13)	p
Age (years)	38.2 ± 7.1	[26.0 - 51.0]	40.3 ± 6.5	36.3 ± 7.4	0.17
BM (kg)	71.3 ± 8.5	[59.0 - 93.0]	70.7 ± 7.9	71.9 ± 9.4	0.721
Height (m)	1.75 ± 0.07	[1.65 - 1.84]	1.75 ± 0.07	1.76 ± 0.08	0.797
BMI (kg·m^{-2})	23 ± 1.1	[20.3 - 24.9]	22.9 ± 1.3	23.1 ± 0.9	0.735
FFM (kg)	55.6 ± 6.2	[45.8 - 71.7]	55.7 ± 7.2	55.4 ± 5.4	0.901
FM (kg)	15.8 ± 4.6	[8.2 - 27.7]	14.9 ± 3.9	16.5 ± 5.2	0.404
FM (%BM)	21.9 ± 4.7	[12.6 - 29.8]	21.1 ± 4.8	22.6 ± 4.7	0.44
$\dot{V}O_2$max (ml·kg^{-1}·min^{-1})	55.4 ± 4	[50.0 - 64.0]	55.6 ± 4.1	55.2 ± 4	0.831
RERmax	1.15 ± 0.04	[1.06 - 1.22]	1.16 ± 0.04	1.14 ± 0.04	0.187
HRmax (bpm)	176 ± 9.1	[160 - 193]	175.6 ± 8.1	176.9 ± 10.2	0.736
vmax (km·h^{-1})	17.6 ± 0.9	[16.1 - 19.6]	17.7 ± 0.8	17.6 ± 0.9	0.849
v$\dot{V}O_2$max (km·h^{-1})	17.2 ± 0.8	[16.1 - 18.9]	17.2 ± 0.7	17.2 ± 0.7	0.797
Gas exchange threshold					
$\dot{V}O_2$ (ml·kg^{-1}·min^{-1})	46.8 ± 3.1	[41.0 - 53.1]	46.7 ± 2.6	46.9 ± 3.6	0.79
$\dot{V}O_2$ (%$\dot{V}O_2$max)	84.6 ± 3.8	[78.9 - 92.4]	84.1 ± 4.2	85.1 ± 3.5	0.528
RER	0.97 ± 0.01	[0.89 - 1.05]	0.97 ± 0.03	0.96 ± 0.04	0.546
RER (%RERmax)	84 ± 2.8	[79.0 - 90.0]	83.6 ± 3	84.6 ± 2.6	0.433
HR (bpm)	166 ± 9.1	[151 - 184]	165.1 ± 9.4	167.8 ± 9.2	0.64
HR (%HRmax)	94.2 ± 1.8	[90.1 - 97.0]	94 ± 1.7	94.4 ± 1.9	0.597
v (km·h^{-1})	15.7 ± 0.5	[14.7 - 16.8]	15.7 ± 0.6	15.7 ± 0.4	0.716
v (%v$\dot{V}O_2$max)	91.4 ± 2.2	[88.0 - 95.8]	91.9 ± 2.3	91 ± 2.1	0.329

Table 2.2. Physiological characteristics of the subjects. All values are means ± s.d. BM: body mass; BMI: body mass index; FFM: fat-free mass; FM: fat mass; $\dot{V}O_2$max: maximal oxygen uptake; RER: respiratory exchange ratio; HR: heart rate; vmax: maximal running speed; v$\dot{V}O_2$max: running speed at $\dot{V}O_2$max. P: unpaired t-test between Control and Exercise groups.

Experimental design

Subjects came into the laboratory three times. During the first visit, the athletes were fully informed regarding the experimental procedures, underwent a medical examination and particularly attention was paid to familiarize them with all the procedures.

During the second visit (PRE), body mass (BM), height, body composition, morphological properties of the GM, triceps surae tendon stiffness (k_{tendon}) and MMP were determined. Then, Cr and spring-mass model parameters at four speeds (8, 10, 12 14 $km \cdot h^{-1}$) were calculated before performing a maximal test on a motorized treadmill. We used this test for determining anaerobic threshold, maximal oxygen uptake ($\dot{V}O_2max$) and the velocity associated with $\dot{V}O_2max$ ($v\dot{V}O_2max$).

After the second visit the subjects were randomly split into two homogenous groups (exercise group, EG, n=13; control group, CG, n=12). The EG added a 12-week SEP training protocol to its normal running training whilst the CG continued its usual running training.

Immediately after the 12-week SEP training protocol, EG and CG came into the laboratory for the third visit (POST) and performed the identical procedures done during the second visit.

Anthropometric characteristics and body composition

Body mass (BM) was measured with a manual weighing scale (Seca 709, Hamburg, Germany). Height was measured on a standardized wall-mounted height board. Body mass index (BMI) was then calculated as body mass (kg)$\cdot$height $(m)^{-2}$. Body composition (fat-free mass, FFM and fat mass, FM) was measured by bioelectrical impedance analysis (BIA, Akern, Florence, Italy) according to the method of Lukaski et al. (1986).

Energy cost of running and maximal oxygen uptake

Metabolic rate at 8, 10, 12 and 14 $km \cdot h^{-1}$ were measured during four steady state steps performed before a maximal test on a motorized treadmill (Saturn, HP Cosmos, Nußdorf, Germany) under medical supervision. Ventilation, oxygen consumption ($\dot{V}O_2$)

and carbon dioxide production ($\dot{V}CO_2$) were measured continuously with a metabolic unit (Quark-b^2, Cosmed, Rome, Italy). The volume and gas analyzers were calibrated before every trial as described elsewhere (Lazzer et al. 2014). Heart rate was measured with a dedicated device (Polar, Kempele, Finland).

The test included a 5 minutes rest period followed by 4 running steps at 8, 10, 12, 14 $km \cdot h^{-1}$ for 5 minutes each; then, the speed was increased by 0.7 $km \cdot h^{-1}$ every minute until the volitional exhaustion.

After subtracting the metabolic rate measured during the standing phase to the gross metabolic rate, the Cr (in $mlO_2 \cdot kg^{-1} \cdot m^{-1}$) at 8, 10, 12 and 14 $km \cdot h^{-1}$ was calculated as the ratio between the net $\dot{V}O_2$ (averaged in the last minute of every step (Lazzer et al. 2014)) and the corresponding running speed. Respiratory exchange ratio (RER= $\dot{V}CO_2/\dot{V}O_2$) was required to be lower than 1.0.

During the incremental test, a leveling off of oxygen uptake (defined as an increase of no more than 1 $ml \cdot kg^{-1} \cdot min^{-1}$) was observed in all subjects during the last one or two minutes indicating that $\dot{V}O_2max$ was attained. $\dot{V}O_2max$ and maximal heart rate (HRmax) were calculated as the average $\dot{V}O_2$ and HR of the last 30 s of the test.

The gas exchange threshold was then determined by the V-slope method (Beaver et al. 1986).

Mechanical measurements

Running mechanics were studied at four different speeds (8, 10, 12, 14 $km \cdot h^{-1}$) using a digital camera with a sample frequency of 400 Hz (Nikon J1, Japan). The camera was placed next to the treadmill and ten subsequent steps between the 4th and the 5th min were analyzed in order to measure contact (t_c, s) and aerial (t_a, s) time. Step frequency (f, $step \cdot s^{-1}$) was then calculated as: $1/(t_a+t_c)$.

Given t_c (s), t_a (s), v ($m \cdot s^{-1}$), subject's BM (kg), and lower limb length (distance between great trochanter and ground during standing, L in m), spring-mass model parameters were calculated using the method proposed by Morin et al. (2005).

Maximal power of the lower limbs

The MMP during a SJ was assessed by means of the Explosive-Ergometer (EXER, University of Udine, Italy), previously described elsewhere (Lazzer et al. 2014). Subjects were asked to perform four all-out SJ starting from a knee angle of 110 degrees with two minutes of rest interval between each trial. The starting position was assured by two blocks, which prevent any counter-movement. The MMP was obtained from the instantaneous product of the developed force (F, N) multiplied by the backward speed (v, $m \cdot s^{-1}$). The SJ with the highest peak of power was taken into account for the analysis.

Further, morphological properties of the gastrocnemius medialis and triceps surae tendon stiffness were measured as previously described (Lazzer et al. 2014).

Training protocol

EG underwent a 12-week training protocol adding three training sessions per week to its usual running training. Athletes performed the training protocol at their home on alternate days avoiding the day after races or after high intensity or long (>2 hours) training sessions.

The training protocol was divided into three 4-week macro-cycles.

The sessions in the first cycle included three exercises for the core, three exercises for the running technique and four strength exercises for the lower limbs.

The sessions in the second and third cycle included two plyometric exercises and five explosive exercises for the lower limbs. In these two cycles, after a familiarization period, three exercises were performed on unstable board (Disc'o'Sit, Ledraplastic, Osoppo, Italy).

Participants underwent 5-8 exercises three times per week. 1-3 sets for 6-15 repetitions were performed for each exercise. The sessions lasted about 25-30 minutes and athletes were free to undergo the workout without rest or with a short rest (<30 seconds) between each exercise. All the exercises were performed without weight loading. During the first two weeks, athletes were supported by a research assistant, who verified that athletes performed the exercises correctly.

Conversely, the CG continued to perform its normal endurance training, including 5-7 running session per week.

Statistical analyses

Statistical analyses were performed using PASW Statistic 18 (SPSS Inc., Chicago, IL, USA) with significance set at $p<0.05$. All results are expressed as means and standard deviation (SD). Normal distribution of the data was tested using the Kolmogorov-Smirnov test. Sphericity (homogeneity of covariance) was verified by the Mauchly's test.

Changes of anthropometrics characteristics, body composition, morphological properties of the gastrocnemius medialis, triceps surae tendon stiffness, MMP, anaerobic threshold and $\dot{V}O_2$max were studied with a general linear model repeated measures considering two factors (time: PRE and POST; group: CG and EG).

Changes of Cr, biomechanical and spring-mass model parameters were studied with a general linear model repeated measures considering three factors (speed: 8, 10, 12, 14 $km \cdot h^{-1}$; time: PRE and POST; group: CG and EG). When significant differences were found, a Bonferroni post hoc test was used to determine the exact location of the difference.

In addition, the relationships between changes in mechanical parameters, MMP, morphological properties of the gastrocnemius medialis and triceps surae tendon variables affecting Cr were investigated using Pearson's product–moment correlation coefficient.

Results

Characteristics of the athletes

All the subjects included in the EG completed more than 95% of the training program (self-reported). The physiological characteristics of the athletes measured at PRE are reported in Table 1. No significant differences were found between CG and EG on anthropometrics (BMI: 22.9±1.3 vs. 23.1±0.9 $kg \cdot m^{-2}$, p=0.735) and body composition (%FM: 21.1±4.8 vs. 22.6±4.7%, p=0.440) characteristics. As well, no significant differences were found in $\dot{V}O_2$max (55.6±4.1 vs. 55.2±4.0 $ml \cdot kg^{-1} \cdot min^{-1}$, p=0.831), $\dot{V}O_2$ at GET (46.7±2.6 vs. 46.9±3.6 $ml \cdot kg^{-1} \cdot min^{-1}$, p=790) and speed at GET (15.7±0.6 vs. 15.7±0.4 $km \cdot h^{-1}$, p=716) between the two groups. There were no significant differences between PRE and POST in the above mentioned parameters both when athletes were analyzed all together and when they were analyzed split in CG and EG.

Further, no differences in mechanical parameters, spring-mass model parameters and MMP were detected between PRE and POST in the CG.

Energy cost of running

At PRE, no significant differences were shown in Cr between CG and EG. Further, when all subjects were analyzed together Cr at 8 km·h^{-1} was significantly higher than Cr at other selected speeds (+6.5±2.0% on average, $p<0.001$). Conversely, Cr was not significantly different between 10, 12 and 14 km·h^{-1} ($p>0.05$).

At POST, Cr decreased significantly in EG at all tested running speeds (-6.4±6.5%, $p=0.005$, at 8 km·h^{-1}; -3.5±5.3%, $p=0.032$, at 10 km·h^{-1}; -4.0±5.5%, $p=0.020$, at 12 km·h^{-1}; -3.2±4.5%, $p=0.022$, at 14 km·h^{-1}, Fig. 2.3). In addition, Cr was significantly lower in EG than CG at every tested speed (-6.2±1.7% on average, $p<0.05$).

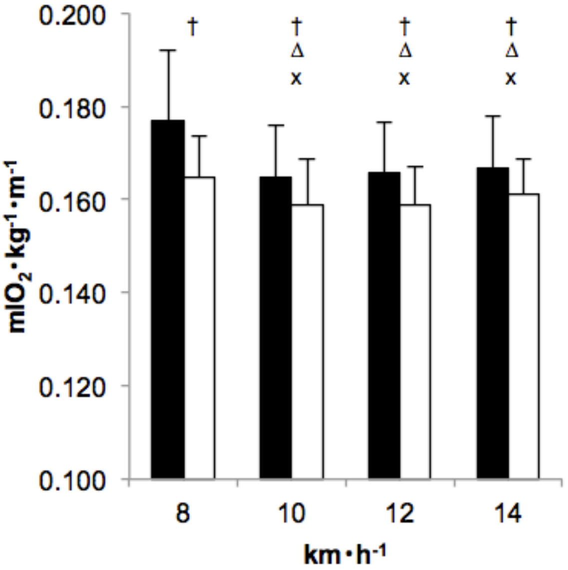


Fig 2.3. Cr (mlO$_2$·kg^{-1}·m^{-1}) as a function of speed (km·h^{-1}) in EG (n=13), before (PRE, black bars) and after (POST, white bars) the training protocol. †: significantly different POST vs. PRE; Δ: significantly different compared with 8 km·h^{-1} PRE; x: significantly different compared with 8 km·h^{-1} POST.

Running mechanics and spring-mass model parameters

At PRE, no significant differences were shown in spring-mass model parameters between CG and EG.

As well, in the EG contact time (t_c, Fig. 2.4A) decreased by mean -8.9±4.2% as a function of speed. Further, in the EG the following mechanical parameters increased as a function of the speed ($p<0.05$): aerial time (t_a, mean +30.1±33.4%, Fig. 2.4B), stride frequency (SF, mean +2.6±0.1% Fig. 2.4C), stride length (SL, mean +17.6±4.0%, Fig. 2.4D), maximal ground reaction force (Fmax, mean +7.1±5.1%, Fig. 2.5), leg length changes (ΔL, mean +12.9±1.6%, Fig. 2.6A) and vertical stiffness (k_{vert}, mean +7.2±1.0%, Fig. 2.6B). While leg stiffness (k_{leg}) decreased as a function of the speed by mean -4.6±5.1% (Fig. 2.6C). At POST, similar changes in the above-mentioned parameters, as a function of speed, were observed in the EG.

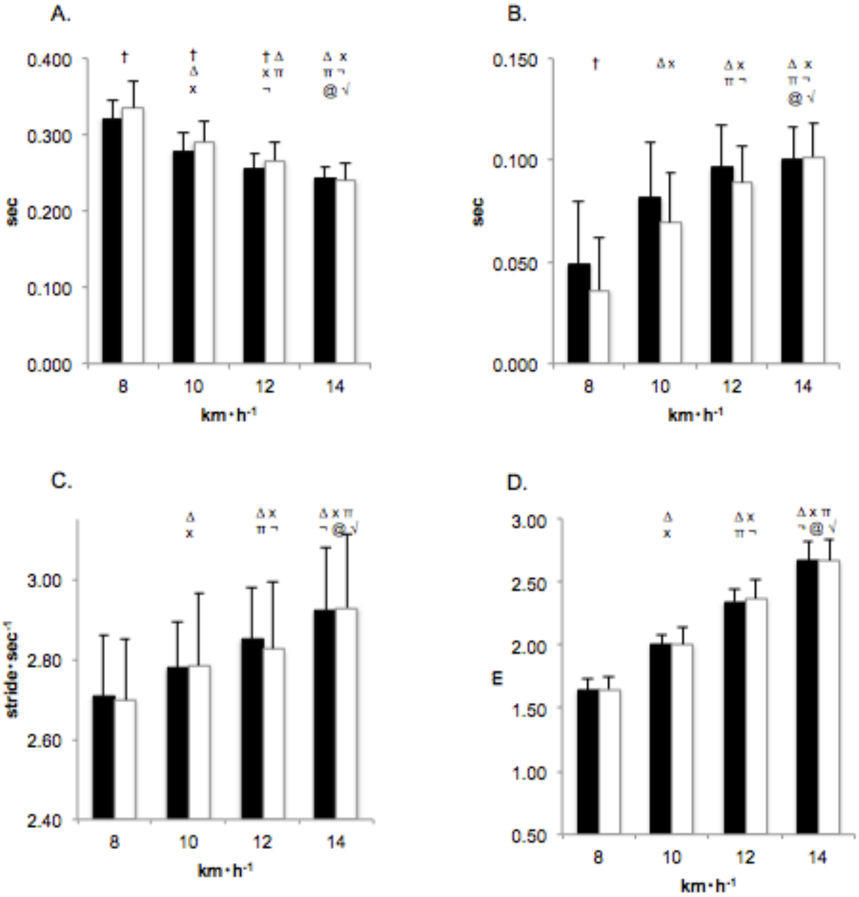


Fig 2.4. t_c (in s, Fig. 2.4A), t_a (in s, Fig. 2.4B), SF (in stride·sec^{-1}, Fig. 2.4C) and SL (in m, Fig. 2.4D) in EG (n=13), before (PRE, black bars) and after (POST, white bars) the training protocol. †: significantly different POST vs. PRE; Δ: significantly different compared with 8 km·h^{-1} PRE; x: significantly different compared with 8 km·h^{-1} POST; π: significantly different compared with 10 km·h^{-1} PRE; ¬: significantly different compared with 10 km·h^{-1} POST; @: significantly different compared with 12 km·h^{-1} PRE; √: significantly different compared with 12 km·h^{-1} POST

After the training protocol, in the EG t_c increased at 8, 10 and 12 km·h^{-1} by mean 4.4±0.1% (Fig. 2.4A) ($p<0.05$) and t_a decreased by -25.6% (p=0.035) at 8 km·h^{-1}, while t_a decreased but not significantly at 10 and 12 km·h^{-1} (-15.2%, p=0.093 and -8.0%, p=0.117, respectively) (Fig. 2.4B). Consequently, Fmax decreased by -3.7% (p=0.032) at 8 km·h^{-1} and it slightly decreased at 10 and 12 km·h^{-1} (-4.4%, p=0.077 and -3.3%, p=0.076, respectively) (Fig. 2.5). Further, k_{leg} decreased at 10 and 12 km·h^{-1} (-9.5%, p=0.034 and -10.1%, p=0.038, respectively), while the decrease was not significant at 8 km·h^{-1} (-7.6%, p=0.054) (Fig. 2.6C). No changes in SF, SL (Fig. 2.6C and 2.6D), Δz and k_{vert} (Fig. 2.6C and 2.6D) were detected in the EG after the training protocol.

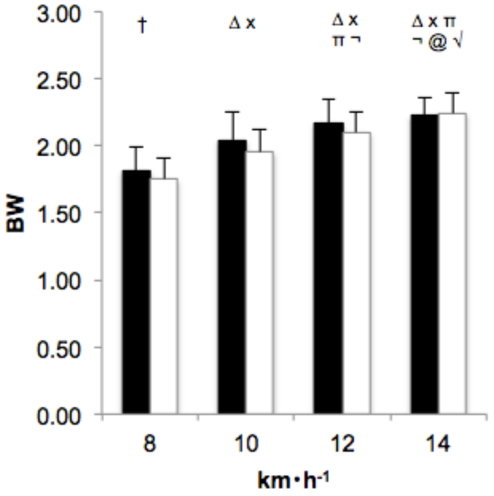


Fig 2.5. Fmax (in BW) in EG (n=13), before (PRE, black bars) and after (POST, white bars) the training protocol. †: significantly different POST vs. PRE; Δ: significantly different compared with 8 km·h^{-1} PRE; x: significantly different compared with 8 km·h^{-1} POST; π: significantly different compared with 10 km·h^{-1} PRE; ¬: significantly different compared with 10 km·h^{-1} POST; @: significantly different compared with 12 km·h^{-1} PRE; √: significantly different compared with 12 km·h^{-1} POST

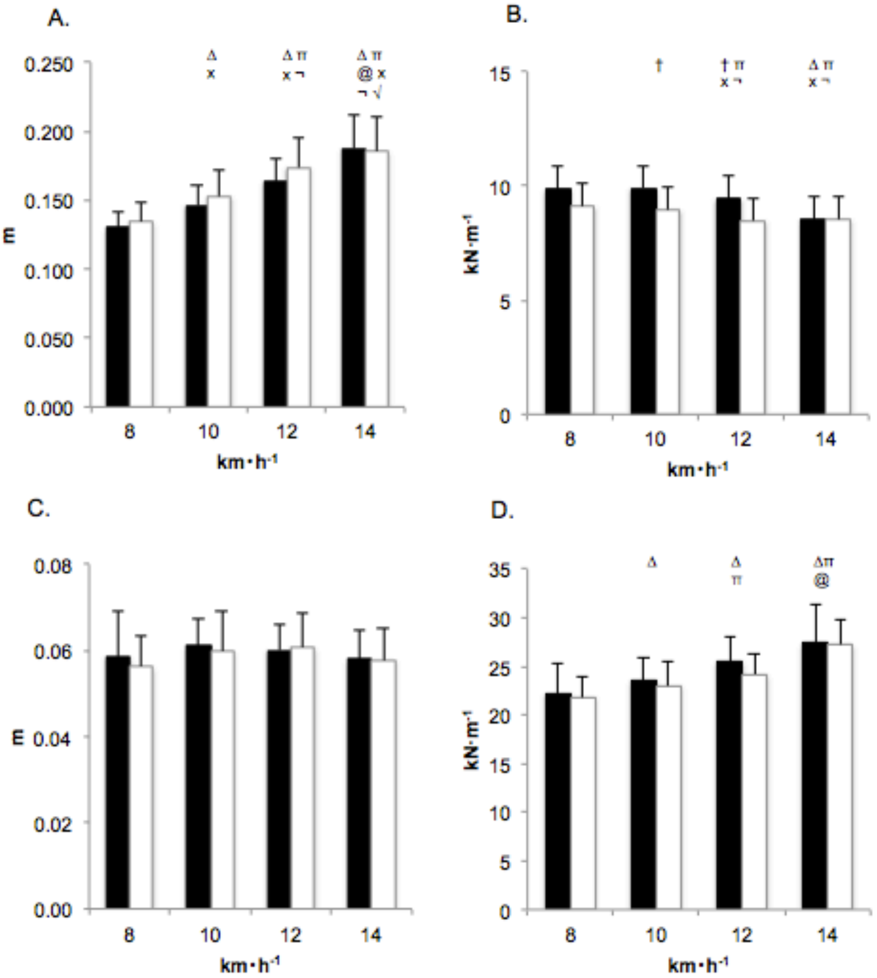


Fig 2.6. ΔL (in m, Fig. 2.6a), k_{leg} (in m, Fig. 2.6b), Δz (in m, Fig. 2.6c) and k_{vert} (in kN·m^{-1}, Fig. 2.6d) in EG (n=13), before (PRE, black bars) and after (POST, white bars) the training protocol. †: significantly different POST vs. PRE; Δ: significantly different compared with 8 km·h^{-1} PRE; x: significantly different compared with 8 km·h^{-1} POST; π: significantly different compared with 10 km·h^{-1} PRE; ¬: significantly different compared with 10 km·h^{-1} POST; @: significantly different compared with 12 km·h^{-1} PRE; √: significantly different compared with 12 km·h^{-1} POST

Maximal muscle power of the lower limbs

At PRE, MMP was not significantly different between CG and EG in absolute (2961±422 vs. 3257±632 W, p=0.186) and relative (42.3±6.72 vs. 43.8±7.4 W·kg^{-1}, p=0.585) values. As well, at POST, MMP increased in the EG, although this increment was not statistically significant (+5.1±12.2%, p=0.174, Table 2.3). Then, in EG inverse relationships between changes in Cr and MMP at 10 (p=0.013; r=-0.67) and 12 km·h^{-1} (p<0.001; r=-0.86) were shown (Fig. 2.7).

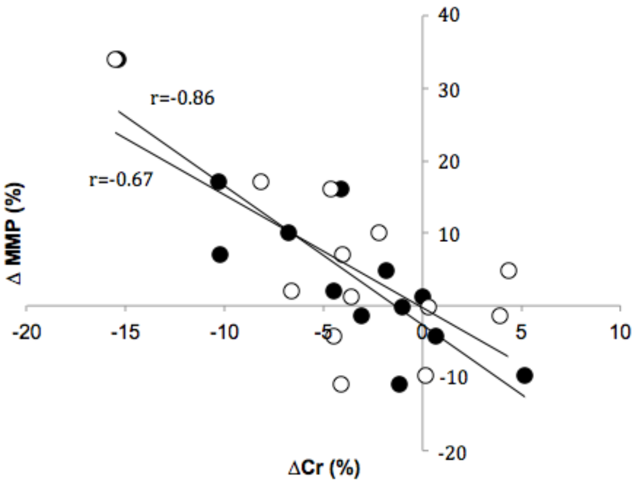


Fig 2.7. Relationships between changes in energy cost of running (ΔCr, %) and maximal muscle power (ΔMMP, %) in the experimental group (EG) at 10 km/h (white dots) and 12 km/h (black dots)

Gastrocnemius medialis and triceps surae tendon proprieties

At PRE and POST, no significant differences were shown in proprieties of GM and triceps surae tendon between CG and EG. Moreover, these proprieties did not change significantly between PRE and POST training protocol both in CG and in EG (Table 2.3).

	Control Group		Exercise Group		p		
	Pre	Post	Pre	Post	Group	Time	G x T
MMP	42.3 ± 6.7	42.1 ± 4.9	43.8 ± 7.4	45.6 ± 6.7	0.317	0.311	0.23
Triceps surae tendon							
Resting length (L_0, mm)	202.8 ± 12.8		217.1 ± 24.1		0.123		
Elongation (ΔL, mm)	22.5 ± 3.6	22.1 ± 4.4	21.5 ± 3.4	21.5 ± 3	0.569	0.738	0.789
Strain ($\Delta L \cdot L_0^{-1}$, %)	11.1 ± 1.6	10.9 ± 2.4	9.9 ± 1.1	9.9 ± 1.4	0.094	0.884	0.77
Cross-sectional area (mm^2)	59 ± 15.9	57 ± 15.4	69.1 ± 13.2	67.7 ± 16	0.118	0.178	0.803
Force (N)	4865.6 ± 1617.71	4754.2 ± 1846.6	5621.2 ± 497.9	5436.4 ± 954.6	0.191	0.414	0.839
Stiffness ($N \cdot mm^{-1}$)	420.2 ± 202	370.4 ± 169.9	442.9 ± 88.6	409.4 ± 61.3	0.59	0.086	0.668
Young's modulus (Gpa)	1.42 ± 0.38	1.31 ± 0.44	1.4 ± 0.32	1.33 ± 0.29	0.984	0.189	0.737
Gastrocnemius medialis							
Fiber length (mm)	48.9 ± 9.3	49.1 ± 9.3	48.8 ± 9.2	49.9 ± 9.8	0.936	0.22	0.362
Pennation angle (deg)	21.2 ± 2.4	20.9 ± 2.6	20.7 ± 1.8	20 ± 2	0.448	0.052	0.497
Thickness (mm)	17.5 ± 2.5	17.2 ± 2.3	17.1 ± 3.1	16.9 ± 3	0.786	0.16	0.876
Torque ($N \cdot m^{-1}$)	251 ± 62.9	245 ± 73.7	268.8 ± 40.7	267.3 ± 66.9	0.437	0.699	0.819

Table 2.3. Physiological characteristics of the triceps surae tendon and gastrocnemius medialis muscle of athletes, before (PRE) and after (POST) the training period in the control group (n=12) and in the exercise group (n=13). All values are means ± s.d. MMP: maximal muscle power of the lower limbs.
p: Significance by GLM repeated measures with two factors of the main effects of Group (CG vs EG), time (PRE vs POST) and their interaction (G x T).

Discussion

The main findings of the present study showed that in high-level ultra-marathoners a 12-week SEP training protocol led to a lower Cr at submaximal speeds and MMP might have an important role in decreasing Cr.

Conversely to previous works in which recreational runners were involved (Ferrauti et al. 2010; Hakkinen et al. 2003; Turner et al. 2003), we enrolled high-level, non-professional ultra-marathoners. As we hypothesized, SEP training led to a lower Cr (-4.3±1.5%), considering the four selected running speeds altogether. We tested four different running speeds (8, 10, 12, 14 km·h^{-1}) because we aimed to test the range of speeds the athletes likely select during ultra-marathons (on average they ran a 100 km race in 9:00±1:56 hours, which means ~11 km·h^{-1}) or during most of their running training. The improvement in Cr (~4%) might seem a small progress; however, for these athletes a small performance enhancement can lead to an important step forward in the final rankings. According to the equation of di Prampero et al. (1986), where the speed of running is determined by the ratio between metabolic power and Cr, and assuming that athletes run a 100 km race at 70% of their $\dot{V}O_2$max (Davies and Thompson 1979; Davies and Thompson 1986), decreasing Cr by -4% would improve their performance by ~17 minutes (from 7h05' min to 6h48' min). Although this computation does not take into account possible changes in Cr due to the distance covered (Lazzer et al. 2015; Lazzer et al. 2014), it highlights the relevance of Cr in ultra-endurance competitions.

Previous works (di Prampero et al. 1986; Margaria et al. 1963) described that Cr is independent from the speed between 8 and 20 km·h^{-1}. However, at 8 km·h^{-1} we reported higher Cr compared to other speeds (+6.5±2.0%). We suppose that this could be due to the fact that our athletes never run slower than ~10 km·h^{-1}, thus they are not adapted to this slow speed. They can carry out a 100 km running race in less than 10 hours and some of them were able to run more than 240 km in 24 hours running race. Farley et al. (1991) predicted that metabolic rate increases at lower frequency during hopping because the body does not behave in an optimal spring-like manner and some elastic energy is dissipated. If we compare running to a series of subsequent hops, this may explain the higher Cr at 8 km·h^{-1} compared with other "optimal speeds" (Steudel-Numbers and Wall-Scheffler 2009). Also, at such slow speed the relaxation phase is shorter and the constriction during the contraction phase is prolonged, promoting a worse blood flow, thus the access of O_2 and substrates to working muscles (Hoff et al. 1999; Storen et al. 2008; Sunde et al. 2010). Further, Fmax and ΔL increased as a function of the speed but, conversely to previous work (Arellano and Kram 2014), Δz did not change. Consequently, k_{leg} decreased as a function of the speed while k_{vert} increased. We speculate that since they are high-level ultra-marathoners they may be adapted to a different running pattern compared with short and middle distance runners. Nevertheless, new studies comparing these athletes are required in order to define possible differences in running technique.

In the present study SL and SF did not change after the training protocol, as previously shown by Ferrauti et al. (2010). These authors considered recreational marathon runners who underwent to 8-week intervention that consisted in two strength-training sessions per week. However, they showed increased t_c at 8.6 and 10.1 $km \cdot h^{-1}$ by ~3%. Our results agree with this work (Ferrauti et al. 2010), since t_c increased by mean +4.4±0.1% at 8, 10 and 12 $km \cdot h^{-1}$ at POST. According to the cost of generating force hypothesis (Kram 2000; Kram and Taylor 1990), running with a longer t_c should be more economical, since it requires slower and less expensive fibers and the force is applied in a longer period of time (Kram and Taylor 1990; Roberts et al. 1998). In agreement with this hypothesis the increased t_c could in part explain the lower Cr.

We reject our second hypothesis although MMP during the squat jump slightly increased (+5.1±12.1%, $p=0.174$) after the SEP training protocol. Anyway, this increase was not significant as opposed to previous studies' results (Hakkinen et al. 2003; Hoff et al. 2002; Millet et al. 2002). We suppose that for these athletes the training protocol was too light and probably they need to train with maximal loads to improve the MMP. Since ultra-endurance athletes are rarely professionals (no one among our subjects), we proposed a training protocol that athletes could easily perform at home three times per week without renouncing to their usual running training.
However, an inverse relationship between changes in MMP and changes in Cr at 10 and 12 $km \cdot h^{-1}$ (Fig. 2.5) suggests that athletes who slightly improved their MMP also decreased their Cr at submaximal speeds suggesting that MMP is an important parameter in determining Cr, as previously shown (Lazzer et al. 2015; Lazzer et al. 2014). Moreover, our group has reported that lower Cr values were related to higher MMP (Lazzer et al. 2014) and athletes with greater MMP had lower changes (i.e.

increase) in Cr during an uphill marathon (Lazzer et al. 2015). As well, in the same race MMP was inversely related with race time (Giovanelli et al. 2015b), emphasizing the importance of high MMP values in ultra-endurance events.

A number of adaptive mechanisms may be involved in the decrease of the Cr after a concurrent strength training protocol (Aagaard et al. 2011; Millet et al. 2002; Spurrs et al. 2003). We analyzed some aspects of the mechanics of running and the properties of the GM and triceps surae tendon, which did not change at POST. We reject our third hypothesis even if previous studies supported the idea that power training leads to a higher MTS (Millet et al. 2002; Spurrs et al. 2003) that affects positively the Cr (Arampatzis et al. 2006). Probably, high-level athletes need longer and/or heavier training protocol to stimulate tissue changes, whereas in a group of both physically active and untrained subjects 4 weeks of training were enough to increase the synthesis of collagen type I in triceps surae tendon (Langberg et al. 2001).

Conclusion

In conclusion, 12-week SEP training program led to a Cr improvement in high-level ultra-marathoners at different submaximal speeds. Increased t_c and an inverse relationship between changes in Cr and changes in MMP can partially explain the decreased Cr. Even if the mechanisms that led to a lower Cr are not clarified in high-level ultra-marathoners, we suggest to add at least three sessions per week of SEP exercises in the normal endurance training program.

Critique of methods

We acknowledge that our study has some limitations. First, athletes enrolled in this study were well trained and the training protocol they underwent was probably too light to induce significant improvements. We preferred to propose a training protocol that could be easily performed at home by not professional athletes and they could integrate this specific training in their habitual activities.

Second, since many of them had never performed strength or explosive training before, we decided to use the first four weeks of training as “adaptation” period. We assumed that this cycle was important to avoid discomfort or lower the injury risk. Indeed, it has been reported that core training improves endurance performance, likely by reducing Cr (Tong et al. 2014). Moreover, the muscles of the core have a critical role for the transfer of energy from the larger torso to the terminal segments, which may be more involved in the ability to control the position and motion of the trunk over the pelvis during running and allows a better force transfer to the extremities (Kibler et al. 2006). Further, some specific exercises (i.e. high knees, butt kicks…) could affect the running posture, which would lower Cr by moving the ground reaction force application point (Biewener et al. 2004; Lacour and Bourdin 2015). As well, exercises performed on a balance board may reduce the chances for lower back and extremity injuries and would allow exerting greater forces when there is an unstable situation (Behm and Colado 2012; Verhagen et al. 2005), which is common in trail running.

Conclusions of Part I

Our studies confirm that the maximal oxygen uptake, the fraction of it maintained throughout the race and the cost of transport are the main physiological parameters affecting the ultra-endurance performance, both on level and uphill (Lazzer et al. 2015; Lazzer et al. 2014).

Also, during a 6h-running race on a *flat* loop, athletes tend to change their running pattern after a certain time (~4 hours) rather than after a certain distance covered. This confirms previous results in which our group reported similar mechanical behavior during an *uphill* only marathon of similar duration (Giovanelli et al. 2015b). Moreover, when the slope becomes very steep athletes can reduce their energy expenditure by walking rather than running (Giovanelli et al. 2015a).

These results suggest that athletes may improve their performance by adding specific trainings to their training plan and trying to maintain a regular mechanical running pattern during the race. Also, to improve the performance on steep terrain they should add the walking gait during their training.

Further, we found a relationship between MMP and performance. Indeed, higher MMP was related to lower Cr and lower deterioration of the running pattern. Thus, our results suggest that an improvement in the MMP would lead to a more economic running pattern and a better performance.

In conclusion, by adding strength, explosive and power training to the usual endurance training it is possible to improve the cost of running (i.e. lower) without affecting other parameters. Many endurance athletes and coaches often underestimate the SEP training. We suggest to include at least three sessions per week of SEP training in the training program in order to optimize the performance through a lower Cr.

PART II

DEVELOPMENT OF INSOLE SHOE SENSOR FOR GAIT ANALYSIS: A PILOT STUDY

Introduction

Ground reaction forces are often investigated in gait biomechanics studies (Blickhan 1989; Cavanagh and Lafortune 1980; Girard et al. 2013; Gottschall and Kram 2005; Lieberman et al. 2015). The measurements are often limited by the positioning of force plates, which makes difficult to obtain data outdoor, in the field. Different devices have been developed to measure vertical and horizontal ground reaction forces in healthy subjects and patients with different pathologies. Davis et al. (1998) used a thin layer of strain gauge transducer whereas Razian and Pepper (2003) adopted piezoelectric copolymer film. Liedtke et al. (2007) used an instrumented shoe with two sensors mounted beneath the forefoot and rearfoot. However, these devices were limited to its specific purpose and thus were not readily available for other researchers. Further, they increased the height of the effective sole and also the weight of the sole altering the correct gait (Veltink et al. 2005).

Thus, the second part of my thesis is focused on designing, developing and validating a mechatronic device for gait data collection. Our objectives were to develop and validate an insole-shoe sensor to collect the vertical and horizontal GRF and the contact and aerial times. The device should be flexible, comfortable and low cost.

Shoe sensor equipment

The sensor equipment was composed by:

- A plastic or rubber insole
- Five piezoresistive force sensors (FSR, Tekscan Flexiforce A401)
- A s-beam load cell (Futek LSB200)
- A NI myRIO-1900 acquisition device
- A battery case

Insole

The very first prototype of our insole was a leather insole on which we fixed the FSRs (Fig. 3.1). With this model it was possible to collect data about the vertical GRF and the contact time, while we could not fix the load cell for the horizontal GRF because of the thin of the insole. The results of the first prototype are presented in the manuscript “A mechatronic system mounted on insole for analyzing human gait” by Giovanelli D et al.

presented at the International Conference on Robotiscs and Mechatronics in Iran (2014, see below).

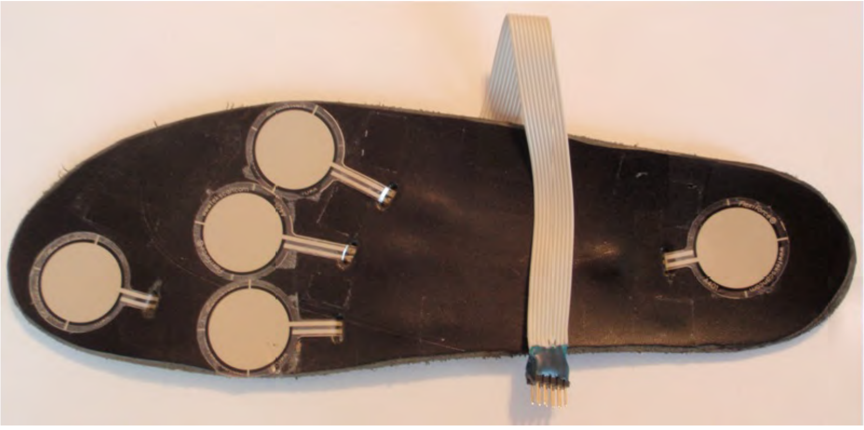


Fig. 3.1 - The leather insole with the 5 PFSs and the cable for the data acquisition

Then, in order to fit the load cell (which has a height of 6.7 mm) we re-designed the prototype using CAD 3D (Fig. 3.2).

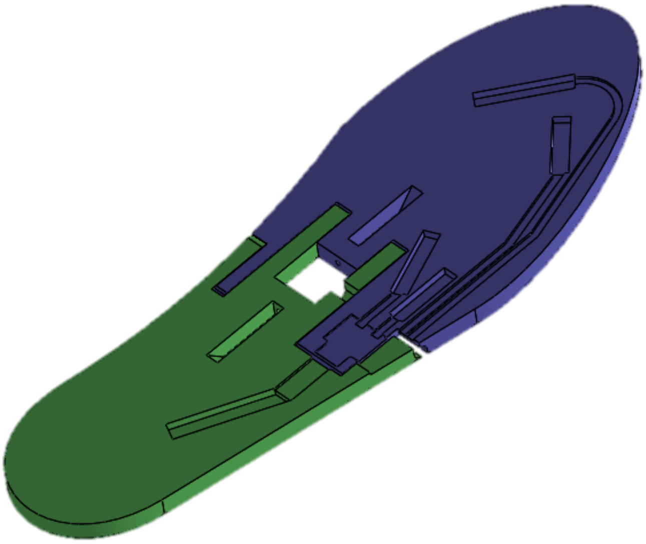


Fig. 3.2 - The 3D model designed with CAD 3D.

This model was composed by two plastic semi-insole (e.g. anterior and posterior, Fig. 3.3 A and B), printed with a 3D printer, which allowed us to fix the load cell in the middle (Fig. 3A).

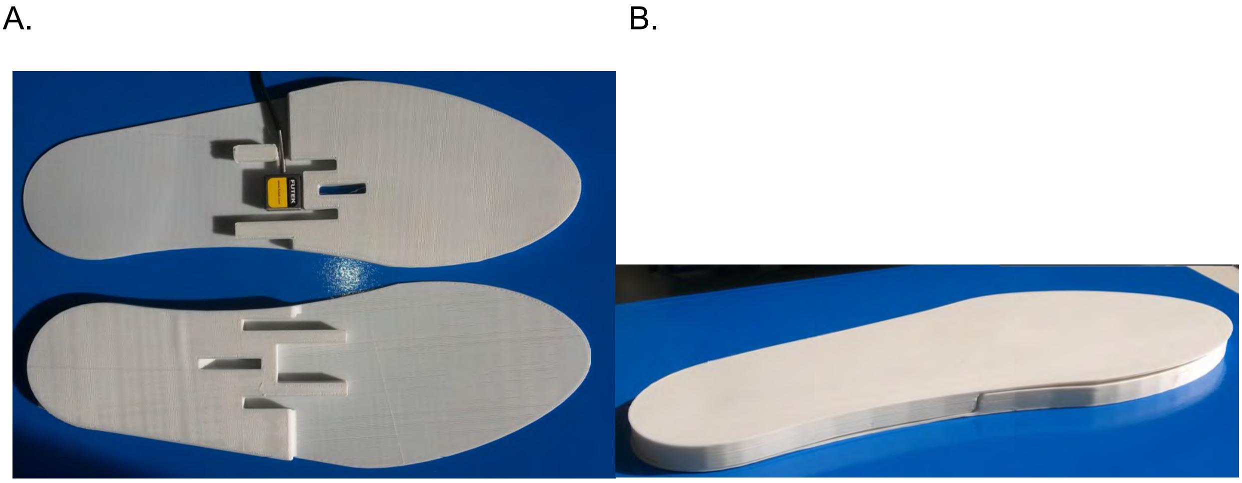


Fig. 3.3 - The two plastic semi-insole with the load cell divided (A) and united (B)

The plastic used in this model was not enough resistant and we had to choose a new material. The new prototype was made by thermoplastic polyurethane (TPU) 70 shore. The two semi-insoles were glued to a carbon sheet (thickness 0.2 mm) to assure a good rigidity. In order to guarantee a good slip we fixed a kapton polyimide film on the surface (Fig 3.4).

Fig 3.4 - The two TPU semi-insole with the kapton polyimide film

Resistive force sensors

In order to minimize the height of the insole we adopted piezoresistive force sensors (Tekscan Flexiforce A401, Fig. 3.5A). These sensors have a sensing area of 506 mm^2 (25.4 mm diameter) and they have a 2-pin connector. We used the sensors with a single voltage source circuit as recommended by the manufacturer (Fig. 3.5B).

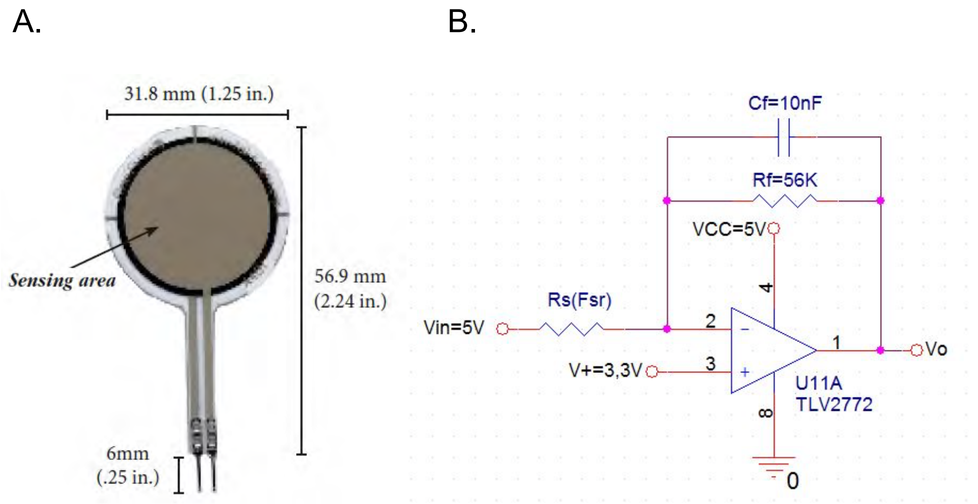


Fig. 3.5 - The piezoresistive force sensor (A) and the circuit adapted for the acquisition (B).

Sensors positioning

Different configurations in positioning the sensors have been attempted by different authors (Bamberg et al. 2008; Edgar 2010; Healy et al. 2012; Howell et al. 2013; Salpavaara 2009; Sazonov et al. 2011; Yan 2010). We adopted the one proposed by Sazonov et al. (2011) with a total of five sensors positioned in coincidence of the first toe, the first, the third and the fifth metatarsus and under the heel (Fig. 3.6).

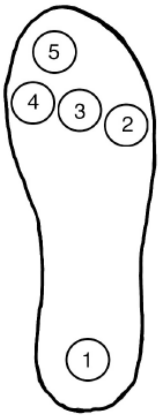


Fig. 3.6 - The sensors position as suggested by Sazanov et al. (2011)

Conversely to other prototypes (Edgar 2010) we preferred to use bigger sensors because after some trials we noted that with the smallest ones we had more noise in the signal processing.

Load cell

In order to measure the horizontal GRF we applied a load cell (JR S-Beam, Futek, LSB200) in the middle of the insole. The load cell was fixed with two screw in the place prepared in the insole. The connection wire was positioned in the right side of the prototype (Fig. 3.7 and 3.8).

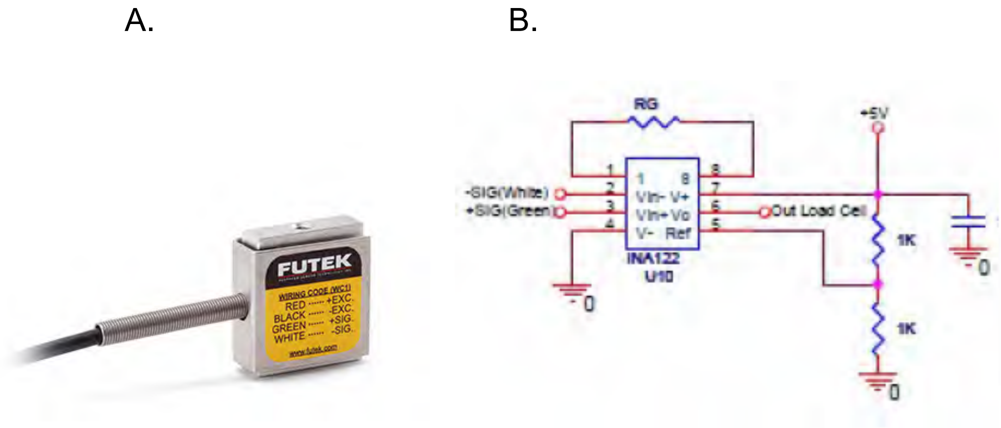
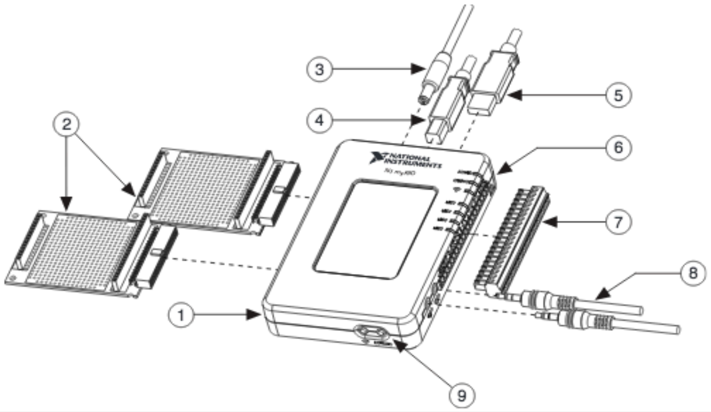


Fig 3.7 - The load cell we used for the acquisition of the horizontal GRF (A) and the amplification circuit adapted for the acquisition (B).

Fig. 3.8 – The whole insole with the five FSRs and the load cell.

Acquisition device and battery case

The data recorded by the load cells and the FSRs were logged into a specific portable acquisition device (NI myRIO-1900, Fig. 3.10), which provides for signal conditioning and data logging. The acquisition device was placed in a belt and was powered by a 4v battery positioned in a battery case. Data were acquired in a flash drive and then copied into a PC and analyzed with a Matlab customized program.



1	NI myRIO-1900	6	LEDs
2	myRIO Expansion Port (MXP) Breakouts (One Included in Kit)	7	Mini System Port (MSP) Screw-Terminal Connector
3	Power Input Cable	8	Audio In/Out Cables (One Included in Kit)
4	USB Device Cable	9	Button0
5	USB Host Cable (Not Included in Kit)		

Fig.3.10 - the NI myRIO-1900

Validation procedures

Participants

Three healthy individuals (27.6±2.5 yr; 69.3±3.5 kg) took part in the study. None of the participants had any history of physical or neurological conditions that might interfere with their respective gait. Written informed consent was obtained from all participants and the experimental protocol was approved by the ethical committee of the University of Udine.

Insole

The TPC insole weighs 175 g. The width was 90 mm and the length was 270 mm (Fig. 3.11). Although the thickness was 6.8 mm it fits good in a shoe half number bigger than usual subjects' shoes.

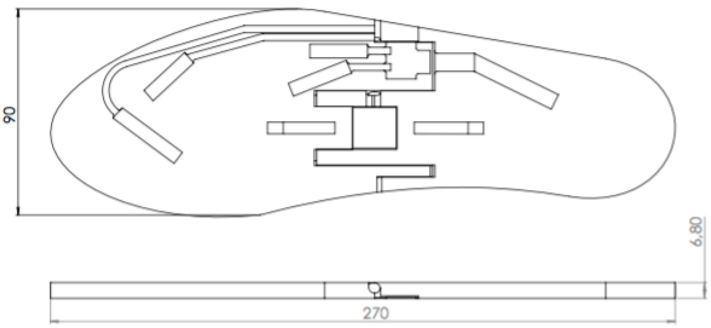


Fig. 3.11 – Details of the insole

Calibration of the sensors

Before every trial the sensors were calibrated using known weights. The calibration of the FSRs was performed after the sensors were fixed on the insole as suggested by the manufacturer. We used eleven different weights (see Table 3.1 and Fig. 3.12) and we averaged the values (in volt, V) obtained from three attempts with a customized LabView program.

Weight (kg)	Newton (N)	FSR output tension [V]			Averaged value [V]
0	0	3.28	3.22	3.26	3.25
0.1	1.4	3.27	3.21	3.25	3.24
1.2	11.4	3.12	3.00	3.09	3.07
2.2	21.2	2.93	2.87	2.91	2.90
3.2	31.2	2.83	2.81	2.83	2.82
4.2	41.4	2.8	2.81	2.76	2.79
5.4	52.7	2.56	2.55	2.56	2.56
6.2	60.9	2.36	2.34	2.39	2.36
7.4	72.4	2.15	2.12	2.16	2.14
8.2	80.8	2.07	2.1	2.04	2.07
10.7	105.4	1.49	1.53	1.56	1.53
12.8	125.2	1.27	1.22	1.25	1.25

Table 3.1 – Calibration of the FSR sensors

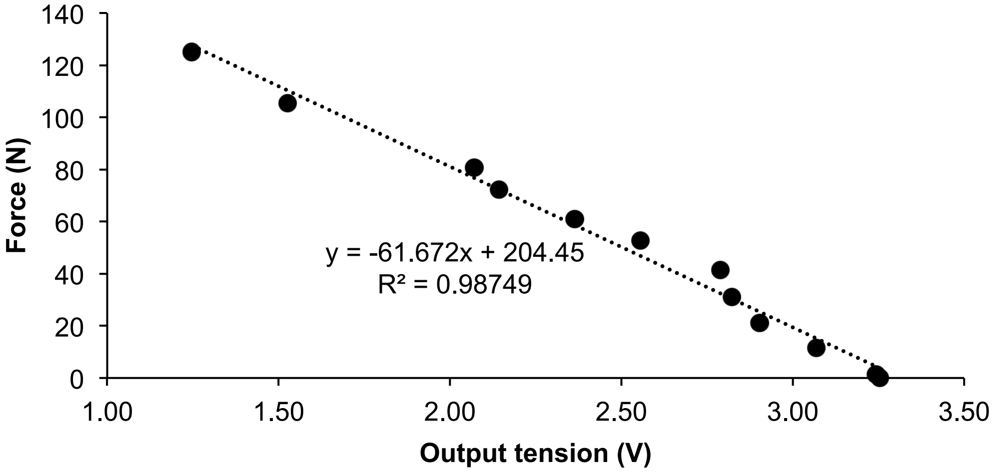


Fig 3.12 – Calibration curve of the FSR sensors

The calibration of the load cell was performed in compression and in traction. We used 16 different weights (Table 3.2 and Fig. 3.13 A and B) and the output tension (in volt, V) was recorded using a customized LabView program.

Weight (kg)	Newton (N)	Output tension Compression (V)	Traction (V)
0.0	0.0	2.59	2.59
1.2	11.4	2.45	2.75
2.2	21.2	2.34	2.85
3.2	31.2	2.22	2.96
4.2	41.4	2.11	3.07
5.4	52.7	1.99	3.20
6.2	61.0	1.88	3.29
7.4	72.4	1.77	3.40
8.2	80.8	1.66	3.52
9.3	91.6	1.55	3.64
10.4	101.7	1.44	3.77
11.4	111.7	1.33	3.87
12.4	121.7	1.22	3.99
13.4	131.8	1.11	4.10
14.5	141.8	0.99	4.22
15.5	151.8	0.88	4.34
16.5	161.8	0.77	4.45

Table 3.2 – Calibration of the load cell

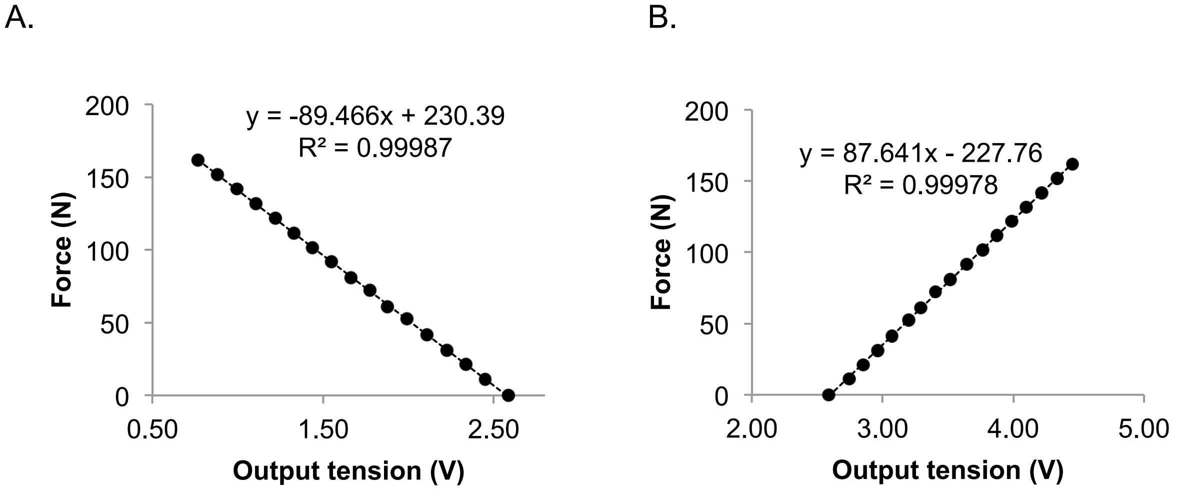


Fig. 3.13 – Calibration curve of the load cell in compression (A) and in traction (B)

Experimental protocol

Every participant performed three runs along a 30 m-runway in which a force plate was positioned. They were instructed to run at three different speeds (10, 12 and 14 $km \cdot h^{-1}$, respectively). Practice trials were permitted to allow subjects to become familiar with the running speeds. A successful trial was defined when the foot in which the sensorized insole was placed (the right) fell within borders of the force plate from initial contact to toe-off, and the running speed was within ±5% of the target speed (Teng and Powers 2015). At every condition 10 steps per subject were analyzed and a total of 30 steps for each speed was used for the validation. Data were continuously registered at 1000 Hz in a flash drive and then analyzed with a customized Matlab program. The data obtained were compared with those collected by a force plate (Kistler, 9281 E) positioned in the track field.

Statistical analysis

Mean and standard deviation were calculated independently for every gait parameter measured at each speed. Bland-Altman test was performed in order to validate the obtained Fmax and t_c, $p<0.05$ was defined as statistically significant.

Results

Contact times recorded with the RFSs were higher than t_c collected with the force plate (+2.47%, p=0.001; Fig. 3.14 A and B). Further, contact time decreased by increasing the speed by mean -9.02% (p<0.05) when compared with 10 km·h^{-1}.

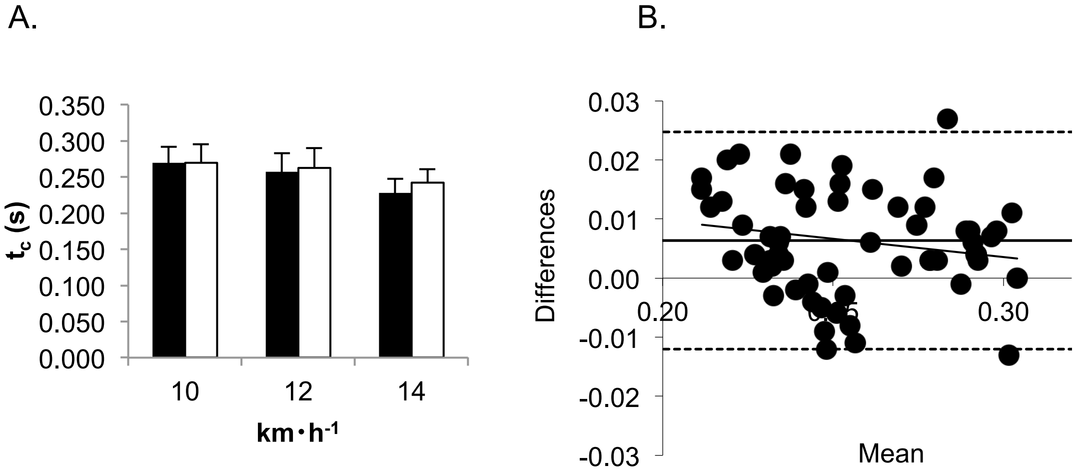


Fig. 3.14 – A. Contact time (tc, in s) measured with the force plate (black bars) and with the insole sensor (white bars) at 10, 12 and 14 km·h^{-1}. B, the Bland-Altman plot for the contact time.

No differences in Fmax were detected at the three speeds analyzed between the force plate and the insole sensors (p>0.05, Fig. 3.15 A and B). Further, Fmax increased by increasing the speed by mean +4.61% (p<0.05) when compared with 10 km·h^{-1}.

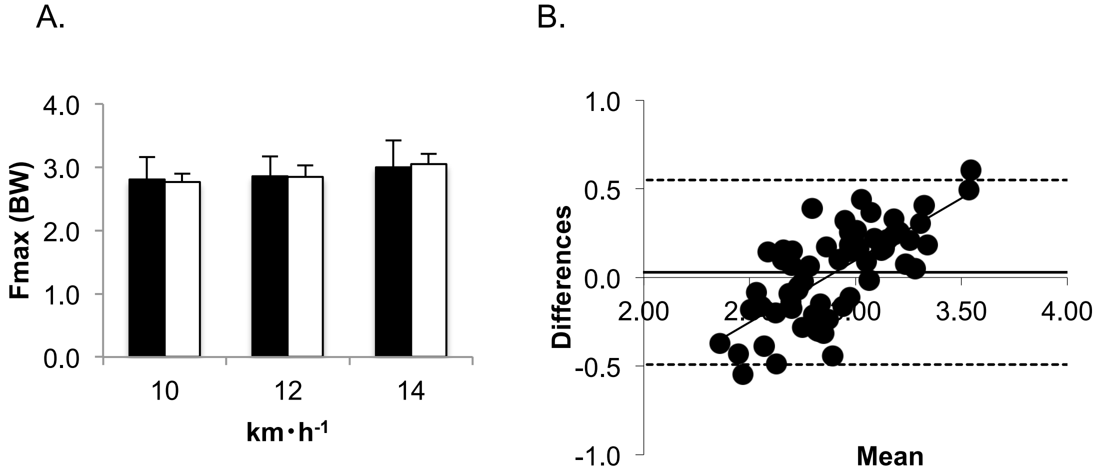


Fig. 3.15 – A. Maximal vertical ground reaction force (Fmax, in BW) measured with the force plate (black bars) and with the insole sensor (white bars) at 10, 12 and 14 km·h^{-1}. B, the Bland-Altman plot for the Fmax.

Discussion

The aim of the present study was to develop and validate an insole shoe sensor for detecting vertical and horizontal ground reaction forces and contact times. Our prototype allows the measurements of the Fmax and t_c with a small error compared with the gold standard. Although the difference between the t_c measured with the sensors is statistically significant, the error is ~2% which is accetable when the running gait is analyzed (Giovanelli et al. 2015b; Lazzer et al. 2015; Morin et al. 2011b).

With this device we can obtain the Fmax and t_c and we can compute other parameters by using the formula proposed by Morin et al. (2005). With this method we can calculate the vertical displacement of the centre of mass and the vertical stiffness, which are parameters commonly used in the gait analysis.

We developed our prototype by trying different solutions in materials and sensors positioning. At the end we obtained a confortable tool which could be worn by the subjects with no trouble during running.

Limitations

This study has several limitations, one being the low number of participants. However, this was a pilot study to clarify if it was possible to develop a sensor for measuring some mechanical parameters.

However, during our experiments we attempted to collect the horizontal GRF with a small load cell. After few trials we identified some issues with the load cell. Although we tried to use some slippery materials in order to obtain two semi-insole which were able to slip on each other, this was not enough and the load cell experienced some problems in recording the data. After trying different solutions we decided to renounce in collecting the horizontal GRF.

Conclusion

We developed a device which can be used in detecting the vertical GRF and contact time during overground running without using force platforms. This prototype is comfortable and can be worn by athletes during running.

REFERENCES

Aagaard P et al. (2011) Effects of resistance training on endurance capacity and muscle fiber composition in young top-level cyclists Scand J Med Sci Sports 21:e298-307 doi:10.1111/j.1600-0838.2010.01283.x

Abe D, Fukuoka Y, Horiuchi M (2015) Economical Speed and Energetically Optimal Transition Speed Evaluated by Gross and Net Oxygen Cost of Transport at Different Gradients PLoS One 10:e0138154 doi:10.1371/journal.pone.0138154

Arampatzis A, Bruggemann GP, Metzler V (1999) The effect of speed on leg stiffness and joint kinetics in human running J Biomech 32:1349-1353 doi:S0021-9290(99)00133-5 [pii]

Arampatzis A, De Monte G, Karamanidis K, Morey-Klapsing G, Stafilidis S, Bruggemann GP (2006) Influence of the muscle-tendon unit's mechanical and morphological properties on running economy J Exp Biol 209:3345-3357 doi:209/17/3345 [pii] 10.1242/jeb.02340

Arellano CJ, Kram R (2014) Partitioning the metabolic cost of human running: a task-by-task approach Integr Comp Biol 54:1084-1098 doi:10.1093/icb/icu033

Bamberg SJ, Benbasat AY, Scarborough DM, Krebs DE, Paradiso JA (2008) Gait analysis using a shoe-integrated wireless sensor system IEEE Trans Inf Technol Biomed 12:413-423 doi:10.1109/TITB.2007.899493

Barnes KR, Kilding AE (2015) Strategies to improve running economy Sports Med 45:37-56 doi:10.1007/s40279-014-0246-y

Bassett DR, Jr., Howley ET (2000) Limiting factors for maximum oxygen uptake and determinants of endurance performance Med Sci Sports Exerc 32:70-84

Beaver WL, Wasserman K, Whipp BJ (1986) A new method for detecting anaerobic threshold by gas exchange J Appl Physiol 60:2020-2027.

Behm D, Colado JC (2012) The effectiveness of resistance training using unstable surfaces and devices for rehabilitation Int J Sports Phys Ther 7:226-241

Biewener AA, Farley CT, Roberts TJ, Temaner M (2004) Muscle mechanical advantage of human walking and running: implications for energy cost J Appl Physiol (1985) 97:2266-2274 doi:10.1152/japplphysiol.00003.2004

Billat V, Renoux JC, Pinoteau J, Petit B, Koralsztein JP (1994) Times to exhaustion at 100% of velocity at VO2max and modelling of the time-limit/velocity relationship in elite long-distance runners Eur J Appl Physiol Occup Physiol 69:271-273

Billat VL, Flechet B, Petit B, Muriaux G, Koralsztein JP (1999) Interval training at VO2max: effects on aerobic performance and overtraining markers Med Sci Sports Exerc 31:156-163

Blickhan R (1989) The spring-mass model for running and hopping J Biomech 22:1217-1227 doi:0021-9290(89)90224-8 [pii]

Bramble DM, Lieberman DE (2004) Endurance running and the evolution of Homo Nature 432:345-352 doi:10.1038/nature03052

Brueckner JC et al. (1991) The energy cost of running increases with the distance covered Eur J Appl Physiol Occup Physiol 62:385-389.

Cavagna GA, Heglund NC, Taylor CR (1977) Mechanical work in terrestrial locomotion: two basic mechanisms for minimizing energy expenditure Am J Physiol 233:R243-261

Cavagna GA, Kaneko M (1977) Mechanical work and efficiency in level walking and running J Physiol 268:467--481

Cavagna GA, Saibene FP, Margaria R (1964) Mechanical Work in Running J Appl Physiol 19:249-256

Cavagna GA, Thys H, Zamboni A (1976) The sources of external work in level walking and running J Physiol 262:639-657

Cavanagh PR, Kram R (1989) Stride length in distance running: velocity, body dimensions, and added mass effects Med Sci Sports Exerc 21:467-479

Cavanagh PR, Lafortune MA (1980) Ground reaction forces in distance running J Biomech 13:397-406

Cavanagh PR, Williams KR (1982) The effect of stride length variation on oxygen uptake during distance running Med Sci Sports Exerc 14:30-35

Cohen J (1992) A power primer Psychol Bull 112:155-159

Craig CL et al. (2003) International physical activity questionnaire: 12-country reliability and validity Med Sci Sports Exerc 35:1381-1395.

Dalleau G, Belli A, Bourdin M, Lacour JR (1998) The spring-mass model and the energy cost of treadmill running Eur J Appl Physiol Occup Physiol 77:257-263

Davies CT, Thompson MW (1979) Aerobic performance of female marathon and male ultramarathon athletes Eur J Appl Physiol Occup Physiol 41:233-245

Davies CT, Thompson MW (1986) Physiological responses to prolonged exercise in ultramarathon athletes J Appl Physiol 61:611-617

Davis BL, Perry JE, Neth DC, Waters KC (1998) A Device for Simultaneous Measurement of Pressure and Shear Force Distribution on the Plantar Surface of the Foot J Appl Biomech 14:93-104

Degache F, Guex K, Fourchet F, Morin JB, Millet GP, Tomazin K, Millet GY (2013) Changes in running mechanics and spring-mass behaviour induced by a 5-hour hilly running bout J Sports Sci 31:299-304 doi:10.1080/02640414.2012.729136

Dempsey JA, Hanson PG, Henderson KS (1984) Exercise-induced arterial hypoxaemia in healthy human subjects at sea level J Physiol 355:161-175

di Prampero PE (1986) The energy cost of human locomotion on land and in water Int J Sports Med 7:55-72.

di Prampero PE (2003) Factors limiting maximal performance in humans Eur J Appl Physiol 90:420-429. Epub 2003 Aug 2009.

di Prampero PE, Atchou G, Bruckner JC, Moia C (1986) The energetics of endurance running Eur J Appl Physiol Occup Physiol 55:259-266.

di Prampero PE, Salvadego D, Fusi S, Grassi B (2009) A simple method for assessing the energy cost of running during incremental tests J Appl Physiol 107:1068-1075. Epub 2009 Aug 1066.

Dierks TA, Davis IS, Hamill J (2010) The effects of running in an exerted state on lower extremity kinematics and joint timing J Biomech 43:2993-2998 doi:10.1016/j.jbiomech.2010.07.001

Dutto DJ, Smith GA (2002) Changes in spring-mass characteristics during treadmill running to exhaustion Med Sci Sports Exerc 34:1324-1331

Edgar SRS, T.; Fulk, G.; Sazonov, E.S. Wearable Shoe-Based Device for Rehabilitation of Stroke Patients. In: Proceeding of 2010 Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), Buenos Aires, Argentina, 2010.

Ekblom B, Wilson G, Astrand PO (1976) Central circulation during exercise after venesection and reinfusion of red blood cells J Appl Physiol 40:379-383

Enoksen E, Shalfawi SA, Tonnessen E (2011) The effect of high- vs. low-intensity training on aerobic capacity in well-trained male middle-distance runners J Strength Cond Res 25:812-818 doi:10.1519/JSC.0b013e3181cc2291

Farley CT, Blickhan R, Saito J, Taylor CR (1991) Hopping frequency in humans: a test of how springs set stride frequency in bouncing gaits J Appl Physiol (1985) 71:2127-2132

Farley CT, Ferris DP (1998) Biomechanics of walking and running: center of mass movements to muscle action Exerc Sport Sci Rev 26:253-285

Farley CT, Gonzalez O (1996) Leg stiffness and stride frequency in human running J Biomech 29:181-186

Fenn WO (1930) Work against gravity and work due to velocity changes in running. Am J Physiol 93:433-462

Fernstrom M et al. (2007) Reduced efficiency, but increased fat oxidation, in mitochondria from human skeletal muscle after 24-h ultraendurance exercise J Appl Physiol (1985) 102:1844-1849 doi:10.1152/japplphysiol.01173.2006

Ferrauti A, Bergermann M, Fernandez-Fernandez J (2010) Effects of a concurrent strength and endurance training on running performance and running economy in recreational marathon runners J Strength Cond Res 24:2770-2778 doi:10.1519/JSC.0b013e3181d64e9c

Fourchet F, Girard O, Kelly L, Horobeanu C, Millet GP (2015) Changes in leg spring behaviour, plantar loading and foot mobility magnitude induced by an exhaustive treadmill run in adolescent middle-distance runners J Sci Med Sport 18:199-203 doi:10.1016/j.jsams.2014.01.007

Fourchet F et al. (2012) Effects of a 5-h hilly running on ankle plantar and dorsal flexor force and fatigability Eur J Appl Physiol 112:2645-2652 doi:10.1007/s00421-011-2220-9

Gimenez P, Kerherve H, Messonnier LA, Feasson L, Millet GY (2013) Changes in the Energy Cost of Running during a 24-h Treadmill Exercise Med Sci Sports Exerc 45:1807-1813 doi:10.1249/MSS.0b013e318292c0ec

Giovanelli N, Ortiz AL, Henninger K, Kram R (2015a) Energetics of vertical kilometer foot races; is steeper cheaper? J Appl Physiol (1985):jap 00546 02015 doi:10.1152/japplphysiol.00546.2015

Giovanelli N, Taboga P, Rejc E, Simunic B, Antonutto G, Lazzer S (2015b) Effects of an Uphill Marathon on Running Mechanics and Lower Limb Muscles Fatigue Int J Sports Physiol Perform doi:10.1123/ijspp.2014-0602

Girard O, Millet GP, Slawinski J, Racinais S, Micallef JP (2013) Changes in running mechanics and spring-mass behaviour during a 5-km time trial Int J Sports Med 34:832-840 doi:10.1055/s-0032-1329958

Gledhill N (1982) Blood doping and related issues: a brief review Med Sci Sports Exerc 14:183-189

Gledhill N (1985) The influence of altered blood volume and oxygen transport capacity on aerobic performance Exerc Sport Sci Rev 13:75-93

Gottschall JS, Kram R (2005) Ground reaction forces during downhill and uphill running J Biomech 38:445-452 doi:10.1016/j.jbiomech.2004.04.023

Hakkinen K et al. (2003) Neuromuscular adaptations during concurrent strength and endurance training versus strength training Eur J Appl Physiol 89:42-52 doi:10.1007/s00421-002-0751-9

Hanon C, Lepretre PM, Bishop D, Thomas C (2010) Oxygen uptake and blood metabolic responses to a 400-m run Eur J Appl Physiol 109:233-240 doi:10.1007/s00421-009-1339-4

Hawley JA, Hopkins WG (1995) Aerobic glycolytic and aerobic lipolytic power systems. A new paradigm with implications for endurance and ultraendurance events Sports Med 19:240-250

Healy A, Burgess-Walker P, Naemi R, Chockalingam N (2012) Repeatability of WalkinSense(R) in shoe pressure measurement system: A preliminary study Foot (Edinb) 22:35-39 doi:10.1016/j.foot.2011.11.001

Heise GD, Martin PE (1998) "Leg spring" characteristics and the aerobic demand of running Med Sci Sports Exerc 30:750-754

Hobara H, Inoue K, Gomi K, Sakamoto M, Muraoka T, Iso S, Kanosue K (2010) Continuous change in spring-mass characteristics during a 400 m sprint J Sci Med Sport 13:256-261 doi:10.1016/j.jsams.2009.02.002

Hoff J, Gran A, Helgerud J (2002) Maximal strength training improves aerobic endurance performance Scand J Med Sci Sports 12:288-295 doi:001140 [pii]

Hoff J, Helgerud J, Wisloff U (1999) Maximal strength training improves work economy in trained female cross-country skiers Med Sci Sports Exerc 31:870-877

Hoffman MD (2014) Pacing by winners of a 161-km mountain ultramarathon Int J Sports Physiol Perform 9:1054-1056 doi:10.1123/ijspp.2013-0556

Horowitz JF, Sidossis LS, Coyle EF (1994) High efficiency of type I muscle fibers improves performance Int J Sports Med 15:152-157.

Howell AM, Kobayashi T, Hayes HA, Foreman KB, Bamberg SJ (2013) Kinetic Gait Analysis Using a Low-Cost Insole IEEE Trans Biomed Eng 60:3284-3290 doi:10.1109/TBME.2013.2250972

Kibler WB, Press J, Sciascia A (2006) The role of core stability in athletic function Sports Med 36:189-198

Kram R (2000) Muscular force or work: what determines the metabolic energy cost of running? Exerc Sport Sci Rev 28:138-143

Kram R, Taylor CR (1990) Energetics of running: a new perspective Nature 346:265-267 doi:10.1038/346265a0

Lacour JR, Bourdin M (2015) Factors affecting the energy cost of level running at submaximal speed Eur J Appl Physiol 115:651-673 doi:10.1007/s00421-015-3115-y

Langberg H, Rosendal L, Kjaer M (2001) Training-induced changes in peritendinous type I collagen turnover determined by microdialysis in humans J Physiol 534:297-302

Laursen PB, Rhodes EC (2001) Factors affecting performance in an ultraendurance triathlon Sports Med 31:195-209.

Laursen PB, Shing CM, Peake JM, Coombes JS, Jenkins DG (2002) Interval training program optimization in highly trained endurance cyclists Med Sci Sports Exerc 34:1801-1807 doi:10.1249/01.MSS.0000036691.95035.7D

Lazzer S, Salvadego D, Rejc E, Buglione A, Antonutto G, di Prampero PE (2012) The energetics of ultra-endurance running Eur J Appl Physiol 112:1709-1715 doi:10.1007/s00421-011-2120-z

Lazzer S, Salvadego D, Taboga P, Rejc E, Giovanelli N, di Prampero PE (2015) Effects of the Etna uphill ultramarathon on energy cost and mechanics of running Int J Sports Physiol Perform 10:238-247 doi:10.1123/ijspp.2014-0057

Lazzer S et al. (2014) Factors affecting metabolic cost of transport during a multi-stage running race J Exp Biol 217:787-795 doi:10.1242/jeb.091645

Lieberman DE, Warrener AG, Wang J, Castillo ER (2015) Effects of stride frequency and foot position at landing on braking force, hip torque, impact peak force and the metabolic cost of running in humans J Exp Biol 218:3406-3414 doi:10.1242/jeb.125500

Liedtke C, Fokkenrood SA, Menger JT, van der Kooij H, Veltink PH (2007) Evaluation of instrumented shoes for ambulatory assessment of ground reaction forces Gait Posture 26:39-47 doi:10.1016/j.gaitpost.2006.07.017

Lukaski HC, Bolonchuk WW, Hall CB, Siders WA (1986) Validation of tetrapolar bioelectrical impedance method to assess human body composition J Appl Physiol 60:1327-1332.

Margaria R (1938) Sulla fisiologia e specialmente sul consumo energetico della marcia e della corsa a varia velocita` ed inclinazione del terreno vol 7.

Margaria R, Cerretelli P, Aghemo P, Sassi G (1963) Energy cost of running J Appl Physiol 18:367-370

Martin V et al. (2010) Central and peripheral contributions to neuromuscular fatigue induced by a 24-h treadmill run J Appl Physiol (1985) 108:1224-1233 doi:10.1152/japplphysiol.01202.2009

McMahon TA, Cheng GC (1990) The mechanics of running: how does stiffness couple with speed? J Biomech 23 Suppl 1:65-78

McMahon TA, Valiant G, Frederick EC (1987) Groucho running J Appl Physiol (1985) 62:2326-2337

Mercier J, Le Gallais D, Durand M, Goudal C, Micallef JP, Prefaut C (1994) Energy expenditure and cardiorespiratory responses at the transition between walking and running Eur J Appl Physiol Occup Physiol 69:525-529

Midgley AW, McNaughton LR, Wilkinson M (2006) Is there an optimal training intensity for enhancing the maximal oxygen uptake of distance runners?: empirical research findings, current opinions, physiological rationale and practical recommendations Sports Med 36:117-132

Millet GP, Jaouen B, Borrani F, Candau R (2002) Effects of concurrent endurance and strength training on running economy and .VO(2) kinetics Med Sci Sports Exerc 34:1351-1359

Millet GY (2011) Can neuromuscular fatigue explain running strategies and performance in ultra-marathons?: the flush model Sports Med 41:489-506 doi:5 [pii] 10.2165/11588760-000000000-00000

Millet GY et al. (2011a) Physiological and biological factors associated with a 24 h treadmill ultra-marathon performance Scand J Med Sci Sports 21:54-61 doi:SMS1001 [pii] 10.1111/j.1600-0838.2009.01001.x

Millet GY, Morin JB, Degache F, Edouard P, Feasson L, Verney J, Oullion R (2009) Running from Paris to Beijing: biomechanical and physiological consequences Eur J Appl Physiol 107:731-738. Epub 2009 Sep 2016.

Millet GY et al. (2011b) Neuromuscular consequences of an extreme mountain ultra-marathon PLoS One 6:e17059 doi:10.1371/journal.pone.0017059

Minetti AE, Ardigo LP, Saibene F (1994a) Mechanical determinants of the minimum energy cost of gradient running in humans J Exp Biol 195:211-225

Minetti AE, Ardigo LP, Saibene F (1994b) The transition between walking and running in humans: metabolic and mechanical aspects at different gradients Acta Physiol Scand 150:315-323 doi:10.1111/j.1748-1716.1994.tb09692.x

Minetti AE, Moia C, Roi GS, Susta D, Ferretti G (2002) Energy cost of walking and running at extreme uphill and downhill slopes J Appl Physiol 93:1039-1046 doi:10.1152/japplphysiol.01177.2001

Morin JB, Dalleau G, Kyrolainen H, Jeannin T, Belli A (2005) A simple method for measuring stiffness during running J Appl Biomech 21:167-180

Morin JB, Samozino P, Millet GY (2011a) Changes in running kinematics, kinetics, and spring-mass behavior over a 24-h run Med Sci Sports Exerc 43:829-836 doi:10.1249/MSS.0b013e3181fec518

Morin JB, Tomazin K, Edouard P, Millet GY (2011b) Changes in running mechanics and spring-mass behavior induced by a mountain ultra-marathon race J Biomech 44:1104-1107 doi:S0021-9290(11)00070-4 [pii] 10.1016/j.jbiomech.2011.01.028

Novacheck TF (1998) The biomechanics of running Gait Posture 7:77-95

Paavolainen L, Hakkinen K, Hamalainen I, Nummela A, Rusko H (1999) Explosive-strength training improves 5-km running time by improving running economy and muscle power J Appl Physiol 86:1527-1533

Padulo J, Annino G, Migliaccio GM, D'Ottavio S, Tihanyi J (2012) Kinematics of running at different slopes and speeds J Strength Cond Res 26:1331-1339 doi:10.1519/JSC.0b013e318231aafa

Rabita G, Slawinski J, Girard O, Bignet F, Hausswirth C (2011) Spring-mass behavior during exhaustive run at constant velocity in elite triathletes Med Sci Sports Exerc 43:685-692 doi:10.1249/MSS.0b013e3181fb3793

Razian MA, Pepper MG (2003) Design, development, and characteristics of an in-shoe triaxial pressure measurement transducer utilizing a single element of piezoelectric copolymer film IEEE Trans Neural Syst Rehabil Eng 11:288-293

Rejc E, Lazzer S, Antonutto G (2010) Energy expenditure and dietary intake of athletes during an ultraendurance event developed by hiking, cycling and mountain climbing J Sports Med Phys Fitness 50:296-302 doi:R40102769 [pii]

Renoux JC, Petit B, Billat V, Koralsztein JP (2000) Calculation of times to exhaustion at 100 and 120% maximal aerobic speed Ergonomics 43:160-166 doi:10.1080/001401300184530

Roberts TJ, Kram R, Weyand PG, Taylor CR (1998) Energetics of bipedal running. I. Metabolic cost of generating force J Exp Biol 201:2745-2751

Ronnestad BR, Kojedal O, Losnegard T, Kvamme B, Raastad T (2012) Effect of heavy strength training on muscle thickness, strength, jump performance, and endurance performance in well-trained Nordic Combined athletes Eur J Appl Physiol 112:2341-2352 doi:10.1007/s00421-011-2204-9

Ronnestad BR, Mujika I (2014) Optimizing strength training for running and cycling endurance performance: A review Scand J Med Sci Sports 24:603-612 doi:10.1111/sms.12104

Rubenson J, Heliams DB, Lloyd DG, Fournier PA (2004) Gait selection in the ostrich: mechanical and metabolic characteristics of walking and running with and without an aerial phase Proc Biol Sci 271:1091-1099 doi:10.1098/rspb.2004.2702

Salpavaara TV, J.; Lekkala, J.; Halttunen, J. Wireless Insole Sensor System for Plantar Force Measurements during Sport Events. In: Proceedings of IMEKO XIX World Congress on Fundamental and Applied Metrology, Lisbon, Portugal, 2009.

Saugy J, Place N, Millet GY, Degache F, Schena F, Millet GP (2013) Alterations of Neuromuscular Function after the World's Most Challenging Mountain Ultra-Marathon PLoS One 8:e65596 doi:10.1371/journal.pone.0065596

Saunders PU, Pyne DB, Telford RD, Hawley JA (2004) Factors affecting running economy in trained distance runners Sports Med 34:465-485 doi:3475 [pii]

Sazonov ES, Fulk G, Hill J, Schutz Y, Browning R (2011) Monitoring of posture allocations and activities by a shoe-based wearable sensor IEEE Trans Biomed Eng 58:983-990 doi:10.1109/TBME.2010.2046738

Schena F, Pellegrini B, Tarperi C, Calabria E, Salvagno GL, Capelli C (2014) Running economy during a simulated 60-km trial Int J Sports Physiol Perform 9:604-609 doi:10.1123/ijspp.2013-0302

Snyder KL, Farley CT (2011) Energetically optimal stride frequency in running: the effects of incline and decline J Exp Biol 214:2089-2095 doi:10.1242/jeb.053157

Snyder KL, Kram R, Gottschall JS (2012) The role of elastic energy storage and recovery in downhill and uphill running J Exp Biol 215:2283-2287 doi:10.1242/jeb.066332

Spurrs RW, Murphy AJ, Watsford ML (2003) The effect of plyometric training on distance running performance Eur J Appl Physiol 89:1-7 doi:10.1007/s00421-002-0741-y

Steudel-Numbers KL, Wall-Scheffler CM (2009) Optimal running speed and the evolution of hominin hunting strategies J Hum Evol 56:355-360 doi:10.1016/j.jhevol.2008.11.002

Storen O, Helgerud J, Stoa EM, Hoff J (2008) Maximal strength training improves running economy in distance runners Med Sci Sports Exerc 40:1087-1092 doi:10.1249/MSS.0b013e318168da2f

Sunde A, Storen O, Bjerkaas M, Larsen MH, Hoff J, Helgerud J (2010) Maximal strength training improves cycling economy in competitive cyclists J Strength Cond Res 24:2157-2165 doi:10.1519/JSC.0b013e3181aeb16a

Teng HL, Powers CM (2015) Influence of trunk posture on lower extremity energetics during running Med Sci Sports Exerc 47:625-630 doi:10.1249/MSS.0000000000000436

Tong TK, McConnell AK, Lin H, Nie J, Zhang H, Wang J (2014) 'Functional' inspiratory and core muscle training enhances running performance and economy J Strength Cond Res doi:10.1519/JSC.0000000000000656

Turner AM, Owings M, Schwane JA (2003) Improvement in running economy after 6 weeks of plyometric training J Strength Cond Res 17:60-67

Veltink PH, Liedtke C, Droog E, van der Kooij H (2005) Ambulatory measurement of ground reaction forces IEEE Trans Neural Syst Rehabil Eng 13:423-427 doi:10.1109/TNSRE.2005.847359

Verhagen EA, van Tulder M, van der Beek AJ, Bouter LM, van Mechelen W (2005) An economic evaluation of a proprioceptive balance board training programme for the prevention of ankle sprains in volleyball Br J Sports Med 39:111-115 doi:10.1136/bjsm.2003.011031

Vernillo G, Rinaldo N, Giorgi A, Esposito F, Trabucchi P, Millet GP, Schena F (2015a) Changes in lung function during an extreme mountain ultramarathon Scand J Med Sci Sports 25:e374-380 doi:10.1111/sms.12325

Vernillo G et al. (2015b) Energy cost and kinematics of level, uphill and downhill running: fatigue-induced changes after a mountain ultramarathon J Sports Sci 33:1998-2005 doi:10.1080/02640414.2015.1022870

Vernillo G, Savoldelli A, Zignoli A, Trabucchi P, Pellegrini B, Millet GP, Schena F (2014) Influence of the world's most challenging mountain ultra-marathon on energy cost and running mechanics Eur J Appl Physiol 114:929-939 doi:10.1007/s00421-014-2824-y

Yan CM, Z. Measurement of In-Shoe Plantar Triaxial Stresses in High-Heeled Shoes. In: Proceeding of 2010 3rd International Conference on Biomedical Engineering and Informatics (BMEI), Yantai, China, 2010.

Zaryski C, Smith DJ (2005) Training principles and issues for ultra-endurance athletes Curr Sports Med Rep 4:165-170

FACTORS AFFECTING METABOLIC COST OF TRANSPORT DURING A MULTI-STAGE RUNNING RACE

Stefano Lazzer[1,2], Paolo Taboga[1,2], Desy Salvadego[1,2], Enrico Rejc[1,2], Bostjan Simunic[3], Marco V. Narici[4], Antonio Buglione[5], Nicola Giovanelli[1,2], Guglielmo Antonutto[1,2], Bruno Grassi[1,2], Rado Pisot[3], and Pietro E. di Prampero[2]

1. Department of Medical and Biological Sciences, University of Udine, Udine, Italy
2. School of Sport Sciences, University of Udine, Udine, Italy
3. Institute of Kinesiology Research, University of Primorska, Koper, Slovenia
4. School of Graduate Entry Medicine and Health, University of Nottingham, Derby, UK
5. School of Sport and Exercise Sciences, University Tor Vergata, Rome, Italy

Running title: Energetic and biomechanics of running

Corresponding Author:
Dr. Stefano Lazzer, PhD
University of Udine - Department of Biomedical Sciences and Technology
P.le Kolbe 4 - 33100 Udine, Italy
Phone: +39 0432 494333 - Fax: +39 0432 494301 - e-mail: stefano.lazzer@uniud.it

ABSTRACT

Purpose: to investigate: 1) the role of $\dot{V}O_{2max}$, fraction of it (*F)* and metabolic cost of transport (CoT) in determining performance during an ultra-endurance competition and 2) the effects of the race on several biomechanical and morphological parameters of the lower limbs that are likely to affect CoT. **Methods:** Eleven runners (age: 29-54 years) participated in an ultra-endurance competition consisting of three running stages of 25, 55 and 13 km on three consecutive days. Anthropometric characteristics, body composition, morphological properties of the gastrocnemius medialis, maximal explosive power of the lower limb and $\dot{V}O_{2max}$ were determined before the competition. In addition, biomechanics of running and CoT was determined, before and immediately after each running stage. **Results**: Performance was directly proportional to $\dot{V}O_{2max}$ (r=0.77), and *F* (r=0.36) and inversely proportional to CoT (r=-0.30). Low CoT values were significantly related to high maximal power of the lower limbs (r=-0.74), vertical stiffness (r=-0.65); and low foot-print index (FPI, r=0.70), step frequency (r=0.62) and external work (r=0.60). About 50% of the increase in CoT during the stages of the competition was accounted for by changes in FPI, which represents a global evaluation of medio-lateral displacement of the foot during the whole stance phase, which in turn are associated with the myotendinous characteristics of the lower limb. **Conclusions:** lower CoT values were related to greater muscular power and lower FPI, suggesting that a better ankle stability is likely to achieve better performance in ultra endurance running competition.

Key Words: maximal oxygen uptake; ultra-marathon; kinematics; stiffness; energy cost of running

INTRODUCTION

Middle- and long-distance running performances depend on several physical, physiological, biomechanical, metabolic, psychological and social factors (di Prampero, 2003; di Prampero et al., 1986). In particular, the three most important physiological factors determining high level performances are: 1) a large value of maximal oxygen uptake ($\dot{V}O_{2max}$, $mlO_2 \cdot kg^{-1} \cdot min^{-1}$), 2) a large fraction ($F$, %) of $\dot{V}O_{2max}$ that can be sustained throughout the competition and 3) a small value of metabolic cost of transport (CoT, $mlO_2 \cdot kg^{-1} \cdot m^{-1}$). As shown by di Prampero et al., 1986 , the endurance speed (v_{end}, $m \cdot min^{-1}$) in long distance running can be predicted, for any given runner, provided that his values of CoT, $\dot{V}O_{2max}$, and F are known:

$$v_{end} = F \cdot \dot{V}O_{2max} \cdot CoT^{-1} \tag{1}$$

Indeed, strong correlations were found between $\dot{V}O_{2max}$ and running performance in heterogeneous-level runners (Billat et al., 2003; Maughan and Leiper, 1983). Additionally, several studies showed that, in elite distance runners, F, which is linked primarily to adaptations resulting from prolonged training (Holloszy and Coyle, 1984), is a crucial parameter to determine performance (Maughan and Leiper, 1983). Finally, at the metabolic intensity imposed by the product $F \cdot \dot{V}O_{2max}$, the running velocity is determined by the individual’s ability to translate energy into performance (Daniels, 1985), i.e. to the energy expenditure per unit of mass and distance (CoT).

CoT is generally expressed as the amount of energy spent above resting to transport 1 kg body mass (BM) over 1 meter distance. CoT is independent of speed, at least for speeds ranging from 2.2 $m \cdot s^{-1}$ (8 $km \cdot h^{-1}$) to about 5 $m \cdot s^{-1}$ (18 $km \cdot h^{-1}$) wherein the air resistance is negligible (Jones and Doust, 1996). When normalized per unit of BM, CoT above resting, on flat compact terrain, shows a variability among subjects of 10-20%; its average value reported by di Prampero et al., 1986 amounts to 0.182 ± 0.014 $mlO_2 \cdot kg^{-1} \cdot m^{-1}$. CoT in trained runners depends on several physiological and biomechanical factors, including metabolic adaptations, the ability of the muscle-tendon complex to store and release elastic energy, and more efficient mechanics leading to less energy wasted for accelerating-decelerating and lifting-lowering the body at each stride (Lichtwark and Wilson, 2007; Saunders et al., 2004).

Previous study showed the relevant role of CoT in determining performance in middle and long distance running (di Prampero, 2003). It was also proposed that an increase of CoT throughout the event could explain the worse performance observed in some runners compared with others with similar $\dot{V}O_{2max}$ and *F* (Lazzer et al., 2012; Scrimgeour et al., 1986). Indeed, Brueckner et al., 1991 , reported an increased CoT throughout a marathon, although to a relatively minor extent (0.142% per km of distance), leading to an average increment of CoT at the end of the marathon of ~5%. However, these authors observed that the increase of CoT was widely different among runners with similar characteristics in terms of $\dot{V}O_{2max}$, *F*, training level, age, etc., being essentially negligible at one extreme of the sample, and twice the average for some other athletes. Davies and Thompson, 1986 , observed a linear increase of $\dot{V}O_2$ with time from the 50th to 240th minutes during a 4-hours race on a treadmill at constant speed, the rise becoming significant ($p< 0.01$) after 110 min of exercise. In addition, Gimenez et al., 2013, and Millet et al., 2011 , observed that the ability to maintain a high *F* over a 24 hours running on treadmill is mainly related to a low CoT, and Morin et al., 2011 , in the same study participants, observed significant changes in running biomechanics such as higher oscillation frequency, lower vertical stiffness and lower ground reaction force.

Indeed, interventions to reduce CoT are constantly sought after by athletes, coaches and sport scientists. Strength (Storen et al., 2008) and plyometric (Spurrs et al., 2003) training allow muscles and tendons to utilize more elastic energy and to reduce the amount of energy wasted in braking forces. In addition, the most economical runners display a higher triceps-surae tendon stiffness (k_{tendon}) compared to less economical ones (Arampatzis et al., 2006), thus suggesting that the functionality of the muscle tendon unit at submaximal running speeds is not only dependent on the stiffness of the series elastic elements but also on the maximal strength of the contractile element (Hof et al., 2002).

The primary purpose of the present study was to investigate the role of $\dot{V}O_{2max}$, *F* and CoT in determining the performance of runners who participated in a 93 km trail over three consecutive days, named “Magraid”. The second aim was to evaluate the relationship between CoT, k_{tendon} and the morphological properties of the gastrocnemius medialis (GM). The third aim was to investigate the effects of the race fatigue on several biomechanical parameters that are likely to affect CoT.

RESULTS

Characteristics of subjects

The anthropometric characteristics of the 11 subjects who completed the race are reported in Table 1. Their average $\dot{V}O_2max$ and maximal explosive power of the lower limb were 55.2±6.7 ml·min^{-1}·kgBM^{-1} and 1759±202 W, respectively. The characteristics of triceps surae muscle-tendon complex are reported in Table 2.

Factors determining performance

The role of the three factors of equation 9 individually evaluated with a simple linear regressions, showed that $\dot{V}O_2max$ (ml·min^{-1}·kg^{-1}BM) had the largest role in determining the mean speed (r=0.79) followed by the mean value of CoT throughout the race (CoT_{mean}, r=-0.64) and *F* (r=0.58).

When the multiple regression between them and v_{end}-mean (equation 9) was considered, the overall r increased to 0.91, whereas r values for $\dot{V}O_2max$, *F* and CoT_{mean} were 0.77, 0.36 and -0.30, respectively, the multiple regression being described by:

$$\log v_{end\text{-mean}} = 0.708 \cdot \log \dot{V}O_{2max} + 0.979 \cdot \log F - 1.559 \cdot \log CoT_{mean} - 3.067 \qquad (2)$$

($R^2 = 0.83$, SE = 0.035 km·h^{-1})

One of the main topic of this study was to investigate the role of several biomechanical factors in determining CoT. Therefore the relationships between CoT, measured before the first stage, and the biomechanical variables, showed an inverse relationship between CoT and 1) the peak power of the lower limb (Pmax, r= -0.74, $P<0.001$) and 2) the vertical stiffness (k_{vert}, r= -0.65, $P<0.05$). In addition, direct relationships between CoT and food print index (FPI, r= 0.70, $P<0.05$), step frequency (f, r= 0.62, $P<0.05$) and external work per unit distance (Wext, r= 0.60, $P<0.05$) were found.

In view of these data, a multiple linear regression among these 5 biomechanical parameters and CoT was performed. Only 3 retained a significant role in affecting CoT: 1) the Pmax (r = -0.74, $P<0.001$) followed by 2) Wext (r = 0.42, $P<0.05$) and 3) f (r = 0.38, $P<0.05$). The resulting overall relationship was described by the equation that follows:

$$CoT = -0.000004 \cdot Pmax + 0.195193 \cdot Wext + 0.022361 \cdot f + 0.115432 \qquad (3)$$

($R^2 = 0.86$, SE = 0.003 ml·kgBM^{-1}·m^{-1})

Physiological and biomechanical responses to the race

Running time, v_{mean} and mean heart rate (HR) of the three stages are reported in Table 3. Mean cumulative running time was 8:15:08±1:36:49 hours:min:sec, mean speed was 12.8±2.0 $km \cdot h^{-1}$ and mean %HRmax was 85.0±3.2% (corresponding to 76.2±4.6% of $\dot{V}O_2max$). As shown in Table 4, there was not a chronic stage effect (i.e: P=0.124) on BM, conversely, an acute stage effect (i.e.: P<0.001) on BM was observed after the first and second stage (by -1.3±1.1 and -3.9±3.0 kg, P<0.001, respectively). The mean CoT of the individual stages did not increase significantly with stage number (P=0.135), thus ruling out any chronic stage effect. However, a statistically significant acute stage effect on CoT was observed at the end of the first, second and third stages (+4.3±5.1, +6.6±4.1 and +4.2±4.0 %, respectively, P<0.05). Finally, no chronic or acute stage effect on respiratory exchange ratio (RER) was observed.

No significantly changes on biomechanical parameters were observed before and after the three stages (Tab. 4), with the exception of the FPI which increased significantly at the end of the first, second and third stages (11.9±9.1, 31.6±24.6 and 22.2±21.2 %, respectively, P<0.001) and for the maximal ground reaction force (GRF) which decreased significantly at the end of the first and second stage (-4.0±4.6 and -3.8±4.9 %, respectively, P<0.05).

In order to identify the main factors affecting CoT during an ultra-endurance running race, the effects of the relative changes of the biomechanical parameters before and after the three stages on the corresponding relative changes of CoT were investigated as follows. The relative changes of each variable (X) were calculated as $[(X_b - X_a) \cdot X_{bc}^{-1}] \cdot 100$ where X denotes any given variable before (b) or after (a) the stage considered divided by the corresponding X measured before competition (X_{bc}). To this aim, only the biomechanical parameters which were significantly correlated with CoT before the race (k_{vert}, FPI, f and Wext) were considered.

Considering all the stages together, the results of multiple regression showed that changes in FPI (r = 0.59, P<0.001) had the largest role in determining ΔCoT followed by changes in f (r = 0.39, P<0.001) and changes in k_{vert} (r = - 0.35, P<0.05). The resulting overall relationship was described by the following equation:

$$\Delta CoT = 0.09 \cdot \Delta FPI + 0.69 \cdot \Delta f - 0.09 \cdot \Delta k_{vert} + 33.97 \qquad (4)$$

($R^2 = 0.62$, SE = 4.73 %)

It is worth to note that an analysis of the second stage only, which corresponded to the longest and the hardest one, showed the same trend as reported above for the entire competition. However, the multiple regression analysis showed that the role of the ΔFPI in determining ΔCoT increased substantially ($r = 0.74$, $P<0.001$). Whereas, that of f decreased ($r = 0.32$, $P<0.05$) and that of k_{vert} ($r = -0.38$, $P<0.05$) remained essentially unchanged. The corresponding overall multiple regression between these three variables and ΔCoT (%) is described by the following equation:

$$\Delta CoT = 0.12 \cdot \Delta FPI + 0.41 \cdot \Delta f - 0.10 \cdot \Delta k_{vert} + 59.10 \qquad (5)$$

($R^2 = 0.79$, SE = 3.35 %).

Since ΔFPI had the greatest role in setting ΔCoT during the hardest stage of the race ($r = 0.74$, $P<0.001$), a further statistical analysis was performed to investigate the physiological characteristic of the lower limbs which had the largest correlation with FPI.
The results showed that FPI was inversely related to Pmax ($r= -0.69$, $P<0.05$), k_{vert} ($r= -0.63$, $P<0.05$), k_{tendon} ($r= -0.76$, $P<0.05$) and tendon force (F_{tendon}, $r= -0.69$, $P<0.05$). Conversely, F_{tendon} was directly related to morphological properties of the GM as pennation angle ($r= 0.73$, $P<0.001$), fiber length ($r= 0.74$, $P<0.001$) and muscle thickness ($r= 0.70$, $P<0.001$).

DISCUSSION

The main results of the present study showed that 1) high level performance in long-distance running depends on high $\dot{V}O_{2max}$ ($r=0.77$), high *F* ($r=0.36$) and low CoT_{mean} ($r=-0.30$); 2) low CoT values before the race are related to high Pmax and k_{vert}, and low FPI, f and W_{ext}; and 3) about 50% of the increase in CoT during the stages of the competition is related to changes in FPI which in turn is associated with the myotendinous characteristics of the lower limb.

Factors determining performance

As previously observed by di Prampero et al., 1986 , high level performance in long-distance running depends on, 1) a large value of $\dot{V}O_{2max}$, 2) a large fraction of it, *F,* that can be sustained throughout the competition and, 3) a small value of CoT. Indeed, high correlations have been demonstrated between $\dot{V}O_{2max}$ and running performance in groups of runners of quite different abilities (Maughan and Leiper, 1983). Also in the present study, $\dot{V}O_{2max}$ was found to be the single variable having the largest role in determining performance (r=0.79). However, when groups of athletes with a relatively narrow range of $\dot{V}O_{2max}$ are studied, $\dot{V}O_{2max}$ becomes a less sensitive predictor of performance, *F* and CoT becoming crucial for performance in distance running (Maughan and Leiper, 1983).

Saunders et al., 2004 , showed that a number of physiological and biomechanical factors appear to influence CoT in trained runners. In the present study, a low CoT value was significantly related to high Pmax (r= -0.74), high k_{vert} (r= -0.65) and low FPI (r= 0.70), confirming previous studies which underline the role of the muscle-tendon complex stiffness in storing and releasing elastic energy (Spurrs et al., 2003). Particularly, a low FPI indicates that the trajectory of the foot center of pressure remains close to the foot axis, thus suggesting that a better ankle stability (Huang et al., 2011; Willems et al., 2005) allows better elastic energy absorption along the foot axis (Ker et al., 1987). It should be noted that, Arellano and Kram, 2011 , reported that step width in running is near zero and that running with relatively wide steps is mechanically and energetically wasteful since the goal of running is to move the body in the forward direction. After prolonged exercise, the subjects may experience difficulty balancing due to fatigue (Lepers et al., 1997). Even though it was not measured, it is reasonable to infer that, in order to maintain balance, the subjects increased step width. This would bring about greater medio-lateral forces and hence a higher FPI.

As previously described by Saunders et al., 2004 and also showed in the present study, low W_{ext} and low f were directly related to low CoT. A significant positive correlation between CoT and W_{ext} was in fact found also by Bourdin et al., 1995 , who showed that the W_{ext} could explain a large part of the variations of CoT among subjects at a given velocity.

In addition, Cavanagh and Williams, 1982 , showed that in well trained athletes the aerobic demand of running at a given speed is lowest at a self-selected stride length and step frequency due to the fact that runners naturally acquire an optimal value of these variables over time, based on perceived exertion. It should also be noted that, whereas lowering step frequency would be beneficial in terms of lowering CoT (Gimenez et al., 2013; Morin et al., 2011), it might also cause greater muscular damage which could have negative consequences in long races (Millet et al., 2012).

Physiological and biomechanical responses to the race

In the present study, CoT increased significantly at the end of the first (+4.3%), second (+6.6%) and third (+4.2%) stage. The paragraphs that follows are therefore devoted to a discussion of the factors associated with the increase of CoT. The above mentioned increases in CoT are greater than observed over classical marathons (Brueckner et al., 1991), probably because of the peculiarities of the race terrain, the characteristics of which will substantially add to the physiological, biomechanical and metabolic demands of the performing athlete. On the average, only two biomechanical parameters changed significantly at the end of each stage: FPI and maximal GRF. These results are in line with the study of Morin et al., 2011 , who considered running biomechanics over a 24-h treadmill run and observed changes in biomechanics parameters only after 4 hours. Kyrolainen et al., 2000 , showed that the increase of CoT cannot be explained by changes in running mechanics after a marathon for the entire group of subjects, since they observed significant interindividual variations inside the group. This suggest that other parameters, such as the differences of internal work between pre- and post- race could explain the increased CoT. Our measurements, even if the internal work was not directly measured, showed a slight increase in step frequency, which can suggest an increase in internal work (Cavagna et al., 1991).

To underline interindividual differences and considering the fact that an increase of CoT throughout the event could explain the worse performance observed in some runners, we compared the relative changes of CoT (ΔCoT, %) with the relative changes on biomechanical parameters during the three stages. When considering only the second stage, i. e. the hardest of the present study, a multiple linear regression showed that FPI changes (ΔFPI) has the largest role ($r = 0.74$) in determining ΔCoT, followed by changes on k_{vert} (Δk_{vert}, $r = -0.38$) and f (Δf, $r = 0.32$).

A significantly increase in FPI observed at the end of each stage underlines a reduction in ankle control and then an increase on ankle instability as shown by previous authors (Huang et al., 2011; Willems et al., 2005). This information suggests that the increased ankle instability brings about a reduction of the fraction of elastic energy recovered thanks to the arch of the foot, which, as shown by Ker et al., 1987 , is responsible for about 30% of the overall elastic energy recovery. This hypothesis, is coherent with our results showing that lower FPI was related to higher Pmax, k_{vert}, k_{tendon} and F_{tendon}.

In this connection, it is interesting to point out that the k_{tendon} observed in our runners (463 $N \cdot mm^{-1}$, Tab. 2) is greater than observed in sedentary subjects (319 $N \cdot mm^{-1}$, Rosager et al., 2002). However, this higher k_{tendon} is associated with a greater cross sectional area (CSA) which in our runners turned out to be 92 mm^2 (Tab. 2) as compare to 73 mm^2 to sedentary subjects (Rosager et al., 2002). Thus when normalizing k_{tendon} for the corresponding tendon length and CSA, the obtained results (i.e. the Young modulus, 1.07 GPa, Tab. 2) is essentially equal to that reported in the literature for sedentary subjects (1.02 GPa, Rosager et al., 2002). It can be concluded that long term endurance training leads to a greater k_{tendon}. In particular, in our group of runners, the increased stiffness is due to a hypertrophy of the tendon (i.e. to an increased CSA) without any change of its material properties, as shown by the unchanged Young modulus. It should be considered that an excessive increase of k_{tendon} may lead to the opposite effect, i.e. a decreased recovery of elastic energy (Lichtwark and Wilson, 2008; Magnusson et al., 2003) and hence a higher CoT, a fact that probably did not occur in our subjects.

In addition, the greater F_{tendon} was related to greater pennation angles (r= 0.73) and greater thickness (r= 0.70) of the GM muscle, both suggesting a greater packing of contractile material (Kawakami et al., 1993) an hence an increased number of sarcomeres in parallel (Abe et al., 1997). Moreover, the observed increased in fibre length, likely enabling the sarcomers to operate closer to optimal length, as suggested by Narici and Maganaris, 2007 , may be an additional factor contributing to the observed great F_{tendon}. As observed previously (Fletcher et al., 2010), these observations emphasize the importance of the lower limb muscle characteristics to maximizing gastrocnemius efficiency during running and reducing CoT (Lichtwark and Wilson, 2008).

Finally, we would like to point out that the analysis of the relationship between the biomechanical and bioenergetics characteristics of endurance running man, help us to better understand the evolutionary history of this remarkable form of human locomotion (Bramble and Lieberman, 2004).

In conclusion, performance was directly proportional to $\dot{V}O_{2max}$ and *F* and inversely proportional to CoT_{mean}. Particularly, we have shown that low CoT values before the race are related to high Pmax and k_{vert}, and low FPI, f and W_{ext}. Finally, for the first time to our knowledge we have shown that the increases of CoT during the stages of the competition can be predicted by the changes in FPI which are responsible for about 50% of its changes which in turn are associated with myotendinous characteristics of the lower limb. Taken as a whole, our results suggest that athletes with better ankle stability will achieve better performance in ultra endurance running competitions.

RESEARCH DESIGN AND METHODS

Subjects

Fifteen healthy Caucasian male runners (age range 29-54 years) participated in the ultra-endurance competition named “Magraid”. The experimental protocol was approved by the Ethics Committee of the University of Udine, Italy. Before the study, the purpose and objectives were carefully explained to each subject and written informed consent was obtained from all of them. Subjects having overt metabolic and/or endocrine diseases and those taking medications regularly or using drugs known to influence energy metabolism were excluded. The participants were recruited among experienced ultraendurance runners who filled questionnaires on physical exercise activity. All the participants of this study had run at least one race longer than 100 km. On average, their training experience amounted to (mean ± standard deviation) 12 ± 5 years, of which 6 ± 3 years of ultra-endurance running. They reported to run on average 75.8 ± 16.8 km$\cdot$week^{-1}. Fifteen athletes who were eligible for the study began the race, and the 11 ones who completed the entire competition were considered for the data analysis.

Experimental protocol

One week before the race, the subjects came to the laboratory, where anthropometric characteristics, body composition, triceps surae k_{tendon} and morphological properties of the GM were performed. Furthermore, the maximal explosive jumping muscle power of the lower limb was measured, and a graded exercise test to exhaustion on a treadmill was performed. The subjects were asked to refrain from any vigorous physical activity during the two days preceding the test and on a preliminary testing session they were thoroughly familiarized with all the different measurements.

The competition “Magraid” took place in summer. It consisted of three stages of 25, 55 and 13 km on three consecutive days in the North-East of Italy. The geologic texture of the terrain is an unusual soil in respect the vast majority of ultra-endurance competitions; it is characterized by a gravel (locally named “Magredi”) from the braided river “Cellina-Meduna”. The first day, stage 1 began at 6.00 p.m. with temperature and relative humidity of 26°C and 77%. The second and third days, stages 2 and 3 began at 10.00 a.m. with temperature and relative humidity of 22 and 20°C and 80 and 85%, respectively.

Before and immediately after (mean time interval: 5±3 min) each running stage, BM, CoT, RER, running biomechanics and mechanical work were measured. In addition, HR and GPS coordinates were continuously recorded throughout the three stages (Garmin Forerunner 305 GPS, Kansas City, USA).

Physiological measurements before the race

Anthropometric characteristics and body composition

BM was measured to the nearest 0.1 kg with a manual weighing scale (Seca 709, Hamburg, Germany), stature was measured to the nearest 0.001 m on a standardized wall-mounted height board. Body mass index (BMI) was calculated as BM (kg) · stature^{-2} (m). Body composition was measured by bioelectrical impedance (BIA, Human IM Plus; DS Dietosystem, Milan, Italy) according to the method of Lukaski et al., 1986 . Body composition (fat-free mass, FFM, and fat mass, FM) was obtained from the software provided by the manufacturer.

Triceps surae tendon stiffness.

Maximal voluntary torque (MVT) of plantarflexors was measured during an isometric maximal voluntary contraction (MVC) with the participant laying prone. His right foot was tightened around the adapter of an isokinetic dynamometer (Cybex Norm, CSMi, MA, USA). Straps were also tightened around the hips to prevent a forward displacement of the body during maximal plantarflexions. Participants were positioned with the knee fully extended and an ankle angle of -20° with the lateral malleolus aligned with the axis of rotation of the dynamometer (Maganaris, 2002; Maganaris, 2003). Before MVCs, the participants performed five submaximal plantarflexions and dorsiflexions as a warm up. MVCs were elicited by requesting the subject to increase the plantarflexion moment gradually over a 5-s period. The plantarflexors torque, was obtained adding the torque generated by the activation of the (antagonist) tibialis anterior, to the overall measured torque. In turn, the tibialis anterior torque was estimated from its electromyographic (EMG) activity, as described below.
EMG activity of the tibialis anterior was recorded while performing maximal isometric plantarflexions and dorsiflexions by pre-gelled surface EMG electrodes (circular contact area of 1 cm diameter, BIOPAC Systems, Inc., USA) placed at one-third of muscle length to avoid the motor point with an inter-electrode distance equal to 20 mm. The reference electrode was placed on the lateral femoral condyle. Before placement of the electrodes, the skin was shaved to remove hair, and the recording sites were rubbed lightly using abrasive gel and cleansed with alcohol swabs to reduce interelectrode impedance. The raw EMG activity was acquired at sampling frequency of 2000 Hz and processed with a multichannel analog-to-

digital converter (Biopac Systems, Santa Barbara, CA, US). The raw EMG signal was filtered with band-pass filters set at 10-500 Hz, and amplified with a gain of 2000. This allowed us to determine the relationship between EMG amplitude and torque exerted by the tibialis anterior as determined in the relaxed state and during two submaximal ankle dorsiflexion contractions. The dorsiflexion torque exerted by the tibialis anterior, as estimated from its EMG activity, was then added to the net MVC plantarflexion torque, thus allowing us to obtain the contribution of the triceps surae (Morse et al., 2008). The triceps surae tendon moment arm of the ankle joint was measured as the distance from the center of rotation of the ankle joint to the tendon axis (Morse et al., 2008). In addition, the foot moment arm of the ankle joint was measured as the distance from the center of rotation of the ankle joint to the distal head of the first metatarsal bone. Then, the triceps surae F_{tendon} was calculated by multiplying the force measured at the footplate by the ratio of foot moment arm to tendon moment arm. The compensation of moments due to gravitational forces was done for all subjects before each ankle plantar flexion contraction.

Tendon elongation measurements were taken using a 7.5 MHz, linear, B-mode ultrasound probe (Esaote Biomedica, AU3Partner, Florence, Italy). Details of the methodology employed have been described elsewhere (Maganaris and Paul, 2000). First, consecutive axial-plane scans were taken along the belly of the gastrocnemius medialis muscle with a 2 cm interscan gap. The medial and lateral borders of the muscle in each scan were identified, and the midpoint between the two borders was marked on the skin. Sagittal-plane scans were then taken at the level of the heel to identify the insertion point of the triceps surae tendon in the calcaneus, which was also marked on the skin. A straight line connecting the Achilles tendon insertion with all midpoints marked along the muscle was assumed to be the midlongitudinal mid-sagittal axis of the muscle–tendon unit. The scanning probe was displaced along this axis to locate the distal myotendinous junction of the muscle, and subsequently the probe was placed over a marker fixed to the skin, which cast a line on the ultrasound image and served as a reference position to measure tendon tensile displacement. The relevant scans were identified, and tendon displacement was measured using digitizing software (Kinovea – version 0.8.7 - Joan Charmant & Contrib).

The length and CSA of the triceps surae tendon were quantified from sonographs recorded at rest with the probe described above. The distance between the tendon's origin and insertion along the mid-sagittal axis of the muscle–tendon unit was measured manually to the nearest millimeter and considered to be the tendon's original length. The triceps surae tendon cross-sectional area was digitized in axial-plane scans recorded 1, 2 and 3 cm above the tendon insertion point in the calcaneus.

For each subject, the triceps surae tendon elongation was quantified during the MVC that generated the highest plantarflexion moment. The elongation of the tendon at loads corresponding to 0–100% of the plantarflexion moment generated was measured at 10% intervals. First, the time points corresponding to the above loads were identified from the moment-time relationship, and then the scans corresponding to those time-points were stored in a computer and further processed. The approach followed for identifying the scans corresponding to the loads examined assumes that the moment generated by the triceps surae muscle during a ramp isometric contraction with the knee fully extended changes linearly with the gross plantarflexion moment measured. Evidence for the validity of this assumption has previously been obtained from EMG measurements (Magnusson et al., 2001).

Force-elongation data (i.e. tendon force *vs*. tendon length) were fitted with second-order polynomials. k_{tendon} data were calculated from the slope of the force-elongation curve over 10% force-intervals (Maganaris, 2002). The corresponding tendon Young's modulus data were calculated by multiplying the stiffness values by the ratio of tendon length to tendon cross-sectional area.

Morphological properties of the Gastrocnemius Medialis.

The participants laid prone, with the foot secured at -20° dorsiflexion. Fiber fascicle length (L) and pennation angle (°) were measured using B-mode ultrasound probe (Esaote Biomedica, AU3Partner, Florence, Italy). Images were obtained along the midsagittal plane of the GM, at the mid-distance between the proximal and distal tendon insertion identified by ultrasound (7.5-MHz linear-array probe). The head of the probe was held perpendicular to the dermal surface to provide an image including both superficial and deep aponeuroses, and a number of clearly visible fascicles that could be followed between the aponeuroses. To improve acoustic coupling, water-soluble transmission gel was placed over the scan head. Ultrasound scans were recorded at 25 Hz and analyzed offline with digitizing software (ImageJ 1.44p, National Institute of Health, USA). Pennation angle was measured as the angle of fascicle insertion into the deep aponeurosis, L was defined as the length of the fascicle between the deep and superficial aponeuroses (Narici et al., 1996).

Maximal explosive jumping muscle power of the lower limb.

The maximal explosive jumping muscle power of the lower limbs during very short all-out efforts was assessed by means of the Explosive-Ergometer (EXER, University of Udine, Italy, Fig. 1), previously described in detail elsewhere (Lazzer et al., 2009). Briefly, the EXER consists of a metal frame supporting one rail inclined at 20° to the horizontal. The subject sitting on a seat, fixed to a carriage free to move on the rail, accelerates himself and the carriage seat backward pushing on two force platforms (LAUMAS PA 300, Parma, Italy). The velocity along the direction of motion is continuously recorded by a wire tachometer (LIKA SGI, Vicenza, Italy). The analogue outputs of the force and velocity transducers are digitalized and recorded by a data acquisition system (MP 100 BIOPAC, USA). The subjects were asked to perform four all-out efforts with the right leg and four with the left one, starting from the same knee angle (110°). The requested starting knee angle was obtained by adjusting the position of the mechanical blocks which also prevented the motion of the carriage seat towards the platforms, thus impeding any counter movement. To prevent fatigue, after each push the subjects rested for 2 min with their feet placed on a support. The mechanical power (P, W) developed by the single lower limb was obtained from the instantaneous product of the developed Force (N) times the backward speed (v, $\text{m}\cdot\text{s}^{-1}$):

$$P\ (t) = \text{Force}\ (t) \cdot v\ (t) \qquad (6)$$

Analysis of the time course of P allowed us to assess its peak (Pmax, W).

Graded exercise test to exhaustion.

$\dot{V}O_{2max}$ and maximal heart rate (HR_{max}) were determined by a graded exercise test on a treadmill (Saturn, HP Cosmos, Germany) under medical supervision. During the experiment, ventilatory and gas exchange responses were measured continuously with a metabolic unit (Quark-b^2, Cosmed, Italy). The volume and gas analysers were calibrated using a 3-L calibration syringe and calibration gas (16.00% O_2; 4.00% CO_2), respectively. During the tests, electrocardiogram was continuously recorded and displayed on line for visual monitoring, and HR was measured with a dedicated device (Polar, Finland). The tests were performed one week before the race and comprised a 5-min rest period followed by running at 10 $\text{km}\cdot\text{h}^{-1}$ for 5 min (on a slope of 1%); the speed was then increased by 0.7 $\text{km}\cdot\text{h}^{-1}$ every minute until volitional exhaustion. A leveling off of oxygen uptake (defined as an increase of no more than 1 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was observed in all subjects during the last one or two minutes of the exercise test indicating that $\dot{V}O_{2max}$ had been attained. $\dot{V}O_{2max}$ and HR_{max} were calculated as the average oxygen uptake and HR of the last 20 s of the test. RER was calculated as $\dot{V}CO_2 \cdot \dot{V}O_2^{-1}$. The gas exchange threshold (GET) was determined by the v-slope method (Beaver et al., 1986).

Metabolic cost of transport and biomechanical measurements during the race.

Before and immediately after (mean time interval: 5±3 min) each stage of the competition, the subjects ran for 6 min on a treadmill (Zebris Medical GmbH, Isny, Germany) at a constant speed of 10 $km \cdot h^{-1}$, close to the actual speed that athletes had maintained during the race. The treadmill was positioned near the arrival line, integrated with a series of high quality capacitive force sensors underneath the treading surface. The treadmill was connected to a personal computer integrated with running software analysis (Win FDM-T, v 2.1.1. Zebris Medical GmbH, Isny, Germany) yielding contact (tc, s) and aerial (ta, s) times at a sampling rate of 100 Hz; duty factor (%), obtained dividing tc by step time; step frequency (f, Hz); maximal vertical GRF (N); and FPI (cm^2). FPI is a modified version of medio-lateral trajectory of the centre of pressure (CoP) with respect to the foot axis as a function of time during the stance phase used by Willems et al., 2005 , and Huang et al., 2011 ; it was obtained from the area between the foot axis (a line connecting the center of heel to the midpoint of 2nd and 3rd metatarsal heads) and the CoP trajectory (see Fig. 2). This index is a global evaluation of medio-lateral displacement during the whole stance phase: i.e. a FPI equal or close to zero indicating that the trajectory of the CoP remains close to the foot axis, higher values indicating large oscillations in the medio-lateral direction. For each subject, 10 subsequent "representative" steps (i.e. without anomalous movements of limbs, torsion of head or trunk etc.) were analyzed and FPI was calculated by means of a custom-made Matlab program.

CoT and RER were measured continuously with a metabolic unit (Quark-b^2, Cosmed, Italy), as follows. The analyzer, calibrated prior to each testing session, provided breath-by-breath data. The average of the final 2 min of sampling was used for further analysis. This averaging phase did not start until the following two conditions had been met: 1) at least 4 min of running had passed and 2) real-time plots of $\dot{V}O_2$, HR, and RER indicated that metabolic steady state had been achieved. Net $\dot{V}O_2$ was obtained by subtracting pre-exercise standing values, as measured before each stage, from gross $\dot{V}O_2$ at constant speed. This same procedure was repeated before and after each running stage, on the implicit assumption that pre-exercise resting $\dot{V}O_2$ was not affected by the preceding running stage. CoT was then obtained by dividing net energy expenditure ($mlO_2 \cdot kg^{-1} \cdot s^{-1}$) by speed ($m \cdot s^{-1}$). As mentioned in the introduction, CoT in running, in the range of speeds where the air resistance is negligible (i.e. < 18 $km \cdot h^{-1}$), is independent of the speed. Hence the so obtained value applies throughout the investigated speeds. RER was always below 1.0 confirming that aerobic metabolism was the main metabolic pathway.

The biomechanics of treadmill running was studied using two digital cameras at 210 fps (Basler - Pilot, Ahrensburg, Germany). The video sequences were recorded between the fourth and the fifth minute because in an earlier study (Karamanidis et al., 2003) found that after 2–3 min running on a treadmill the running characteristics are very reproducible.

The cameras were placed symmetrically 5 m behind the treadmill, spaced 6 m one from the other, and were calibrated using a square frame (1 m · 1 m). To improve the quality of the video analysis, seven reflective markers (radius 10·mm) were used to identify joint positions. The markers were fixed on the following landmarks (left side): metatarsal head V, lateral malleolus, calcaneous, femur lateral epicondyle, spina iliaca (right and left) and over the second lumbar vertebra. The video recordings were digitized using a software (Simi Reality Motion System, GmbH Max-Planck-Str., Germany) and three-dimensional position of each marker was reconstructed.

The data were smoothed through a moving-average filter (Radius = 1) and the position of centre of mass (CoM) was calculated as the mean position of the markers placed over the spina iliaca (right and left) and over the second lumbar vertebra (Bourdin et al., 1995; Myers and Steudel, 1985; Taboga et al., 2012). For each subject, 10 subsequent "representative" steps (i.e. without anomalous movements of limbs, torsion of head or trunk etc.) were analyzed by means of a custom-made Matlab program. External mechanical work was calculated from the positive variations of total mechanical energy (potential and kinetic) of CoM, as described by Cavagna et al., 1976 . Total external mechanical work per unit of distance (W^T_{ext}, $mlO_2 \cdot kg^{-1} \cdot m^{-1}$) was then calculated as:

$$W^T_{ext} = \frac{W^{tot}_{ext}}{d} \qquad (7)$$

where W^{tot}_{ext} is the total external mechanical work and d is the distance travelled during the analyzed steps. Mass-specific external mechanical work per unit distance W_{ext} ($mlO_2 \cdot kg^{-1} \cdot m^{-1}$) was then calculated dividing W^T_{ext} by the BM of the subject.

In addition, total stiffness (k_{vert}, N · mm^{-1}) was calculated as the ratio between peak GRF and the vertical displacement of the CoM during the stance phase.
During these ten steps, stride cycle (between two consecutive heel strikes of the same left foot) was analyzed. The joint angles of the knee were also measured at maximal extension (Ext_{max}, °), maximal load ($Load_{max}$, °) and maximal flexion ($Flex_{max}$, °).

Statistical analyses

Statistical analyses were performed using PASW Statistic 18 (SPSS Inc., IL, USA) with significance set at $P<0.05$. All results are expressed as means and standard deviation (SD). Changes of BM, CoT, RER and biomechanical parameters during the race were studied with General Linear Model repeated measures with two factors considering chronic stage effect (called "Stage": Stage 1 *vs* Stage 2 *vs* Stage 3) and the acute stage effect (called "Time": before *vs* after). When significant differences were found, a Bonferroni post hoc test was used to determine the exact location of the difference.
Equation 1 yields v_{end} values in long distance running; however, as written, it does not take into account the increase of CoT that may occur during the competition, as observed previously (Lazzer et al., 2012). Therefore, the mean value of CoT throughout the race (CoT_{mean}) was estimated as follows:

$$CoT_{mean} = [[(CoT_{Ib} + CoT_{Ia}) \cdot 0.5 \cdot d_I] + [(CoT_{IIb} + CoT_{IIa}) \cdot 0.5 \cdot d_{II}] + [(CoT_{IIIb} + CoT_{IIIa}) \cdot 0.5 \cdot d_{III}]] \cdot (d_I + d_{II} + d_{III})^{-1} \quad (8)$$

where the suffix I, II, and III refer to the first, second and third stage, b and a indicate the CoT value assessed immediately before (b) and after (a) the appropriate stage, the distance of which are indicate by d ($d_I = 25000$ m; $d_{II} = 50000$ m; $d_{III} = 13000$ m). Therefore, applying equation 1 to the overall competition and taking into account the average value of CoT_{mean}, as from equation 8, one obtains:

$$v_{end\text{-}mean} = F \cdot \dot{V}O_{2max} \cdot CoT_{mean}^{-1} \quad (9)$$

In turn, *F* was estimated from the mean HR determined throughout each individual stage and expressed as a fraction of the corresponding maximal HR. It should be noted that *F* may be better predicted by considering the ratio of the mean HR increase above resting, throughout each stage, to the HR reserve. However, in view of the fact that resting HR is somewhat difficult to assess precisely, we preferred to stick to absolute values, as described above. The role of each of the three factors of equation 9 was evaluated with a simple linear regressions determined between each individual variable and endurance speed; Pearson's correlation coefficients were used to analyze the association between variables. Subsequently, in order to calculate multiple regression coefficients of all three factors combined as in equation 9, it is convenient to use the logarithmic transformation:

$$\log v_{end\text{-}\mathrm{mean}} = \log (F \cdot \dot{V}O_{2\mathrm{max}} \cdot \mathrm{CoT}_{\mathrm{mean}}^{-1}) = \log F + \log \dot{V}O_{2\mathrm{max}} - \log\mathrm{CoT}_{\mathrm{mean}} \qquad (9')$$

This multivariate analysis enabled us to assess the role of each variable in setting the athletes' performance.

In addition, the relationships between biomechanical variables affecting CoT were investigated using Pearson's product–moment correlation coefficient.

LIST OF SYMBOLS AND ABBREVIATIONS

BM: body mass
BMI: body mass index
CoM: centre of mass
CoP: centre of pressure
CoT : metabolic cost of transport
CSA: cross sectional area
d: distance travelled during the analyzed steps
EMG: electromyography
$\mathrm{Ext}_{\mathrm{max}}$: maximal extension joint angle of the knee
$\mathrm{Flex}_{\mathrm{max}}$: maximal flexion joint angle of the knee
f: step frequency
F: fraction of $\dot{V}O_{2\mathrm{max}}$
FFM: fat-free mass
FM: fat mass
F_{tendon}: tendon force
FPI: Foot-print index
GM: gastrocnemius medialis

GRF: ground reaction force

HR: heart rate

k_{vert}: total stiffness

$Load_{max}$: maximal load joint angle;

TST: triceps surae tendon

k_{tendon}: tendon stiffness

L: fiber fascicle length

MVT: maximal voluntary torque

MVC: maximal voluntary contraction

P: mechanical power

°: degrees

RER: respiratory exchange ratio

RMS: root mean square

tc: contact

ta: aerial

$\dot{V}O_{2max}$: maximal O_2 intake

$\dot{V}CO_2$: CO_2 uptake

v : speed

X: variable

v_{end}: endurance speed

W^{T}_{ext}: external mechanical work per unit of distance

W^{tot}_{ext}: total external mechanical work

W_{ext}: mass-specific external mechanical work per unit distance

ACKNOWLEDGEMENTS

We are grateful to the athletes for their kind collaboration, to Mr A. Iossa, Mr P. Tedeschi, and the A.S.D. Triathlon Team – Pordenone, Italy, for their valuable contribution in carrying out measurement during the race. We are grateful to Dr. S. Poser, M. Viotto, M. Conforto for their kind assistance during the study. The financial support of the Lions Club, Udine-Duomo, Italy, is gratefully acknowledged.

DISCLOSURES

No competing interests, financial or otherwise, are declared by the author(s).

REFERENCES

Abe, T., Kawakami, Y., Suzuki, Y., Gunji, A. and Fukunaga, T. (1997). Effects of 20 days bed rest on muscle morphology. *J Gravit Physiol* **4**, S10-4.

Arampatzis, A., De Monte, G., Karamanidis, K., Morey-Klapsing, G., Stafilidis, S. and Bruggemann, G. P. (2006). Influence of the muscle-tendon unit's mechanical and morphological properties on running economy. *J Exp Biol* **209**, 3345-57.

Arellano, C. J. and Kram, R. (2011). The effects of step width and arm swing on energetic cost and lateral balance during running. *J Biomech* **44**, 1291-5.

Beaver, W. L., Wasserman, K. and Whipp, B. J. (1986). A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol*. **60**, 2020-7.

Billat, V., Lepretre, P. M., Heugas, A. M., Laurence, M. H., Salim, D. and Koralsztein, J. P. (2003). Training and bioenergetic characteristics in elite male and female Kenyan runners. *Med Sci Sports Exerc* **35**, 297-304; discussion 305-6.

Bourdin, M., Belli, A., Arsac, L. M., Bosco, C. and Lacour, J. R. (1995). Effect of vertical loading on energy cost and kinematics of running in trained male subjects. *J Appl Physiol* **79**, 2078-85.

Bramble, D. M., Lieberman D.E. (2004). Endurance running and the evolution of Homo. *Nature* **18**, 345-52.

Brueckner, J. C., Atchou, G., Capelli, C., Duvallet, A., Barrault, D., Jousselin, E., Rieu, M. and di Prampero, P. E. (1991). The energy cost of running increases with the distance covered. *Eur J Appl Physiol Occup Physiol*. **62**, 385-9.

Cavagna, G. A., Thys, H. and Zamboni, A. (1976). The sources of external work in level walking and running. *J Physiol* **262**, 639-57.

Cavagna, G. A., Willems, P. A., Franzetti, P. and Detrembleur, C. (1991). The two power limits conditioning step frequency in human running. *J Physiol* **437**, 95-108.

Cavanagh, P. R. and Williams, K. R. (1982). The effect of stride length variation on oxygen uptake during distance running. *Med Sci Sports Exerc* **14**, 30-5.

Daniels, J. T. (1985). A physiologist's view of running economy. *Med Sci Sports Exerc*. **17**, 332-8.

Davies, C. T. and Thompson, M. W. (1986). Physiological responses to prolonged exercise in ultramarathon athletes. *J Appl Physiol* **61**, 611-7.

di Prampero, P. E. (2003). Factors limiting maximal performance in humans. *Eur J Appl Physiol*. **90**, 420-9. Epub 2003 Aug 9.

di Prampero, P. E., Atchou, G., Bruckner, J. C. and Moia, C. (1986). The energetics of endurance running. *Eur J Appl Physiol Occup Physiol*. **55**, 259-66.

Fletcher, J. R., Esau, S. P. and MacIntosh, B. R. (2010). Changes in tendon stiffness and running economy in highly trained distance runners. *Eur J Appl Physiol* **110**, 1037-46.

Gimenez, P., Kerherve, H., Messonnier, L. A., Feasson, L. and Millet, G. Y. (2013). Changes in the Energy Cost of Running during a 24-h Treadmill Exercise. *Med Sci Sports Exerc* **45**, 1807-13.

Hof, A. L., Van Zandwijk, J. P. and Bobbert, M. F. (2002). Mechanics of human triceps surae muscle in walking, running and jumping. *Acta Physiol Scand* **174**, 17-30.

Holloszy, J. O. and Coyle, E. F. (1984). Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J Appl Physiol*. **56**, 831-8.

Huang, P. Y., Lin, C. F., Kuo, L. C. and Liao, J. C. (2011). Foot pressure and center of pressure in athletes with ankle instability during lateral shuffling and running gait. *Scand J Med Sci Sports* **21**, e461-7.

Jones, A. M. and Doust, J. H. (1996). A 1% treadmill grade most accurately reflects the energetic cost of outdoor running. *J Sports Sci*. **14**, 321-7.

Karamanidis, K., Arampatzis, A. and Bruggemann, G. P. (2003). Symmetry and reproducibility of kinematic parameters during various running techniques. *Med Sci Sports Exerc* **35**, 1009-16.

Kawakami, Y., Abe, T. and Fukunaga, T. (1993). Muscle-fiber pennation angles are greater in hypertrophied than in normal muscles. *J Appl Physiol* **74**, 2740-4.

Ker, R. F., Bennett, M. B., Bibby, S. R., Kester, R. C. and Alexander, R. M. (1987). The spring in the arch of the human foot. *Nature* **325**, 147-9.

Kyrolainen, H., Pullinen, T., Candau, R., Avela, J., Huttunen, P. and Komi, P. V. (2000). Effects of marathon running on running economy and kinematics. *Eur J Appl Physiol* **82**, 297-304.

Lazzer, S., Pozzo, R., Rejc, E., Antonutto, G. and Francescato, M. P. (2009). Maximal explosive muscle power in obese and non-obese prepubertal children. *Clin Physiol Funct Imaging* **29**, 224-8.

Lazzer, S., Salvadego, D., Rejc, E., Buglione, A., Antonutto, G. and di Prampero, P. E. (2012). The energetics of ultra-endurance running. *Eur J Appl Physiol* **112**, 1709-15.

Lepers, R., Bigard, A. X., Diard, J. P., Gouteyron, J. F. and Guezennec, C. Y. (1997). Posture control after prolonged exercise. *Eur J Appl Physiol Occup Physiol* **76**, 55-61.

Lichtwark, G. A. and Wilson, A. M. (2007). Is Achilles tendon compliance optimised for maximum muscle efficiency during locomotion? *J Biomech* **40**, 1768-75.

Lichtwark, G. A. and Wilson, A. M. (2008). Optimal muscle fascicle length and tendon stiffness for maximising gastrocnemius efficiency during human walking and running. *J Theor Biol* **252**, 662-73.

Lukaski, H. C., Bolonchuk, W. W., Hall, C. B. and Siders, W. A. (1986). Validation of tetrapolar bioelectrical impedance method to assess human body composition. *J Appl Physiol*. **60**, 1327-32.

Maganaris, C. N. (2002). Tensile properties of in vivo human tendinous tissue. *J Biomech* **35**, 1019-27.

Maganaris, C. N. (2003). Force-length characteristics of the in vivo human gastrocnemius muscle. *Clin Anat* **16**, 215-23.

Maganaris, C. N. and Paul, J. P. (2000). In vivo human tendinous tissue stretch upon maximum muscle force generation. *J Biomech* **33**, 1453-9.

Magnusson, S. P., Aagaard, P., Dyhre-Poulsen, P. and Kjaer, M. (2001). Load-displacement properties of the human triceps surae aponeurosis in vivo. *J Physiol* **531**, 277-88.

Magnusson, S. P., Hansen, P. and Kjaer, M. (2003). Tendon properties in relation to muscular activity and physical training. *Scand J Med Sci Sports* **13**, 211-23.

Maughan, R. J. and Leiper, J. B. (1983). Aerobic capacity and fractional utilisation of aerobic capacity in elite and non-elite male and female marathon runners. *Eur J Appl Physiol Occup Physiol*. **52**, 80-7.

Millet, G. Y., Banfi, J. C., Kerherve, H., Morin, J. B., Vincent, L., Estrade, C., Geyssant, A. and Feasson, L. (2011). Physiological and biological factors associated with a 24 h treadmill ultra-marathon performance. *Scand J Med Sci Sports* **21**, 54-61.

Millet, G. Y., Hoffman, M. D. and Morin, J. B. (2012). Sacrificing economy to improve running performance--a reality in the ultramarathon? *J Appl Physiol* **113**, 507-9.

Morin, J. B., Samozino, P. and Millet, G. Y. (2011). Changes in running kinematics, kinetics, and spring-mass behavior over a 24-h run. *Med Sci Sports Exerc* **43**, 829-36.

Morse, C. I., Tolfrey, K., Thom, J. M., Vassilopoulos, V., Maganaris, C. N. and Narici, M. V. (2008). Gastrocnemius muscle specific force in boys and men. *J Appl Physiol* **104**, 469-74.

Myers, M. J. and Steudel, K. (1985). Effect of limb mass and its distribution on the energetic cost of running. *J Exp Biol* **116**, 363-73.

Narici, M. V., Binzoni, T., Hiltbrand, E., Fasel, J., Terrier, F. and Cerretelli, P. (1996). In vivo human gastrocnemius architecture with changing joint angle at rest and during graded isometric contraction. *J Physiol* **496 (Pt 1)**, 287-97.

Narici, M. V. and Maganaris, C. N. (2007). Plasticity of the muscle-tendon complex with disuse and aging. *Exerc Sport Sci Rev* **35**, 126-34.

Rosager, S., Aagaard, P., Dyhre-Poulsen, P., Neergaard, K., Kjaer, M. and Magnusson, S. P. (2002). Load-displacement properties of the human triceps surae aponeurosis and tendon in runners and non-runners. *Scand J Med Sci Sports* **12**, 90-8.

Saunders, P. U., Pyne, D. B., Telford, R. D. and Hawley, J. A. (2004). Factors affecting running economy in trained distance runners. *Sports Med* **34**, 465-85.

Scrimgeour, A. G., Noakes, T. D., Adams, B. and Myburgh, K. (1986). The influence of weekly training distance on fractional utilization of maximum aerobic capacity in marathon and ultramarathon runners. *Eur J Appl Physiol Occup Physiol* **55**, 202-9.

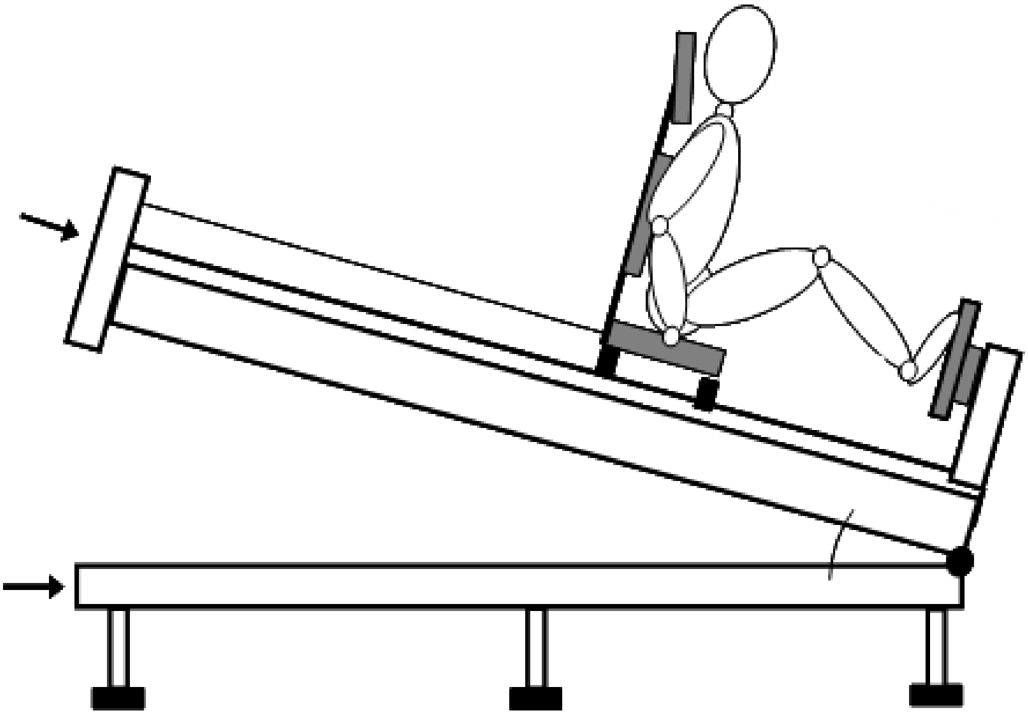
Spurrs, R. W., Murphy, A. J. and Watsford, M. L. (2003). The effect of plyometric training on distance running performance. *Eur J Appl Physiol* **89**, 1-7.

Storen, O., Helgerud, J., Stoa, E. M. and Hoff, J. (2008). Maximal strength training improves running economy in distance runners. *Med Sci Sports Exerc* **40**, 1087-92.

Taboga, P., Lazzer, S., Fessehatsion, R., Agosti, F., Sartorio, A. and di Prampero, P. E. (2012). Energetics and mechanics of running men: the influence of body mass. *Eur J Appl Physiol* **112**, 4027-33.

Willems, T., Witvrouw, E., Delbaere, K., De Cock, A. and De Clercq, D. (2005). Relationship between gait biomechanics and inversion sprains: a prospective study of risk factors. *Gait Posture* **21**, 379-87.

Figure 1. Schematic view of the Explosive Ergometer (EXER).



WT: wire tachometer; CS: carriage seat; FP: force platform. Rail system (R) and lower frame (LF) are hinged (Hi).

Figure 2. Foot-print Index (FPI). Thick line (A): foot-axis; thin line (CoP): trajectory of the center of pressure during stance phase. Area between CoP and A (striped) in cm^2 is FPI.

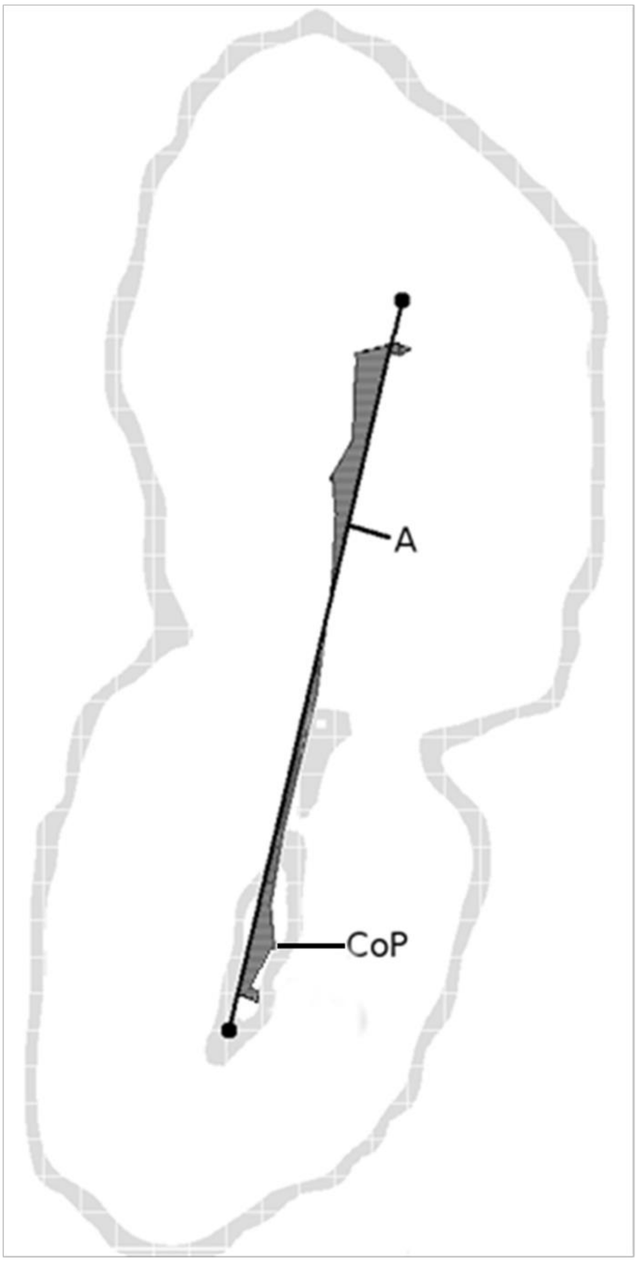


TABLE 1. Physiological characteristics of subjects (n = 11) before the race.

Age (yr)	40.5	±	8.4	[29.5 ; 54.0]
Body mass (kg)	68.6	±	8.2	[57.0 ; 81.0]
Stature (m)	1.72	±	0.06	[1.62 ; 1.80]
Body mass index ($kg \cdot m^{-2}$)	23.2	±	1.9	[20.8 ; 26.4]
Fat-free mass (kg)	56.3	±	5.4	[48.4 ; 64.9]
Fat mass (kg)	12.3	±	4.3	[7.6 ; 22.8]
Fat mass (%)	17.6	±	4.4	[12.2 ; 28.1]
P_{max} (W)	1759	±	202	[1319 ; 1980]
$\dot{V}O_{2max}$ ($ml \cdot min^{-1}$)	3755	±	467	[2969 ; 4387]
$\dot{V}O_{2max}$ ($ml \cdot min^{-1} \cdot kgBM^{-1}$)	55.2	±	6.7	[40.0 ; 62.7]
RER	1.08	±	0.04	[1.02 ; 1.13]
HR_{max} (bpm)	175.5	±	13.7	[147.2 ; 194.9]
v_{max} ($km \cdot h^{-1}$)	17.8	±	1.6	[14.2 ; 19.8]
CoT ($mlO_2 \cdot kg^{-1} \cdot m^{-1}$)	0.190	±	0.008	[0.182 ; 0.197]
Gas exchange threshold				
$\dot{V}O_2$ ($ml \cdot min^{-1}$)	3251	±	488	[2741 ; 4091]
$\dot{V}O_2$ (% $\dot{V}O_{2max}$)	86.6	±	6.5	[75.9 ; 90.0]
RER	0.93	±	0.05	[0.83 ; 1.02]
RER (% RER_{max})	86.4	±	4.0	[81.2 ; 93.2]
HR (bpm)	154.1	±	11.9	[122.4 ; 168.2]
HR (%HR_{max})	87.9	±	5.1	[81.1 ; 92.2]
v ($km \cdot h^{-1}$)	14.1	±	1.7	[10.7 ; 16.3]
v (% v_{max})	79.2	±	4.6	[71.2 ; 85.3]

All values are mean ± standard deviation (SD). Range in square brackets. Pmax: maximal explosive muscle power of the one leg; $\dot{V}O_2$: oxygen uptake; RER: respiratory exchange ratio; HR: heart rate; *v*: velocity; CoT: metabolic cost of transport.

TABLE 2. Physiological characteristics of the triceps surae tendon (TST) and gastrocnemius medialis (GM) of subjects (n = 11) before the race.

TST- cross sectional area (mm^2)	92	±	14	[	79	;	130	]
TST- resting length (L_0, mm)	212	±	21	[	190	;	250	]
TST- strain ($\Delta L \cdot L_0^{-1}$, %)	7.9	±	0.8	[	7.0	;	9.2	]
TST- force (N)	4758	±	828	[	3240	;	5421	]
TST- stiffness (N·mm^{-1})	463	±	85	[	357	;	612	]
TST- Young module (GPa)	1.07	±	0.13	[	0.90	;	1.30	]
GM - fiber length (mm)	61.0	±	6.5	[	49.0	;	68.9	]
GM - pennation angle (°)	19.3	±	2.6	[	15.0	;	23.0	]
GM – thickness (mm)	18.2	±	2.9	[	14.6	;	25.0	]

All values are mean ± standard deviation (SD). Range in square brackets.

TABLE 3. Running time, mean velocity (v_{mean}) and mean heart rate (HR_{mean}) of the three stages (n = 11).

	Stage 1 (25 km)	Stage 2 (55 km)	Stage 3 (13 km)	Total
Running time (hh:mm:ss)	1:45:40 ± 0:14:00 [1:29:12; 2:09:31]	5:30:43 ± 1:12:15 [4:09:31; 7:44:33]	0:58:49 ± 0:12:28 [0:48:29; 1:30:43]	8:15:08 ± 1:36:49 [6:27:00; 11:24:41]
v_{mean} ($km \cdot h^{-1}$)	14.4 ± 1.8 [11.6; 16.8]	10.4 ± 2.1 [7.1; 13.2]	13.7 ± 2.2 [8.6; 16.2]	12.8 ± 2.0 [9.1; 15.4]
HR_{mean} (% of HR_{max})	90.6 ± 3.7 [82.5; 96.0]	78.1 ± 4.8 [69.0; 86.0]	86 ± 4 [79.0; 92.0]	85.0 ± 3.2 [81.3; 91.3]

All values are mean ± standard deviation (SD). Range in square brackets.

TABLE 4. Body mass (BM), metabolic cost of transport (CoT), gas exchange ratio (RER) and biomechanical parameters determined before and immediately after each Stage.

	Stage 1 (25 km)		Stage 2 (55 km)		Stage 3 (13 km)		p		
	Before	After	Before	After	Before	After	Stage	Time	S x T
BM (kg)	68.8 ± 7.7	67.9 ± 7.9	68.8 ± 7.6	66.1 ± 7.7	68.1 ± 7.7	67.5 ± 8.1	0.124	0.001	0.087
CoT ($mlO_2 \cdot kg^{-1} \cdot m^{-1}$)	0.190 ± 0.008	0.197 ± 0.008	0.192 ± 0.006	0.205 ± 0.012	0.196 ± 0.009	0.203 ± 0.013	0.135	0.001	0.338
RER	0.83 ± 0.04	0.82 ± 0.12	0.80 ± 0.05	0.79 ± 0.07	0.79 ± 0.08	0.81 ± 0.11	0.092	0.276	0.096
Contact Time (sec)	0.131 ± 0.014	0.134 ± 0.016	0.135 ± 0.016	0.133 ± 0.013	0.135 ± 0.011	0.135 ± 0.009	0.582	0.898	0.491
Aerial time (sec)	0.221 ± 0.025	0.213 ± 0.018	0.217 ± 0.025	0.214 ± 0.014	0.209 ± 0.017	0.215 ± 0.015	0.328	0.469	0.046
Duty factor (%)	37.4 ± 4.5	38.5 ± 4.2	38.6 ± 5.1	38.1 ± 3.2	39.4 ± 3.1	38.7 ± 2.7	0.336	0.931	0.187
Step frequency ($step \cdot s^{-1}$)	2.85 ± 0.15	2.87 ± 0.12	2.86 ± 0.10	2.87 ± 0.10	2.89 ± 0.11	2.89 ± 0.12	0.191	0.549	0.692
Foot-print Index (cm^2)	13.64 ± 4.72	14.95 ± 4.61	13.38 ± 5.15	16.93 ± 5.28	13.90 ± 4.18	16.51 ± 3.99	0.497	0.001	0.061
CoM (m)	0.054 ± 0.016	0.052 ± 0.011	0.050 ± 0.013	0.050 ± 0.013	0.049 ± 0.012	0.051 ± 0.013	0.262	0.826	0.690
GRF (N)	1473 ± 181	1409 ± 138	1458 ± 163	1400 ± 159	1454 ± 150	1474 ± 139	0.154	0.029	0.075
W_{ext} ($mlO_2 \cdot kg^{-1} \cdot m^{-1}$)	0.087 ± 0.013	0.088 ± 0.014	0.082 ± 0.009	0.085 ± 0.015	0.078 ± 0.009	0.078 ± 0.016	0.093	0.588	0.733
k_{vert} ($N \cdot m^{-1}$)	28360 ± 5734	27315 ± 5001	29120 ± 5868	29644 ± 5492	30771 ± 6324	30848 ± 5836	0.083	0.726	0.517
Ext_{max} (°)	169 ± 5	169 ± 3	170 ± 4	170 ± 6	170 ± 4	171 ± 4	0.362	0.899	0.558
$Load_{max}$ (°)	143 ± 5	143 ± 5	145 ± 5	146 ± 6	145 ± 5	145 ± 5	0.221	0.461	0.304
$Flex_{max}$ (°)	89 ± 8	88 ± 7	93 ± 7	91 ± 8	94 ± 9	90 ± 7	0.073	0.142	0.884

All values are mean ± standard deviation (SD). Range in square brackets.

BM: body mass; CoT: cost of transport; RER: respiratory exchange ratio; CoM: centre of mass; GRF: maximal vertical ground reaction force; W: external mechanical work; k_{vert}: vertical stiffness; Ext_{max}: maximal extension joint angle of the knee; $Load_{max}$: maximal load joint angle; $Flex_{max}$: maximal flexion joint angle of the knee.

Significance by GLM Repeated Measures with two factors of the main effects of Stage (S), Time (T, before *vs* after) and Interaction (S x T).

Effects of the Etna uphill ultra-marathon on energy cost and mechanics of running

Stefano Lazzer[1,2], Desy Salvadego[1,2], Paolo Taboga[1,2], Enrico Rejc[1,2], Nicola Giovanelli[1,2] and Pietro E. di Prampero[2]

1. Department of Medical and Biological Sciences, University of Udine, Italy
2. School of Sport Sciences, University of Udine, Italy

Abbreviated title: Energetics and mechanics of running

Corresponding Author:
Dr. Stefano Lazzer, PhD
University of Udine
Department of Medical and Biological Sciences
P.le Kolbe 4
33100 Udine, Italy
Phone: +39 0432 494333 - Fax: +39 0432 494301
e-mail: stefano.lazzer@uniud.it

ABSTRACT

Purpose: To investigate the effects of an extreme uphill marathon on the mechanical parameters that are likely to affect the energy cost of running (Cr).

Methods: Eleven runners (27-59 years) participated in the “Etna SuperMarathon” (43 km, 0-3063 m a.s.l.). Anthropometric characteristics, maximal explosive power of the lower limb (P_{max}) and $V'O_{2max}$ were determined before the competition. In addition, before and immediately after the race, Cr, contact (t_c) and aerial (t_a) times, step frequency (f) and running velocity (v) were measured at constant self-selected speed. Then, peak vertical ground reaction force (F_{max}), vertical downward displacement of the centre of mass (Δz), leg length change (ΔL), vertical (k_{vert}) and leg (k_{leg}) stiffness were calculated.

Results: Direct relationship between Cr, measured before de race, and race time was shown ($r= 0.61$; $p<0.001$). Cr increased significantly at the end of the race by 8.7%. Immediately after the race, the subjects showed significantly lower t_a (-58.6%), f (-11.3%), F_{max} (-17.6%), k_{vert} (-45.6%) and k_{leg} (-42.3%) and higher t_c (+28.6%), Δz (+52.9%) and ΔL (+44.5%) than before the race. The increase of Cr was associated with a decrement in F_{max} ($r=-0.45$), k_{vert} ($r=-0.44$) and k_{leg} ($r=-0.51$). Finally, an inverse relationship between P_{max} measured before the race and ΔCr during race was found ($r=-0.52$).

Conclusions: Lower Cr was related with better performance, and athletes characterized by the greater P_{max} showed lower increases in Cr during the race. This suggests that specific power training of the lower limbs may lead to better performance in ultra-endurance running competition.

Key Words: maximal oxygen uptake; cost of transport; trail; kinematics; stiffness.

INTRODUCTION

The energy cost of running (Cr), together with the maximal aerobic power (V'O$_2$max), its fraction (*F*) sustained throughout the competition and the maximal capacity of the anaerobic stores (AnS) represent the main factors determining running performances [1]. Cr, defined as the amount of energy spent above resting to transport 1 kg body mass over 1 m distance (expressed in $J \cdot kg^{-1} \cdot m^{-1}$ or $mlO_2 \cdot kg^{-1} \cdot m^{-1}$), plays a relevant role in determining the performance among middle and long distance runners with the same V'O$_2$max and *F* [2]. Its average value amounting to 0.182 ± 0.014 $mlO_2 \cdot kg^{-1} \cdot m^{-1}$ (3.75 ± 0.29 $J \cdot kg^{-1} \cdot m^{-1}$) [1], with an inter-individual variability of about 10%, and with lower values in endurance runners than in middle distance runners.

Cr is unaffected by the speed from about 2.2 $m \cdot s^{-1}$ to 5 $m \cdot s^{-1}$ [1], where the air resistance plays a minor role, less than 5% of the total energy cost [3]. In long distance runners, Cr increases with the distance covered because of the fatigue effects. Brueckner et al.[4] observed an increment of Cr of about 0.142% per km of distance during a marathon, with a total increase greater than 5%. Indeed, Gimenez et al. [5], in subjects who ran 24h on a motorized treadmill, observed a substantial increases in Cr after 8 hours; in addition, the subjects who maintained the highest speed (expressed in percentage of the velocity attained at V'O$_2$max) were those having the smallest Cr increase over the 24. Furthermore, several authors [6,7] have shown that, in mountain ultra-marathons, the changes of Cr are brought about by changes of the mechanics of running. The principal aim of which is to minimize damage to lower limb tissue, muscular fatigue, and symptoms associated with prolonged running over irregular terrain with a large positive/negative elevation variation along their race [8,9].

The mechanics of running in different conditions has been frequently investigated using the spring mass model [10], i.e., representing the leg in contact with the ground as a simple linear spring. In this model, the parameters most frequently studied are the leg (k_{leg}) and vertical

(k_{vert}) stiffness coefficients, associated with leg spring deformation (ΔL) and with the vertical displacement (Δz) of the centre of mass, respectively. Thus, whereas, k_{vert} is a measure of the resistance of the body to vertical displacement after application of ground reaction forces, k_{leg} is the resistance to change in leg length after application of internal or external forces.

The effects of long and ultra-long races on running mechanics have recently been investigated. Morin et al.[11], considering a mountain ultra-marathon race (166 km, total positive and negative elevation of 9500 m) showed that athletes significantly reduced ($P<0.001$) aerial time (t_a), peak vertical ground reaction force (F_{max}), Δz, with an increment in step frequency (f). On the other hand, the contact time (t_c) was not different as compared to before the race. Furthermore, there was a nearly significant ($P=0.053$) change in k_{vert}, which increased by 6% after the race. This study supports previous findings [12], where the same behaviour of f, brought about by a shorter t_a with no changes in t_c was reported. Conversely, after 24 hours of treadmill running on the level, Morin et al.[13] observed a reduction in F_{max}, Δz, ΔL, and an increment in k_{vert} and f, but with lower t_c and constant t_a. This discrepancy in changes of t_c and t_a compared to previous studies could be due to the different mechanics of uphill and downhill mountain running, as compared to treadmill running. As evidenced by Fourchet et al. [14], a 5h long hilly run induces different effects on ankle muscles, as compared to flat running; in particular only plantar flexor muscles are affected by neuromuscular alterations, likely leading to a different running mechanics between mountain and flat runs.

Indeed, interventions to reduce Cr are constantly sought after by athletes, coaches and sport scientists. Strength [15] and plyometric [16] training allow muscles and tendons to utilize more elastic energy and to reduce the amount of energy wasted in braking forces thus reducing Cr.

Therefore, the purpose of the present study was to investigate the effects of an extreme uphill marathon on several mechanical parameters that are likely to affect C_r.

RESEARCH DESIGN AND METHODS

Participants

Sixteen healthy Italian male runners (age range 27-59 years) were enrolled in this study as participants in the uphill marathon named “Etna SuperMarathon”. The experimental protocol was approved by the Ethics Committee of the University of Udine. Before the study began, the purpose and objectives were carefully explained to each subject and written informed consent was obtained from all of them. Subjects having overt metabolic and/or endocrine diseases and those taking medications regularly or using drugs known to influence energy metabolism were excluded. The participants were recruited among experienced ultra-endurance runners who filled out questionnaires on physical exercise activity, demographics, medical history and lifestyle [17]. All the participants of this study had run at least one ultra-endurance race in their career. On average, subjects had 9.3±5.4 and 5.8±5.6 years of training history and of ultra-endurance running race, respectively. They reported to run on average 69.2 ± 23.5 km every week. Sixteen athletes who were eligible for the study began the race, and the 11 who completed the entire competition were taken into account for data analysis.

Experimental protocol

One week before the race, the subjects came to the exercise physiology laboratory, where anthropometric characteristics, mechanical power of the lower limbs and a graded exercise test to exhaustion on a treadmill were performed. The subjects were asked to refrain from any vigorous physical activity during the day preceding the test and during the preliminary testing session that they performed to familiarize with all the different equipment.

The “Etna SuperMarathon” took place in June 2012. The race started at 8:00 AM from the beach of Marina di Cottone (Catania, Italy), at sea level, with temperature and relative humidity of 29°C and 42%, respectively. Athletes covered about 30 km on the road to the

Etna volcano, while the last part of the race took place on a path of lava rock. After a total distance of about 43 km, athletes reached the finish line covering an altitude difference of 3063 m with a mean slope of about 7% and with peak values reaching 14% (Garmin Forerunner 305 GPS, Kansas City, USA). At the finish, temperature and relative humidity were 21°C and 52%, respectively.

The day before the race and immediately after the end of the race (4 ± 2 min), body mass (BM), Cr, respiratory exchange ratio (RER) and running mechanics were measured.

Physiological measurements before the race

BM was measured to the nearest 0.1 kg with a manual weighing scale (Seca 709, Hamburg, Germany), stature was measured to the nearest 0.001 m on a standardized wall-mounted height board. Body mass index (BMI) was calculated as BM (kg) · stature^{-2} (m).

Maximal power of lower limbs during a counter movement jump was assessed by means of the Bosco et al.[18] test (Ergo Jump, Boscosystem, Italy) .

Maximal oxygen uptake (V'O_2max) and maximal heart rate (HR_{max}) were determined during a graded exercise test on a treadmill (Saturn, HP Cosmos, Germany) under medical supervision. During the experiment, ventilatory and gas exchange responses were measured continuously with a metabolic unit (Quark-b^2, Cosmed, Italy). The volume and gas analysers were calibrated using a 3-L calibration syringe and calibration gas (16.00% O_2; 4.00% CO_2), respectively. During the tests, electrocardiogram was continuously recorded and displayed on line for visual monitoring, and HR was measured with a dedicated device (Polar, Finland).

Before the start of the study, subjects were thoroughly familiarized with treadmill running. The tests were performed one week before the race and consisted in a 5-min rest period followed by running at 10 km·h^{-1} for 5 min (treadmill slope: 1%); the speed was then increased by 0.7 km·h^{-1} every minute until volitional exhaustion. A levelling off of oxygen uptake (defined as an increase of no more than 1 ml·kg^{-1}·min^{-1}) was observed in all subjects during the last one or two minutes of the exercise test, indicating that V'O$_2$max had been attained. V'O$_2$max and HR$_{max}$ were calculated as the average oxygen uptake and HR of the last 20 s of the test.

Energy cost of running and mechanical measurements during the race

The day before and immediately after the race, the subjects ran for 6 min at a constant self-selected speed on two oval compact rock paths situated near the start line (at sea level) and near the finish line (at 3063 m above sea level), respectively. Both compact rock paths were flat and 50 m long.

Cr and RER were measured continuously with a portable metabolic unit (k4, Cosmed, Italy). The analyser, calibrated prior to each testing session, provided breath-by-breath data recording. The last minute of sampling was used for further analysis. For all subjects, real-time plots of V'O$_2$ and RER indicated that metabolic steady state was achieved after 5 minutes. Net V'O$_2$, obtained by subtracting pre-exercise standing V'O$_2$ (measured for 6 min in resting condition before the race) from gross V'O$_2$, was converted to joules using an energetic equivalent for O$_2$ based on the RER. This RER was always below 1.0 confirming that aerobic metabolism was the main metabolic pathway. Cr was then obtained by dividing net energy expenditure (J·kg^{-1}·s^{-1}) by running speed (v, m·s^{-1}); the latter was measured by means of to two photocells placed immediately before and after the video recording zone (see below), at a distance of 10 m between them. In addition, average lap speed was obtained dividing the circuit length by the time needed to cover it. Average lap speed was not significantly different than running speed measured in the video recording zone. All subjects were also requested to maintain the same self-selected speed during the tests before and after the race.

The running mechanics was studied using a digital camera with a sample frequency of 400 Hz (Nikon J1, Japan). The camera was placed perpendicular to the running direction of athletes. For each subject, video was recorded between the fourth and the sixth minute of running. Ten subsequent representative steps were analysed, taking into account t_c (s) and t_a (s).

Step frequency (step·s^{-1}) was calculated as:

$$f = \frac{1}{(t_a + t_c)} \qquad \text{eq. 1}$$

Given t_c (s), t_a (s), v (m·s^{-1}), subject's BM (kg), and lower limb length (distance between great trochanter and ground during standing, L in m), spring mass parameters were calculated using the computation method proposed by Morin et al.[19]. This method, based on modelling of the ground reaction force signal during the contact phase by a sine function, allows the computation of k_{vert} (kN·m^{-1}) as the ratio of the F_{max} (N) to the Δz (m). K_{leg} (kN·m^{-1}) was then calculated as the ratio of F_{max} and the ΔL (m,) during contact of the foot on the ground.

Statistical analyses

Statistical analyses were performed using PASW Statistic 18 (SPSS Inc., IL, USA) with significance set at $p<0.05$. All results are expressed as means and standard deviation (SD).

Normal distribution of the data was tested using the Kolmogorov-Smirnov test.

Changes of BM, Cr, RER and mechanical parameters during the competition were studied with Student's paired t-test.

The relationships between mechanical variables affecting Cr were investigated using Pearson's product–moment correlation coefficient.

RESULTS

The physical characteristics before the race of the 11 subjects who completed the race are reported in Table 1, together with their performance time. Their average V'O_2max, Cr and P_{max} were 49.2±8.8 $mlO_2 \cdot min^{-1} \cdot kgBM^{-1}$, 0.190±0.023 $mlO_2 \cdot m^{-1} \cdot kg^{-1}$ and 1628±212 W, respectively.

As reported in Figure 1, a direct relationship between Cr and race time was observed before ($r = 0.61$, $p<0.001$) as well as after ($r = 0.48$, $p<0.05$) the race.

Immediately after the race, Cr was 8.7% higher ($p<0.001$) than before the race; on the contrary, BM and self-selected running speed were 5.7% and 7.3% lower ($p<0.05$) than before the race (Table 2). In addition, subjects showed significantly lower t_a (-58.6%), f (-11.3%), F_{max} (-17.6%), k_{vert} (-45.6%) and k_{leg} (-42.3%) and higher t_c (+28.6%), Δz (+52.9%) and ΔL (+44.5%) than before the race (Table 2).

In order to identify the main factors affecting Cr during the race, the mechanical parameters measured before and after the race were plotted for all subjects as a function of Cr. Pearson's correlation coefficients were then used to analyse the association between variables entering these equations. This analysis showed an inverse relationships between Cr and F_{max} ($r = -0.45$, Figure 2C), Cr and k_{vert} ($r = -0.44$, Figure 2E) and Cr and k_{leg} ($r = -0.51$, Figure 2F). No significant relationships between Cr and t_c, t_a, step frequency, ΔZ and ΔL were found.

Finally, an inverse relationship between mechanical power of the lower limbs measured before the race and changes in Cr during the race was found (Figure 3; $r = -0.52$).

DISCUSSION

The main results of the present study showed that 1) Cr is directly related with the race time; 2) Cr increased significantly at the end of this extreme uphill race (~9%); 3) the increase in Cr was associated with a decrease in F_{max}, k_{vert} and k_{leg}; and 4) the greater the mechanical power of the lower limbs the lesser the changes in Cr due to the race.

Several authors have shown that Cr is an important part of the success in athletes with comparable V'O_2max and *F* even if conflicting results have also been reported[2]. Millet et al.[20] observed, during a 24 hours treadmill run, that Cr was not directly related to performance but may nevertheless be important to be able to maintain a high %V'O_2max. In addition, Gimenez et al. [5] have shown that Cr measured before the 24 hours treadmill run was negatively correlated with the speed expressed in %V'O_2max. This finding suggests that a low Cr could be important in determining the performance during ''low-intensity'' ultra-endurance events and our results support this view, since Cr was strongly related with race performance (Figure 1).

At the end of this extreme uphill race, Cr was increased by about 9% compared to before the race, as observed in previous studies considering ultra-marathon events [5,6]. This difference was greater than the ones observed during classic flat marathons [4], probably because of the relevant slope and altitude difference covered by subjects and because of the type of road surface. As observed previously[21], the increase in Cr with the slope is related with the increase in total work including internal work. Furthermore, in the last part of the race (~15 km), the subjects ran on a path of lava rock. This terrain can contribute to increasing Cr as compared to compact terrain, and could be attributed to a reduced recovery of potential and kinetic energy at each stride [22]. Indeed, as suggested by Millet et al. [6], during long distance running events, greatly exceeding the marathon, maintaining a high *F* may help reducing damage to lower limb tissue, muscular fatigue and symptoms associated with prolonged running, even if such a strategy may lead to increased Cr values; thus, in the end "sacrificing economy to improve running performance”. **On the other hand, in agreement with our results, some authors [23,24] are of the opinion that Cr in ultra-marathon runners has an important role in setting performance, suggesting that the same phenotype and physiological factors, including Cr, that determine success in marathon running [25] are also likely to determine success in ultra-marathons and this should be even more evident when the level of ultra-endurance athletes increases [23].**

Moreover, we do not think that the increasing altitude (from 0 to 3063 m above sea level) had any effect on Cr, while obviously leading to a fall of about 10-15% on $V'O_2max$. We would like to point out that at sea level, before the race, $V'O_2$ at the speed of 173 $m{\cdot}min^{-1}$ was on the average 42.7 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$, i.e. about 87% of the corresponding $V'O_{2max}$. At altitude, immediately after the race, $V'O_2$ was reduced to 36 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$ at the speed of 161 $m{\cdot}min^{-1}$, i.e. about 80-85% of the corresponding $V'O_{2max}$ estimated at altitude. The O_2 consumption of the respiratory muscles, as obtained from the expiration ventilation (BTPS), according to Coast et al.[26], amounted to 188 and 170 $ml{\cdot}min^{-1}$ at sea level and at altitude, respectively. Thus, the energy cost of running, when subtracting the O_2 consumption of the respiratory muscles and the resting $V'O_2$ (4.4 and 4.6 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$ at sea level and at altitude) amounted to 0.171 and 0.183 $ml{\cdot}kg^{-1}{\cdot}m^{-1}$, respectively. The resultant increase of Cr, about 7%, is therefore essentially equal to that reported above. Then, the observed increase of Cr is independent of the effects of altitude on $V'O_{2max}$ and on ventilation. Which, as is well known, are widely different in different subjects and lead to larger decreases in individual $V'O_2max$ [27], the larger its sea level value [28].

In addition, we would like to point out that the RER amounted to 0.88 and 0.82 at sea level and at altitude, respectively, and that these values are close to that can be expected for the metabolic respiratory quotient for these exercise intensities.

At the end of the race, the following changes of the running mechanics were observed: lower t_a, *f*, F_{max}, k_{vert} and k_{leg} and higher t_c, Δz and ΔL (Table 2). Only the decreases of t_a and F_{max} were in line with previous studies on ultra-endurance events [11,13,29]. These differences could be related to the fact that the subjects ran, before and after the race, at self-selected speed which represented their real optimal running speed. At the end of the race, subjects decreased their self-selected speed during the test by 7.3% on the average, this reduction was related with their degree of fatigue and represents the real effort that subjects were able to sustain after the race. However, the changes in self-selected running speed observed during the test before and after the race had only a partial effect on changes in the mechanical parameters considered in the present study. In fact, as observed previously[30], k_{leg} showed no statistical differences at speeds between 2.5 and 3.5 $m \cdot s^{-1}$; in addition, the speed has no effect on k_{leg} [19]. Indeed, if the speed was reduced from 2.9 to 2.7 $m \cdot s^{-1}$, k_{vert} decreased from 33 to 32 $kN \cdot m^{-1}$ (-4%)[30], which was not statistically significant. Morin et al.[19] did not measure k_{vert} at speeds as low as 2.9 and 2.7 $m \cdot s^{-1}$, even so, we fitted the data points reported in their study with a 2nd order polynomial, obtaining the following equation:

$$k_{vert}=1.512 \cdot s^2 - 6.906 \cdot s + 34.022 \qquad \text{eq. 2}$$

where k_{vert} is expressed in $kN \cdot m^{-1}$ and the speed (s) in $m \cdot s^{-1}$ (N=5 data points, $r^2=0.997$). According to equation 2, at 2.9 and 2.7 $m \cdot s^{-1}$, k_{vert} would be 27 and 26 $kN \cdot m^{-1}$, respectively (-1%). In the present study k_{leg} decreased by 42.3% and k_{vert} by 45.6%, thus suggesting that the changes in these mechanical parameters observed in the present study were largely affected by fatigue and only marginally by speed.

In addition, at the end of the race t_c increased (by ~29%) and t_a decreased (by ~59%), leading to a significant decrease in step frequency (by ~11%). In turn, the observed increase of t_c lead to a significant increase in Δz (by ~53%) and ΔL (by ~45%). Furthermore, k_{vert} and k_{leg}

decreases were strongly related to a reduction in F_{max} and to the increase in vertical displacement (Δz) which can be interpreted as a safer running style, as discussed below. The differences in the changes of the mechanical parameters between the present study and the previous ones on ultra-marathon[11,13,29] can be explained as follow.

1) We considered self-selected speed as representative of the fatigue level of the subjects, which induced different mechanical adaptations, particularly increasing t_c and consequently reducing t_a and f. Dutto and Smith [31] reported decreases in f accompanied by a decrease of k_{vert} in long running trials, suggesting that it is the inability of the system to maintain an optimal stiffness that drives to exhaustion. Furthermore, the decrease in f observed at the end of the race, was probably related to the fact that this ultra-marathon was characterised by a continuous positive work. This condition implies mainly concentric muscle contractions, which induce less muscle damage in knee-extensor and plantar-flexors muscles than the eccentric contractions characterising downhill running generally included in ultra-marathon [8,9]. This condition may lead to lesser changes in running mechanics (aiming at decreasing the load on the muscles) than observed in previous extreme ultra-marathons [11,13,29]. In addition, the decrease in f observed in the present condition is likely associated with a decrease in internal work performance and thus in the corresponding cardiorespiratory responses, which in turn, may be particularly relevant which running uphill at 3000 m above sea level.

2) A greater continuous positive work performance than observed in previous studies [11,29], which did not allow any recovery periods for the athletes during the race.

3) The potential differences between ultra-long distance running on a treadmill and over ground [13,20], which may have induced different adaptations of t_c.

4) The post-race tests were done immediately after the subjects crossing the finishing line which allowed us to examine the real effects of total fatigue on metabolic and mechanical parameters.

In order to identify the main factors affecting Cr during the ultra-endurance running race, the effects of changes on mechanical parameters before and after the race were plotted for all subjects as a function of the corresponding changes on Cr (Figure 2).

In particular, the increases in t_c with decreases in t_a, implied a decreases in F_{max}, which was related with the increase in Cr during the race (Figure 2 C). These changes in running mechanics can be interpreted as a safer running style associated with an overall lower impact, especially during the eccentric phase of each step, to the detriment of an increase of Cr[6].

In particular, the decrease of k_{vert} and k_{leg}, brought about by fatigue, induced each runner to sink further during contact, i.e. increasing t_c and Δz. Furthermore the decreased f likely led runners to a less efficient elastic energy utilization [32], and therefore lower velocity, at the end of the stance phase, resulting in a decreased t_a. Finally, a shorter t_a implies that the runner landed with less downward momentum, thus requiring less upward impulse during the subsequent stance phase, therefore also F_{max} was lower. In addition, a decreased F_{max} can also be due to a reduced force capacity of subjects because of fatigue during the race. Our results are in accordance with Morin et al. [13] and Degache et al. [29] who evidenced a decreased Fmax at the end of long running trials; however, the question if this is a strategy intentionally adopted by runners or the result of fatigue, it remains unsolved.

Indeed, the most powerful athletes showed the lower changes in Cr (Figure 3). These results are in agreement with previous studies in athletes [15,16] which emphasize the importance of the muscle-tendon system and strength training to reduce Cr. In addition, force reduction during the race can lead to ankle instability[33], thus leading to a reduction of the capacity of the foot to utilize all the mechanical energy transmitted by the muscle-tendon complex for forward displacement.

Practical Applications

Cr represents one of the main factors determining performances in ultra-endurance runners and its increase during the competition is related to mechanics of running deterioration and lower Pmax. These data show the importance of the lower limb muscle's characteristics which maximize efficiency and reduce Cr during running. This suggests that coaches and ultra-endurance runners need to strengthen the specific lower limb power training in their preparation.

Conclusion

The increased Cr during the Etna uphill marathon was related to changes in the mechanics of running such as increases in t_c, Δz and ΔL and decreases in t_a, f, F_{max}, k_{vert} and k_{leg}. In addition, lower Cr was related with better performance, and athletes characterized by the greater P_{max} showed lower increases in Cr during the race. This suggests that specific power training of the lower limbs may lead to better performance in ultra-endurance running competition.

ACKNOWLEDGEMENTS

We are grateful to the athletes for their kind collaboration, to Mr M. Malfitana, Mr E. Bertolissi and the Unione Sportiva M. Tosi, Tarvisio (Italy) for their valuable contribution in carrying out measurement during the race. We are grateful to Prof. G Antonutto, Prof. B. Grassi and Dr A. Da Ponte for the assistance during the study. The financial support of the Unione Sportiva M. Tosi, Tarvisio (Italy) and Lions Club, Udine-Duomo (Italy), are gratefully acknowledged.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES

1. di Prampero PE, Atchou G, Bruckner JC, Moia C. The energetics of endurance running. *Eur J Appl Physiol Occup Physiol* 1986;55:259-266.
2. Sjodin B, Svedenhag J. Applied physiology of marathon running. *Sports Med* 1985;2:83-99.
3. Jones AM, Doust JH. A 1% treadmill grade most accurately reflects the energetic cost of outdoor running. *J Sports Sci* 1996;14:321-327.
4. Brueckner JC, Atchou G, Capelli C, et al. The energy cost of running increases with the distance covered. *Eur J Appl Physiol Occup Physiol* 1991;62:385-389.
5. Gimenez P, Kerherve H, Messonnier LA, Feasson L, Millet GY. Changes in the Energy Cost of Running during a 24-h Treadmill Exercise. *Med Sci Sports Exerc* 2013;45:1807-1813
6. Millet GY, Hoffman MD, Morin JB. Sacrificing economy to improve running performance--a reality in the ultramarathon? *J Appl Physiol* 2012;113:507-509
7. Vernillo G, Savoldelli A, Zignoli A, et al. Influence of the world's most challenging mountain ultra-marathon on energy cost and running mechanics. *Eur J Appl Physiol* 2014;114:929-939
8. Millet GY, Tomazin K, Verges S, et al. Neuromuscular consequences of an extreme mountain ultra-marathon. *PLoS One* 2011;6:e17059
9. Saugy J, Place N, Millet GY, et al. Alterations of Neuromuscular Function after the World's Most Challenging Mountain Ultra-Marathon. *PLoS One* 2013;8:e65596
10. Blickhan R. The spring-mass model for running and hopping. *J Biomech* 1989;22:1217-1227

11. Morin JB, Tomazin K, Edouard P, Millet GY. Changes in running mechanics and spring-mass behavior induced by a mountain ultra-marathon race. *J Biomech* 2011;44:1104-1107

12. Morin JB, Samozino P, Peyrot N. Running pattern changes depending on the level of subjects' awareness of the measurements performed: a "sampling effect" in human locomotion experiments? *Gait Posture* 2009;30:507-510

13. Morin JB, Samozino P, Millet GY. Changes in running kinematics, kinetics, and spring-mass behavior over a 24-h run. *Med Sci Sports Exerc* 2011;43:829-836

14. Fourchet F, Millet GP, Tomazin K, et al. Effects of a 5-h hilly running on ankle plantar and dorsal flexor force and fatigability. *Eur J Appl Physiol* 2012;112:2645-2652

15. Storen O, Helgerud J, Stoa EM, Hoff J. Maximal strength training improves running economy in distance runners. *Med Sci Sports Exerc* 2008;40:1087-1092

16. Spurrs RW, Murphy AJ, Watsford ML. The effect of plyometric training on distance running performance. *Eur J Appl Physiol* 2003;89:1-7

17. Craig CL, Marshall AL, Sjostrom M, et al. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 2003;35:1381-1395.

18. Bosco C, Luhtanen P, Komi PV. A simple method for measurement of mechanical power in jumping. *Eur J Appl Physiol Occup Physiol* 1983;50:273-282

19. Morin JB, Dalleau G, Kyrolainen H, Jeannin T, Belli A. A simple method for measuring stiffness during running. *J Appl Biomech* 2005;21:167-180

20. Millet GY, Banfi JC, Kerherve H, et al. Physiological and biological factors associated with a 24 h treadmill ultra-marathon performance. *Scand J Med Sci Sports* 2011;21:54-61

21. Minetti AE, Ardigo LP, Saibene F. Mechanical determinants of the minimum energy cost of gradient running in humans. *J Exp Biol* 1994;195:211-225

22. Zamparo P, Perini R, Orizio C, Sacher M, Ferretti G. The energy cost of walking or running on sand. *Eur J Appl Physiol Occup Physiol* 1992;65:183-187

23. Millet GP. Economy is not sacrificed in ultramarathon runners. *J Appl Physiol (1985)* 2012;113:686; author reply 687

24. Perrey S, Joyner M, Ruiz JR, et al. Commentaries on viewpoint: sacrificing economy to improve running performance--a reality in the ultramarathon? *J Appl Physiol (1985)* 2012;113:510-512

25. Joyner MJ, Ruiz JR, Lucia A. The two-hour marathon: who and when? *J Appl Physiol (1985)* 2011;110:275-277

26. Coast JR, Rasmussen SA, Krause KM, et al. Ventilatory work and oxygen consumption during exercise and hyperventilation. *J Appl Physiol* 1993;74:793-798

27. Cerretelli P. Limiting factors to oxygen transport on Mount Everest. *J Appl Physiol* 1976;40:658-667

28. Ferretti G, Moia C, Thomet JM, Kayser B. The decrease of maximal oxygen consumption during hypoxia in man: a mirror image of the oxygen equilibrium curve. *J Physiol* 1997;498:231-237.

29. Degache F, Guex K, Fourchet F, et al. Changes in running mechanics and spring-mass behaviour induced by a 5-hour hilly running bout. *J Sports Sci* 2013;31:299-304

30. Arampatzis A, Bruggemann GP, Metzler V. The effect of speed on leg stiffness and joint kinetics in human running. *J Biomech* 1999;32:1349-1353

31. Dutto DJ, Smith GA. Changes in spring-mass characteristics during treadmill running to exhaustion. *Med Sci Sports Exerc* 2002;34:1324-1331

32. Snyder KL, Farley CT. Energetically optimal stride frequency in running: the effects of incline and decline. *J Exp Biol* 2011;214:2089-2095

33. Lazzer S, Taboga P, Salvadego D, et al. Factors affecting metabolic cost of transport during a multi-stage running race. *J Exp Biol* 2014;217:787-795

FIGURE CAPTION

Figure 1: Race time (min) plotted for all subjects as a function of energy cost of running (Cr, $mlO_2 \cdot kg^{-1} \cdot m^{-1}$) measured before (●) and immediately after (o) the race.

Figure 2: Contac time (t_c, A), aerial time (t_a, B), maximal vertical ground reaction force (F_{max}, C), downward displacement of centre of mass during contact (Δz, D), vertical stiffness (k_{vert}, E) and leg stiffness (k_{leg}, F) measured before (•) and immediately after the race (o) plotted for all subjects as a function of the measured energy cost of running (C_r, $mlO_2 \cdot kg^{-1} \cdot m^{-1}$) .

Figure 3: Maximal mechanical power of the lower limbs (P, W) measured before the race plotted for all subjects as a function of changes in energy cost of running caused by the race (ΔCr, %) .

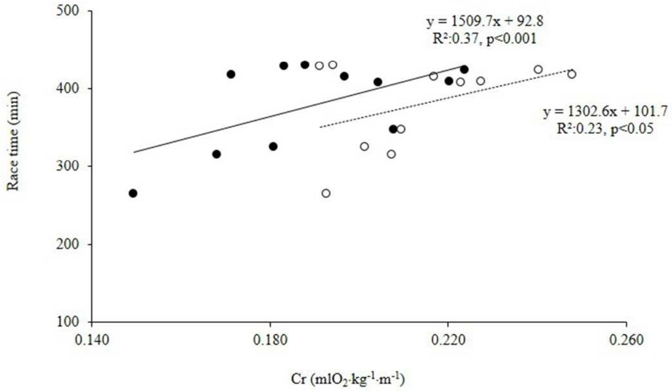
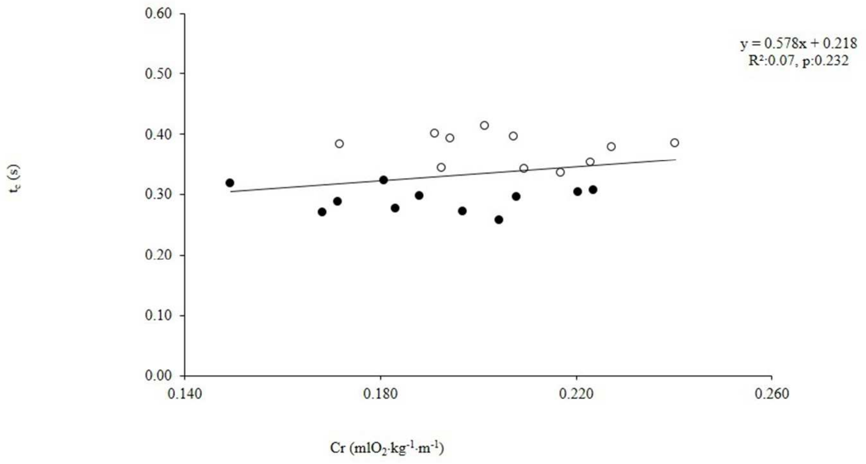
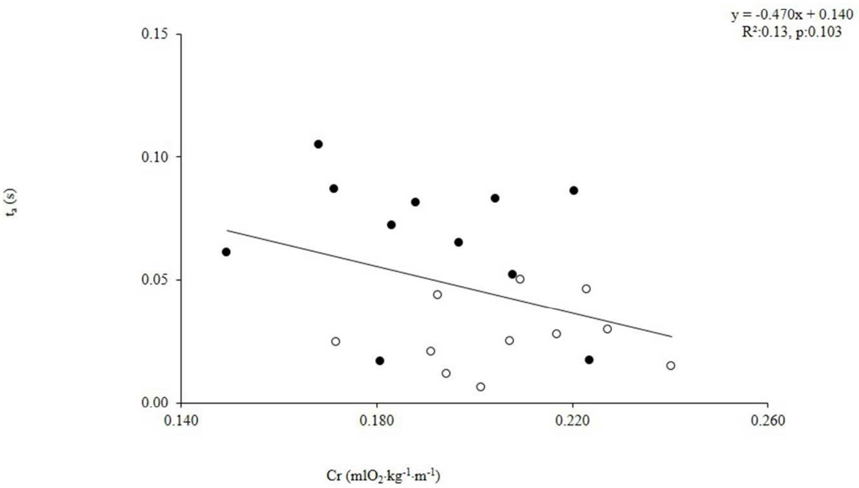


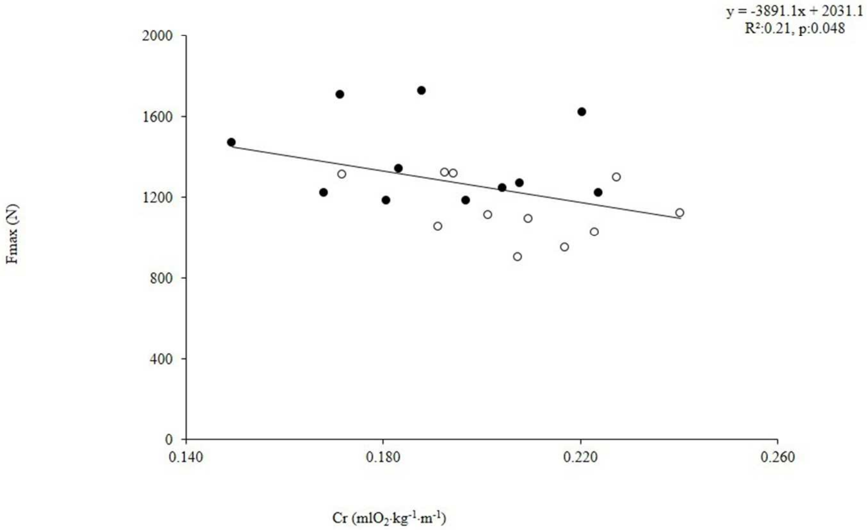
Figure 1
275x190mm (96 x 96 DPI)



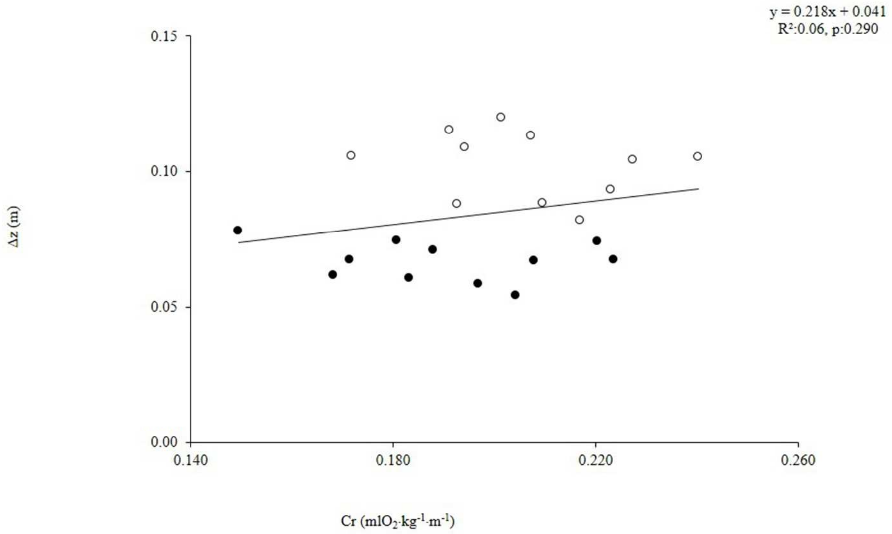
275x190mm (96 x 96 DPI)



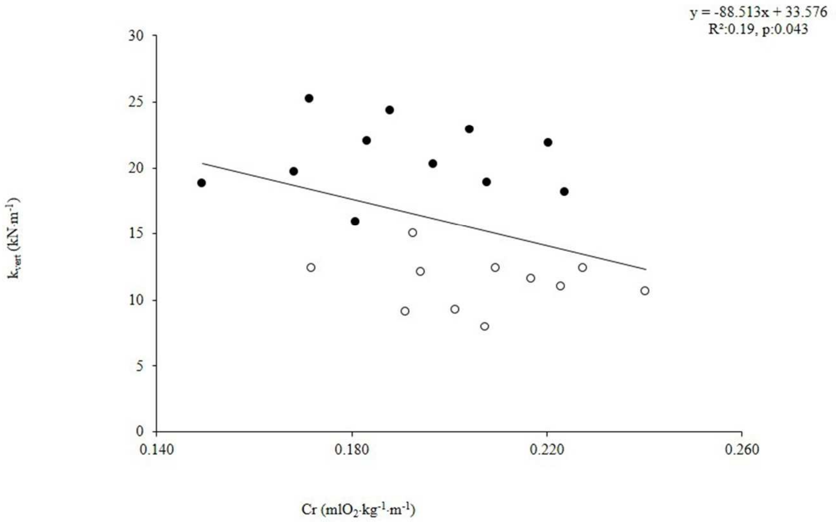
275x190mm (96 x 96 DPI)



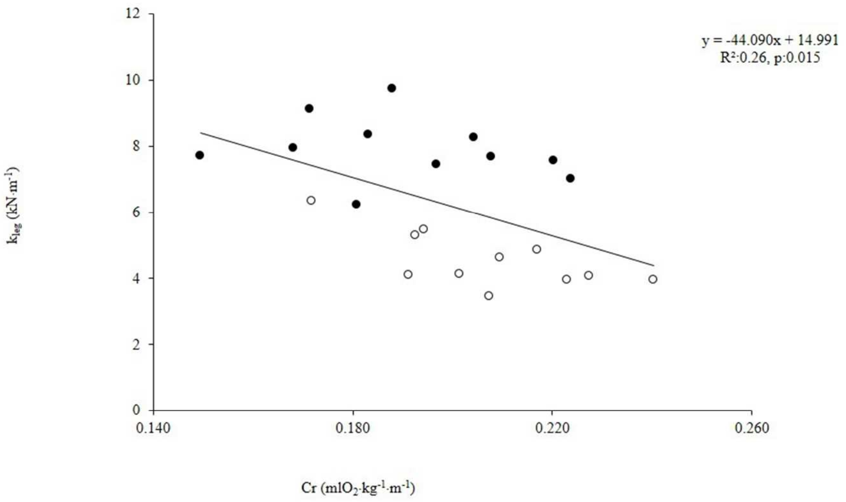
275x190mm (96 x 96 DPI)



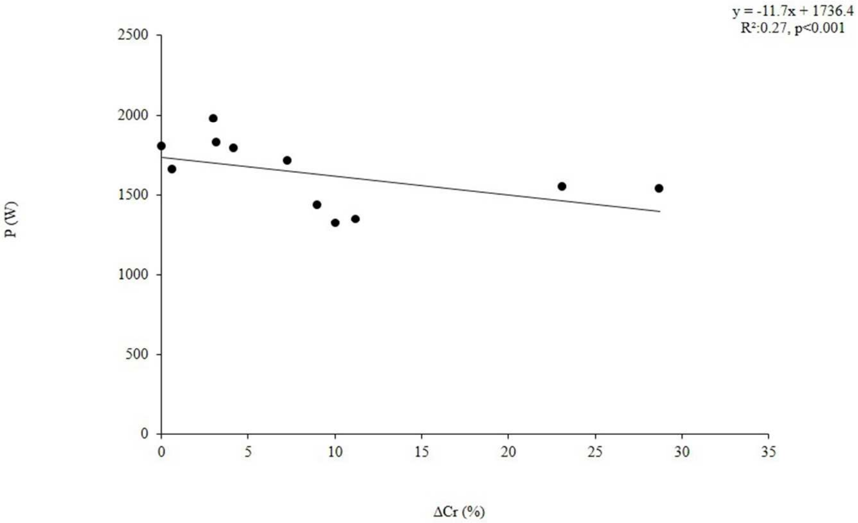
275x190mm (96 x 96 DPI)



275x190mm (96 x 96 DPI)



275x190mm (96 x 96 DPI)



275x190mm (96 x 96 DPI)

TABLE 1. Physical characteristics of subjects (n:11) before the race.

Age (year)	43.2 ± 11.0	[27.0 – 59.0]
Body mass (kg)	72.9 ± 10.2	[57.0 – 88.0]
Stature (m)	1.77 ± 0.07	[1.63 – 1.85]
Body mass index ($kg \cdot m^{-2}$)	23.1 ± 2.4	[20.2 – 27.4]
L (m)	0.89 ± 0.04	[0.81 – 0.94]
$V'O_2max$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	49.2 ± 8.8	[37.9 – 61.5]
HR_{max} (bpm)	176.8 ± 11.0	[161.0 – 193.0]
Cr ($mlO_2 \cdot m^{-1} \cdot kg^{-1}$)	0.190 ± 0.023	[0.149 – 0.224]
P_{max} (W)	1628 ± 212	[1319 – 1971]
Race time (hh:mm:ss)	6:14:01±1:04:29	[4:24:12 - 7:09:36]

All values are mean ± standard deviation (SD). Range in square brackets.

L: lower limb length; $V'O_2max$: maximal oxygen uptake; HR: heart rate; Cr: energy cost of running; P_{max}: maximal mechanical power of the lower limbs.

130 runners started the race, 109 completed it. Of the 11 runners of this study, 4 were classified within the 10th place, 2 between the 30th and 40th, 3 between the 50th and 60th and 2 between the 70th and 80th.

TABLE 2. Body mass, energy cost of running, respiratory exchange ratio and mechanical parameters determined before and immediately after the race.

	Before	After	Changes %	P
BM (kg)	72.9 ± 10.2	68.7 ± 9.8	-5.7	0.001
Cr ($mlO_2 \cdot m^{-1} \cdot kg^{-1}$)	0.190 ± 0.023	0.207 ± 0.019	+8.7	0.001
RER	0.88 ± 0.06	0.82 ± 0.08	-6.6	0.123
Self-selected running speed ($m \cdot s^{-1}$)	2.89 ± 0.17	2.68 ± 0.39	-7.3	0.024
Contact Time (s)	0.291 ± 0.021	0.375 ± 0.027	+28.6	0.001
Aerial time (s)	0.066 ± 0.028	0.027 ± 0.014	-58.6	0.001
Step frequency ($step \cdot s^{-1}$)	2.81 ± 0.18	2.49 ± 0.11	-11.3	0.001
F_{max} (N)	1380.0 ± 213.1	1136.4 ± 152.9	-17.6	0.001
Δz (m)	0.067 ± 0.007	0.102 ± 0.013	+52.9	0.001
ΔL (m)	0.175 ± 0.020	0.253 ± 0.034	+44.5	0.001
k_{vert} ($kN \cdot m^{-1}$)	20.72 ± 2.81	11.26 ± 1.97	-45.6	0.001
k_{leg} ($kN \cdot m^{-1}$)	7.90 ± 0.96	4.56 ± 0.85	-42.3	0.001

All values are mean ± standard deviation (SD).

BM: body mass; Cr: energy cost of running; RER: respiratory exchange ratio; F_{max}: maximal vertical ground reaction force; Δz: downward displacement of centre of mass during contact; ΔL: displacement of the leg spring; k_{vert}: vertical stiffness; k_{leg}: leg stiffness.

P: Significance by Student paired t-test.

EFFECTS OF AN UPHILL MARATHON ON RUNNING MECHANICS AND LOWER LIMB MUSCLES FATIGUE

Submission type: original investigation

Nicola Giovanelli[1,2], Paolo Taboga[1,2], Enrico Rejc[1,2], Bostjan Simunic[3], Guglielmo Antonutto[1,2] and Stefano Lazzer[1,2]

1. Department of Biomedical Sciences and Technology, University of Udine, Italy
2. School of Sport Sciences, University of Udine, Italy
3. University of Primorska, Science and Research Centre, Institute for Kinesiology Research, Slovenia

Corresponding author:
Nicola Giovanelli
University of Udine
Department of Medical and Biological Sciences
P.le Kolbe 4
33100 Udine, Italy
Phone: +39 0432 494330 - Fax: +39 0432 494301
e-mail: nicola.giovanelli@uniud.it

Preferred running head: Running mechanics and muscle fatigue

Abstract word count: 249

Text only-word count: 3623

Number of figures: 3

Number of tables: 4

ABSTRACT

PURPOSE: To investigate the effects of an uphill-marathon (43 km, 3063 m elevation gain) on running mechanics and neuromuscular fatigue in lower limb muscles.

METHODS: Maximal mechanical power of lower limbs (MMP), temporal tensiomyography (TMG) parameters and muscle belly displacement (Dm) were determined in the vastus lateralis muscle before and after the competition in eighteen runners (age: 42.8±9.9 yr; body mass: 70.1±7.3 kg; maximal oxygen uptake: 55.5±7.5 $mL \cdot kg^{-1} \cdot min^{-1}$). Contact ($t_c$) and aerial ($t_a$) times, step frequency (f) and running velocity (v) were measured at 3,14,30 km and after the finish line (POST). Peak vertical ground reaction force (F_{max}), vertical displacement of the centre of mass (Δz), leg length change (ΔL), vertical (k_{vert}) and leg (k_{leg}) stiffness were calculated.

RESULTS: MMP was inversely related with race time (r:-0.56,p:0.016), t_c (r:-0.61,p:0.008), Δz (r:-0.57,p:0.012) and directly related with F_{max} (r:0.59,p:0.010), t_a (r:0.48,p:0.040), k_{vert} (r:0.51,p:0.027). In the fastest sub-group (n:9) the following parameters were lower in POST ($p<0.05$) than at km 3: t_a (-14.1±17.8%), F_{max} (-6.2±6.4%), k_{vert} (-17.5±17.2%), k_{leg} (-11.4±10.9%). The slowest sub-group (n:9) showed changes ($p<0.05$) at km 30 and POST in F_{max} (-5.5±4.9% and -5.3±4.1%), t_a (-20.5±16.2% and -21.5±14.4%), t_c (+5.5±7.5% and +3.2±5.2%), k_{vert} (-14.0±12.8% and -11.8±10.0%), k_{leg} (-8.9±11.5 and -11.9±12%). TMG temporal-parameters decreased in all runners (-27.35±18.0%,$p<0.001$), while Dm increased (+24.0±35.0%,p:0.005), showing lower muscle stiffness and higher muscle sensibility to the electrical stimulus.

CONCLUSIONS: Greater MMP was related with smaller changes in running biomechanics induced by fatigue. Thus, lower limb power training could improve running performance in uphill-marathons.

Key Words: kinematics; stiffness; tensiomyography; post-activation potentiation.

INTRODUCTION

The mechanics of running in different conditions[1-3] have been frequently investigated using the spring mass model (SMM)[4]. This model consists of a point of mass supported by a single mass-less linear spring, which allows to investigate the leg (k_{leg}) and vertical (k_{vert}) stiffness coefficients associated with leg-spring compression (ΔL) and with the vertical displacement (Δz) of the centre of mass (CoM) at the middle of the stance phase[3]. In this model, k_{leg} is defined as the ratio between peak vertical ground reaction force (F_{max}) and ΔL, while k_{vert} is the ratio between F_{max} and Δz [5].

Previous studies[2,5,6] showed a reduction in F_{max}, Δz, ΔL, and an increment in k_{vert} and step frequency (*f*) after many hours of prolonged running (mountain ultra-marathon or MUM, 24-h treadmill run, 5-h hilly running) with different behaviour of contact (t_c) and aerial (t_a) time. Morin et al.[5] hypothesized that these changes in the running pattern could lead to a smoother and safer running style, likely preserving the body structures especially during the braking phase of each step. Moreover, the different changes in t_c and t_a among these studies could be due to the different running conditions (treadmill *vs* over-ground running; level *vs* uphill/downhill running). Some authors suggested that treadmill and over-ground running can be considered similar only when the sample size is sufficiently wide, because large individual differences between the two running conditions were found[7]. In addition, the inclination of the running surface also influences running biomechanics[8]. Indeed, in uphill running, the peak forces recorded are smaller, *f* is greater, and the stride length is shorter as compared to level and downhill running[8]; similarly, the eccentric step phase is reduced. Also, the muscle volume activated in the lower limbs is larger in uphill than in horizontal running. Besides, uphill running requires considerably greater activation of the vastus and soleus and a lesser activation of the rectus femoris, gracilis and semitendinosus compared to horizontal running[9]. It follows that, as showed by Lazzer et al.[3], uphill running may lead to different changes in running mechanics than those observed in previous MUM[2,5,6].

Furthermore, neuromuscular fatigue (i.e. an exercise-related decrease in the maximal voluntary force or power of a muscle group[10]) has been shown to significantly impair the performance of ultra-endurance athletes[10,11]. This potentially involves processes at all levels of the motor pathway from the brain to skeletal muscle.

Muscular fatigue was previously investigated by analysing electromyography together with muscle mechanical output during dynamic and static muscle contractions[11,12]. Recently, the non-invasive technique of tensiomyography (TMG) has been used to examine the contractile properties of skeletal muscle. Simunic et al.[13] also suggested that this methodology could be used to evaluate peripheral fatigue; however, few authors have used TMG to study this phenomenon[14-16].

To the best of our knowledge, no study has already analysed running biomechanics and muscular fatigue during and after an uphill race. This type of event is peculiar because it is characterized by lower impact and lower eccentric phase than a classic “flat” marathon or MUM.

Therefore, the primary purpose of the present study was to investigate the effects of an extreme uphill running marathon on running mechanics and on SMM. The secondary purpose was to evaluate the effect of race-induced fatigue on muscle contractile properties by TMG. The third aim of this study was to examine whether the changes in running mechanics and TMG parameters due to the race-induced fatigue were different between faster and slower runners.

We hypothesized that the changes in SMM induced by the investigated uphill running were different than those brought about by level running or classical MUM; in particular, we expected a decrease in k_{vert} and k_{leg}. Also, we hypothesized that the fastest runners showed smaller changes in running mechanics as compared to the slowest athletes. Finally, we expected different muscle stiffness and sensibility to the electrical stimulus between the two groups.

METHODS

Subjects

Twenty-five healthy Italian male runners were enrolled in this study as participants in the "Supermaratona dell'Etna" (SME), and the 18 athletes who completed the race were considered for data analysis (mean±SD: age= 42.8 ± 9.9 yr, body mass=70.1±7.3 kg, height= 1.71±0.05 m,$V'O_2max$=55.5±7.5 mL·kg^{-1}·min^{-1}, maximal mechanical power (MMP) of the lower limbs=27.6±7.7 W·kg^{-1}, (Table 1)). The experimental protocol was approved by the Ethics Committee of the University of Udine. Before the study began, the purpose and objectives were carefully explained to each subject and written informed consent was obtained from all of them. The participants were recruited among experienced ultra-endurance runners (12.4±8.5 years of training history in running; 6.5±3.5 years of ultra-endurance running race experience and 88.4±39.5 km/week of running training) and were asked to fill out a questionnaire on physical exercise activity, demographics, medical history and lifestyle. Subjects who reported any muscular or metabolic diseases or recent physical injury were excluded from the study.

Experimental protocol

The race took place in June 2013; the starting time was set at 8:00 AM in Marina di Cottone (Catania, Italy), at sea level, the temperature and relative humidity were 27°C and 22% respectively. The first 30-km of the race to Etna North (1810 AMSL), were on paved road, whereas its final part led to the finish line at 3000 AMSL over an all-trail course. The overall distance was 43km with 3063m of elevation gain, a mean slope of about 7% and with peak values reaching 14% (Figure 1). At the finish line, temperature and relative humidity were 16°C and 45% respectively.

During the week before the race, participants were asked to come to the laboratory to perform a graded exercise test on a treadmill to evaluate their maximal oxygen uptake (V'O_2max). Athletes were also asked to refrain from any vigorous physical activity during the day preceding the test and during the preliminary testing session that they performed to familiarize with all the equipment. Moreover, the day before the race and immediately after the end of the race, the jumping test[17] and TMG assessment were performed, and anthropometric characteristics measurements were carried out. Furthermore, running mechanics were evaluated during the race at km 3, 14, 30 and immediately after the athletes reached the finish line (POST). In addition, GPS coordinates were continuously recorded throughout the competition (Garmin Forerunner 305 GPS, Kansas City, USA).

Physiological measurements before and after the race

Body mass (BM) and V'O_2max were assessed the week before the race as described by Lazzer et al.[18]. The day before and immediately after the race, MMP was assessed during a counter movement jump by means of the Bosco test[17] (Ergo Jump, Boscosystem, Italy). In addition, the subjects underwent TMG before the race and immediately after (2-4 min) crossing the finish line, using a protocol previously described by Simunic et al.[13]. From every twitch response, the displacement of muscle belly (Dm), delay time (Td), contraction time ($T_{contraction}$), sustained contraction time (Ts) and relaxation time (Tr) were calculated. Dm was defined as the peak amplitude in the displacement–time curve of the tensiomyographical twitch response. Td was defined as the time between the electrical stimulus and displacement of the sensor to 10% of Dm, $T_{contraction}$ was the time from 10% to 90% of Dm reached, Ts was the time period in which muscle response remains greater than 50%, and Tr was the time from 90% Dm to decline to one-half of the Dm in the relaxation phase[13,15].

Mechanical measurements during the race

Running mechanics were studied using four digital cameras with a sample frequency of 400 Hz (Nikon J1, Japan). The cameras were placed perpendicular to the running direction of athletes at km 3, 14, 30 and immediately after the finish line of the race (POST). The recording zone during the race (km 3, 14 and 30) was selected in order to include at least 15m of flat road (inclination <1%, as measured by means of GPS devices the day before the race). Then, immediately after the race, the athletes were asked to run at a constant self-selected speed, as close as possible to the race speed, for 50 m on a flat compact rock path situated near the finish line. Three attempts were performed, and the one with the running speed closest to that recorded during the race (at the three checkpoints) was used for video analysis. Running speed was measured by means of two photocells placed immediately before and after each video recording zone. Because of the limited space available for placing the camera, only five subsequent steps were analysed in order to measure t_c (s) and t_a (s). Step frequency (f, step·s^{-1}) was calculated as: $1/(t_a+t_c)$.

Given t_c (s), t_a (s), v (m·s^{-1}), subject's BM (kg), and lower limb length (distance between great trochanter and ground during standing, L in m), spring mass parameters were calculated using the computation method proposed by Morin et al.[1]. This method, based on modelling of the ground reaction force during the contact phase by a sine function, allows the computation of k_{vert} (kN·m^{-1}) as the ratio of the F_{max} (N) to the Δz (m) and of k_{leg} (kN·m^{-1}) as the ratio of F_{max} and the ΔL (m). Moreover, in order to identify the effect of MMP on biomechanical parameters during the race, MMP measured before and after the race was plotted as a function of the biomechanical parameters for all athletes.

Statistical analyses

Statistical analyses were performed using PASW Statistic 18 (SPSS Inc., IL, USA) with significance set at $p<0.05$. All results are expressed as means and standard deviation (SD). Normal distribution of the data was tested using the Kolmogorov-Smirnov test.

Median value of the subjects' final ranking was considered to split all subjects into two sub-groups of 9 subjects (the 9 fastest runners and the 9 slowest runners). Changes of speed and mechanical parameters during the race were studied with General Linear Model repeated measures with two factors considering groups (called "G": the 9 fastest runners *vs* the 9 slowest runners) and distance (called "D": 3 km *vs* 14 km *vs* 30 km *vs* POST). As well, changes of BM, maximal mechanical power of the lower limbs and TMG parameters before and after the race were studied with General Linear Model repeated measures with two factors considering groups (called "G": 9 fastest runners *vs* 9 slowest runners) and Time (called "T": pre *vs* post). When significant differences were found, a Bonferroni post-hoc test was run to determine the exact location of the difference.

The relationships between V'O_2max and performance time, MMP and mechanical variables were investigated using Pearson's product–moment correlation coefficient.

RESULTS

Race time and physical characteristics of the athletes measured before the race (PRE) are reported in Table 1. Race time of the winner of the SME was 3:50:38, while the average time of the subjects was 5:29:10 ± 1:01:12 (ranking 1^{st}–101^{st}).

An inverse relationship between V'O_2max and race time (r:-0.85, $p<0.001$) as well as between MMP-PRE and race time (r:-0.56, p:0.016) were observed.

When MMP measured before and after the race was plotted as a function of mechanical parameters, inverse relationships between MMP and t_c (Figure 2A) as well as Δz (Figure 2D) were observed. However, direct relationships between MMP and t_a (Figure 2B), F_{max} (Figure 2C) as well as k_{vert} (Figure 2E) were observed. No significant relationships between MMP and *f*, ΔL and k_{leg} were found.

A further analysis was focused on the comparison between two sub-groups of athletes (n=9), who were divided according to the final ranking. The 9 fastest runners were younger (-21.5% in age, p:0.024), with lower BM (-12.2%, p:0.004) and BMI (-11.7%, p:0.002) and higher V'O_2max (+17.5%, p:0.007) and MMP (+29.5%, p:0.047) as compared to the 9 slowest runners (Table 1).

Mechanical parameters

When the results recorded form all 18 athletes were averaged (Table 2), there was a decrement at km 14 and 30 in speed (-2.4±3.4% and -4.8±7.2%; $p<0.01$), at km 30 and POST in t_a (-14.6±18.2% and -18.0±16.4% respectively, $p<0.01$), F_{max} (-4.2±6.4% and -5.6±5.2% respectively, $p<0.001$) and k_{vert} (-12.1±15.0% and -15.0±14.0% respectively, $p<0.01$). Moreover, k_{leg} decreased only POST (-11.7±11.2%, $p<0.001$). Conversely, at km 30 and POST, an increment in Δz (+7.5±11.8% and 7.5±17.6% respectively, $p<0.05$) and in t_c (+4.8±7.8% and +5.2±9.6% respectively, $p<0.05$) was observed.

When the two sub-groups were analysed separately, the fastest runners did not show any significant change in *v* and mechanical parameters throughout the race (Table 3). On the contrary, immediately after the race (POST), they showed lower t_a (-14.1±17.8%, $p<0.05$), F_{max} (-6.2±6.4%, $p<0.05$), k_{vert} (-17.5±17.2%, $p<0.05$), k_{leg} (-11.4±10.9%, $p<0.05$) and MMP (-23.6±26.2%, $p<0.05$, Table 4). The slowest runners showed a decrease in F_{max} at km 30 and POST (-5.5±4.9% and -5.3±4.1%; $p<0.05$), a t_a decrease at km 30 and POST (-20.5±16.2%

and -21.5±14.4%, respectively, $p<0.005$) and t_c increase at km 30 and POST (+5.5±7.5% and +3.2±5.2%, respectively, $p<0.05$). Consequently, k_{vert} and k_{leg} decreased at km 30 and POST (-14.0±12.8% and -11.8±10.0%; -8.9±11.5 and -11.9±12%, respectively; $p<0.05$) (Table 3). In this group, MMP decreased by -23.2±15.3% after the race ($p<0.005$, Table 4). Moreover, MMP was higher in the fastest runners before and after the race as compared to the slowest ones (+28.9±0.4%, $p<0.05$, Table 4). The gait parameters were not compared between fastest and slowest athletes because of the significant difference in speed at every checkpoint (29.9±5.3%, $p<0.001$).

TMG parameters

Figure 3 shows the TMG responses averaged among all runners that were carried out before (solid line) and immediately after (dashed line) the race. After the race, a significant decrease ($p<0.001$) in $T_{contraction}$ (-12.8±9.7%), Ts (-39.3±31.6%), Tr (-46.2±33.5%) and Td (-11.1±9.5%) was observed, together with an increase of Dm (+24.0±35.0%, $p=0.005$). When these parameters were compared between the fastest and slowest group, no significant differences were found (Table 4).

DISCUSSION

The main results of the present study showed that 1) race time was inversely related with $V'O_2max$ and MMP, 2) running mechanics did not change throughout the race in the fastest runners, while it changed from the 30th km onward in the slowest runners. However, in both groups running mechanics before the race (PRE) was significantly different than immediately after the race (POST). 3) TMG time-parameters ($T_{contraction}$, Ts, Tr, and Td) decreased and Dm increased after the race in both groups.

As previously observed by several authors, strong correlations have been shown between V'O_2max and running performance in subjects with different running levels[18]. However, when groups of athletes with a relatively narrow range of V'O_2max are studied, V'O_2max becomes a less sensitive predictor of performance, while its fraction that can be sustained throughout the race and the energy cost of running (Cr) become more and more important for predicting performance in distance running[18]. Particularly, some authors[18], showed that lower values of Cr in trained runners were related with higher values of MMP, k_{vert} and low foot-print index (i.e. the medio-lateral displacement of the foot during the whole stance phase), supporting previous studies that underlined the role of muscle-tendon complex stiffness in storing and releasing elastic energy[19].

Indeed, in the present study, the athletes with higher values of MMP presented lower t_c and Δz and higher t_a, F_{max} and k_{vert}: these are all factors that could promote higher running velocity[20] and lower energy expenditure because of the lower oscillation of the centre of mass[18,19].

In contrast to previous studies[2,6], no changes in f and an increase in Δz have been observed. This suggests that the lower eccentric phase that is involved in uphill races like SME promoted peculiar adaptations so that the characteristics of the spring mass system rather than the running speed were modulated throughout the race. Indeed, during an uphill running race it may not be necessary to adopt a safer running style because of the peculiarity of the course profile. Furthermore, the increase in Δz observed in the present study could be a consequence of the decrease in k_{vert} and F_{max} as observed previously in exhaustive but much shorter running efforts[20-23], in which spring mass characteristics changed toward a longer contact time[22-24], higher Δz and lower k_{vert}[21].

Furthermore, the fastest runners changed their running pattern only at the last checkpoint, immediately after they crossed the finish line. We can speculate that these athletes changed their running pattern between km 30 and km 43, in the non-paved leg of the race. This part of the race, where the surface stiffness was different compared to the first part, could affect the running mechanics even in the fastest and most trained runners, although previous studies have shown that runners adjust their stiffness to maintain consistent support mechanics across different surfaces[25]. Conversely, the slowest runners changed their spring mass parameters between km 14 and km 30. Interestingly, the transit at km 30 for the slowest athletes occurred after about 4 hours from the race start, while the fastest athletes reached this checkpoint in about 3 hours. Our hypothesis, in accordance with the study of Morin et al.[2], is that the spring mass parameters change after a certain time of exercise performed rather than after a certain amount of distance covered.

Neuromuscular alterations due to fatigue[2] and muscle damage which occur during an ultra-endurance event could affect running mechanics[5]. Millet et al.[12] demonstrated that central fatigue plays the main role in decreasing force after an ultra-marathon. As well, alterations of neuromuscular propagation, excitation-contraction (E-C) coupling failure and modifications of the contractile apparatus may be involved in decreasing force[26]. Hunter et al.[16] used the TMG to asses peripheral fatigue 24 hours after exercise-induced muscle damage and they observed a decrease in Dm and an increase in $T_{contraction,}$ by -31% and +21% respectively. However, a different behaviour of TMG-parameters during various fatigue protocols has been shown by other authors[14,15] even if, to our knowledge, the TMG has been used only once to evaluate muscular fatigue during an ultra-endurance event. After an Ironman triathlon, authors found muscle specific decreased Td in *rectus femoris* and increased $T_{contraction}$, Tr and Dm in *biceps femoris*[14]. In contrast with our hypothesis, in the present study, no differences in TMG parameters between the two sub-groups of athletes before and after the race were found. When all 18 athletes were analysed together, Dm increased by 24% while the other investigated parameters decreased, suggesting that the vastus lateralis muscle was less stiff and reacted faster to the electrical stimulus. Our results are in agreement with Millet et al.[12] who stimulated electrically the femoral nerve before and after a 65-km ultramarathon race, showing greater peak twitch tension and shorter contraction time after the race. The authors hypothesized that these changes could be due to the potentiation of the twitch force after fatigue[12]. In fact, a shift to the left of both torque[27] curve and TMG curve, similar to that observed in the present study after the race (Figure 3), is analogous to the shift usually observed in post-activation potentiation (PAP). PAP is commonly detected after short burst of strength or power exercise[28] and it was also seen in endurance athletes after a maximal isometric contractions[27]. Therefore, we suggest that enhanced PAP may counteract fatigue during endurance exercise which affect the behaviour of the muscle fibres.

Limits of the study

In this study, one issue was related to the running speed, which was self-selected both throughout the race and after its conclusion. However, the difference in speed was -2.4% between the second and the first checkpoint, -4.9% between the third and the first checkpoint and -4.1% between the last and the first checkpoint. As previously observed[5], these differences can be considered acceptable when comparing gait parameters by video analysis. In order to minimize this issue for the POST time point, athletes performed three running attempts, and the one with the speed closest to the average speed value recorded during the race (at km 3, 14 and 30) was taken into account for further analysis. Also in this case, the speed difference was negligible (-4.1%).

A second limit of this study was related to the number of subsequent steps that were analysed in order to calculate the SMM parameters. We considered 5 subsequent steps, the maximum allowed by the camera placement with respect to the environment characteristics. However, other studies analysed running mechanics taking into consideration a similar number of consecutive steps (either 5-8[5] or 10 steps[3,18]), thus supporting our approach.

Finally, muscle contractile properties can be affected by muscle temperature [29]. In order to minimize this issue, in the present study, prior the beginning of the race, athletes underwent TMG measurements after a 10-minute warm-up. This countermeasure conceivably increased intramuscular temperature to values similar to the ones present after the end of the race, seen as this physiological variable shows steep increments in the first 10 minutes, reaching its plateau or values comparable to the ones recorded after prolonged exercise [30].

PRACTICAL APPLICATIONS

The present study shows that greater values of MMP are related to smaller changes in running biomechanics induced by fatigue. Thus, lower limb power training could be important for long-distance uphill running performance. This suggests that coaches and athletes should consider the integration of specific lower limb power training to their training programs in order to enhance long-distance uphill running performance.

CONCLUSIONS

An inverse relationship between race time and V'O$_2$max as well as MMP was found. Higher MMP was related with higher F_{max}, t_a and k_{vert} as well as lower t_c and Δz: all these factors could conceivably promote higher running velocity. These findings suggest that lower limbs' muscle power plays an important role in determining the performance of uphill long-distance runners. Future interventional studies are required to investigate whether lower limb power training can improve running performance in uphill long-distance competitions. TMG analysis showed a decrement in muscle stiffness and higher sensibility of the muscle to the electrical stimulus, suggesting that the potentiation of fast twitch fibres and the fatigue of slow twitch fibres are two parallel mechanisms involved in this type of race.

ACKNOWLEDGEMENTS

We are grateful to the athletes for their kind collaboration, to Mr M. Malfitana, Mr E. Bertolissi and the Unione Sportiva M. Tosi, Tarvisio (Italy) for their valuable contribution in carrying out measurement during the race. We are grateful to Prof. P.E. di Prampero, Prof. N. Maffiuletti, Dr D. Salvadego, Dr A. Da Ponte for their kind assistance during the study and Dr M. Arteni and J. Perrin for the English revision of the paper. The financial support of the Unione Sportiva M. Tosi, Tarvisio (Italy) is gratefully acknowledged.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES

1. Morin JB, Dalleau G, Kyrolainen H, Jeannin T, Belli A. A simple method for measuring stiffness during running. *J Appl Biomech* 2005; 21: 167-180

2. Morin JB, Samozino P, Millet GY. Changes in running kinematics, kinetics, and spring-mass behavior over a 24-h run. *Med Sci Sports Exerc* 2011; 43: 829-836

3. Lazzer S, Salvadego D, Taboga P et al. Effects of the Etna Uphill Ultra-Marathon on Energy Cost and Mechanics of Running. *Int J Sports Physiol Perform* 2014:

4. Blickhan R. The spring-mass model for running and hopping. *J Biomech* 1989; 22: 1217-1227

5. Morin JB, Tomazin K, Edouard P, Millet GY. Changes in running mechanics and spring-mass behavior induced by a mountain ultra-marathon race. *J Biomech* 2011; 44: 1104-1107

6. Degache F, Guex K, Fourchet F et al. Changes in running mechanics and spring-mass behaviour induced by a 5-hour hilly running bout. *J Sports Sci* 2013; 31: 299-304

7. Fellin RE, Manal K, Davis IS. Comparison of lower extremity kinematic curves during overground and treadmill running. *J Appl Biomech* 2010; 26: 407-414

8. Gottschall JS, Kram R. Ground reaction forces during downhill and uphill running. *J Biomech* 2005; 38: 445-452

9. Sloniger MA, Cureton KJ, Prior BM, Evans EM. Lower extremity muscle activation during horizontal and uphill running. *J Appl Physiol (1985)* 1997; 83: 2073-2079

10. Millet GY, Tomazin K, Verges S et al. Neuromuscular consequences of an extreme mountain ultra-marathon. *PLoS One* 2011; 6: e17059

11. Saugy J, Place N, Millet GY et al. Alterations of Neuromuscular Function after the World's Most Challenging Mountain Ultra-Marathon. *PLoS One* 2013; 8: e65596

12. Millet GY, Lepers R, Maffiuletti NA et al. Alterations of neuromuscular function after an ultramarathon. *J Appl Physiol* 2002; 92: 486–492

13. Simunic B, Degens H, Rittweger J et al. Noninvasive estimation of myosin heavy chain composition in human skeletal muscle. *Med Sci Sports Exerc* 2011; 43: 1619-1625

14. Garcia-Manso JM, Rodriguez-Ruiz D, Rodriguez-Matoso D et al. Assessment of muscle fatigue after an ultra-endurance triathlon using tensiomyography (TMG). *J Sports Sci* 2011; 29: 619-625

15. Garcia-Manso JM, Rodriguez-Matoso D, Sarmiento S et al. Effect of high-load and high-volume resistance exercise on the tensiomyographic twitch response of biceps brachii. *J Electromyogr Kinesiol* 2012; 22: 612-619

16. Hunter AM, Galloway SD, Smith IJ et al. Assessment of eccentric exercise-induced muscle damage of the elbow flexors by tensiomyography. *J Electromyogr Kinesiol* 2012; 22: 334-341

17. Bosco C, Luhtanen P, Komi PV. A simple method for measurement of mechanical power in jumping. *Eur J Appl Physiol Occup Physiol* 1983; 50: 273-282

18. Lazzer S, Taboga P, Salvadego D et al. Factors affecting metabolic cost of transport during a multi-stage running race. *J Exp Biol* 2014; 217: 787-795

19. Spurrs RW, Murphy AJ, Watsford ML. The effect of plyometric training on distance running performance. *Eur J Appl Physiol* 2003; 89: 1-7

20. Girard O, Millet GP, Slawinski J, Racinais S, Micallef JP. Changes in running mechanics and spring-mass behaviour during a 5-km time trial. *Int J Sports Med* 2013; 34: 832-840

21. Dutto DJ, Smith GA. Changes in spring-mass characteristics during treadmill running to exhaustion. *Med Sci Sports Exerc* 2002; 34: 1324-1331

22. Avogadro P, Dolenec A, Belli A. Changes in mechanical work during severe exhausting running. *Eur J Appl Physiol* 2003; 90: 165-170

23. Rabita G, Slawinski J, Girard O, Bignet F, Hausswirth C. Spring-mass behavior during exhaustive run at constant velocity in elite triathletes. *Med Sci Sports Exerc* 2011; 43: 685-692

24. Rabita G, Couturier A, Dorel S, Hausswirth C, Le Meur Y. Changes in spring-mass behavior and muscle activity during an exhaustive run at VO2max. *J Biomech* 2013; 46: 2011-2017

25. Ferris DP, Louie M, Farley CT. Running in the real world: adjusting leg stiffness for different surfaces. *Proc Biol Sci* 1998; 265: 989-994

26. Millet GY, Lepers R. Alterations of neuromuscular function after prolonged running, cycling and skiing exercises. *Sports medicine (Auckland, NZ* 2004; 34: 105-116

27. Hamada T, Sale DG, Macdougall JD. Postactivation potentiation in endurance-trained male athletes. *Med Sci Sports Exerc* 2000; 32: 403-411

28. Rixon KP, Lamont HS, Bemben MG. Influence of type of muscle contraction, gender, and lifting experience on postactivation potentiation performance. *J Strength Cond Res* 2007; 21: 500-505

29. Ranatunga KW, Sharpe B, Turnbull B. Contractions of a human skeletal muscle at different temperatures. *J Physiol* 1987; 390: 383-395

30. Magnusson SP, Aagaard P, Larsson B, Kjaer M. Passive energy absorption by human muscle-tendon unit is unaffected by increase in intramuscular temperature. *J Appl Physiol (1985)* 2000; 88: 1215-1220

FIGURE CAPTIONS

Figure 1: Race profile of SME obtained from the GPS device. Black arrows indicate where the videos were taken (km 3-14-30 and POST).

Figure 2: Maximal mechanical power (MMP) plotted for all subjects as a function of contact time (t_c, A), aerial time (t_a, B), maximal vertical ground reaction force (F_{max}, C), downward displacement of centre of mass during contact (Δz, D) and vertical stiffness (k_{vert}, E) measured before (•) and immediately after the race (o).

Figure 3. Muscle response averaged among all runners to an electric stimulus obtained using tensiomyography on the vastus lateralis muscle, measured before (solid line) and immediately after the race (dashed line).

TABLE 1. Physical characteristics of subjects measured before the race in all athletes and in the first and last nine runners of the group.

	All runners		9 fastest runners	9 slowest runners	P
Age (years)	42.8 ± 9.9	[24.0 - 60.0]	37.7 ± 8.4	48.0 ± 8.8	0.024
Body mass (kg)	70.1 ± 7.3	[60.0 - 83.0]	65.5 ± 5.7	74.6 ± 5.8	0.004
Stature (m)	1.72 ± 0.05	[1.65 - 1.84]	1.72 ± 0.05	1.73 ± 0.04	0.720
Body mass index (kg·m^{-2})	23.5 ± 2.2	[20.1 - 28.3]	22.0 ± 1.2	24.9 ± 2.1	0.002
L (m)	0.91 ± 0.05	[0.82 - 1.00]	0.89 ± 0.04	0.93 ± 0.05	0.064
$V'O_2max$ (mL·kg^{-1}·min^{-1})	55.5 ± 7.5	[40.4 - 71.8]	59.9 ± 7.3	51.0 ± 4.6	0.007
MMP (W·kg^{-1})	27.6 ± 7.7	[15.8 - 45.8]	31.2 ± 8.2	24.1 ± 5.5	0.047
Race time (hh:mm:ss)	05:29:10 ± 01:01:12	[03:50:38 - 07:16:28]	04:38:13 ± 00:35:21	06:20:07 ± 00:29:30	0.001

All values are mean ± standard deviation (SD). Range in square brackets.

L: lower limb length; $V'O_2max$: maximal oxygen uptake; MMP: maximal mechanical power of the lower limbs;

p: Significance by ANOVA test (fastest 9 runners *vs* slowest 9 runners).

TABLE 2. Mechanical parameters determined at km 3, 14, 30 and immediately after the race (POST) in all subjects (N:18)

	All subjects			
	3km	14km	30km	POST
v (m/s)	3.69 ± 0.62	3.60* ± 0.61	3.51* ± 0.68	3.54 ± 0.72
tc (s)	0.251 ± 0.030	0.252 ± 0.031	0.263* ± 0.034	0.265* ± 0.030
ta (s)	0.089 ± 0.023	0.086 ± 0.021	0.076* ± 0.027	0.073* ± 0.025
f (Hz)	2.96 ± 0.15	2.96 ± 0.16	2.96 ± 0.16	2.97 ± 0.24
Fmax (BM)	2.14 ± 0.21	2.13 ± 0.20	2.05* ± 0.22	2.02* ± 0.19
Δz (m)	0.053 ± 0.010	0.053 ± 0.010	0.057* ± 0.011	0.057* ± 0.010
ΔL (m)	0.178 ± 0.029	0.173 ± 0.027	0.181 ± 0.033	0.187 ± 0.037
k_{vert} (kN·m^{-1})	28.85 ± 6.77	28.13 ± 6.87	25.37* ± 6.85	24.45* ± 6.34
k_{leg} (kN·m^{-1})	8.48 ± 1.73	8.53 ± 1.55	7.86 ± 1.93	7.46* ± 1.87

All values are mean ± standard deviation (SD).

v: speed; tc: contact time; ta: aerial time; *f*: step frequency; F_{max}: maximal vertical ground reaction force; BM: body mass; Δz: downward displacement of center of mass during contact; ΔL: displacement of the leg spring; k_{vert}: vertical stiffness; k_{leg}: leg stiffness.

*p<0.05 compared to the first checkpoint

TABLE 3. Mechanical parameters determined at km 3, 14, 30 and immediately after the race (POST) in the 9 fastest and 9 slowest runners.

(Continue)

	9 fastest runners			
	3km	14km	30km	POST
v (m·s^{-1})	4.19 ± 0.40	4.08 ± 0.43	4.01 ± 0.56	3.90 ± 0.77
tc (s)	0.229 ± 0.026	0.230 ± 0.028	0.240 ± 0.031	0.248 ± 0.034
ta (s)	0.099 ± 0.025	0.100 ± 0.019	0.091 ± 0.026	0.085 ± 0.026
f (Hz)	3.04 ± 0.09	3.03 ± 0.12	3.03 ± 0.08	3.02 ± 0.27
Fmax (BM)	2.27 ± 0.22	2.27 ± 0.19	2.19 ± 0.23	2.13 ± 0.21
Δz (m)	0.046 ± 0.007	0.046 ± 0.009	0.049 ± 0.009	0.052 ± 0.012
ΔL (m)	0.186 ± 0.022	0.179 ± 0.017	0.192 ± 0.037	0.190 ± 0.020
k_{vert} (kN·m^{-1})	32.71 ± 7.19	32.27 ± 7.37	29.25 ± 7.56	26.98 ± 8.15
k_{leg}(kN·m^{-1})	7.97 ± 1.65	8.16 ± 1.38	7.53 ± 2.37	7.05 ± 1.52

(Continue)

	9 slowest runners				P		
	3km	14km	30km	POST	Group	Distance	G x D
v (m·s^{-1})	3.20 ± 0.32	3.11 ± 0.29	2.99 ± 0.25	3.18 ± 0.48	0.001	0.121	0.844
tc (s)	0.272 ± 0.017	0.275 ± 0.012	0.287 ± 0.015	0.281 ± 0.010	0.001	0.001	0.653
ta (s)	0.078 ± 0.015	0.073 ± 0.014	0.062 ± 0.018	0.061 ± 0.018	0.011	0.005	0.596
f (Hz)	2.87 ± 0.15	2.89 ± 0.18	2.88 ± 0.18	2.93 ± 0.19	0.098	0.970	0.782
Fmax (BM)	2.02 ± 0.10	1.99 ± 0.07	1.91 ± 0.10	1.91 ± 0.10	0.003	0.048	0.369
Δz (m)	0.060 ± 0.006	0.060 ± 0.005	0.064 ± 0.006	0.062 ± 0.005	0.002	0.004	0.405
ΔL (m)	0.170 ± 0.034	0.167 ± 0.035	0.171 ± 0.027	0.182 ± 0.049	0.271	0.323	0.441
k_{vert} (kN·m^{-1})	24.99 ± 3.50	23.99 ± 2.72	21.48 ± 2.92	22.05 ± 2.31	0.007	0.003	0.740
k_{leg}(kN·m^{-1})	8.98 ± 1.74	8.89 ± 1.70	8.19 ± 1.42	7.92 ± 2.17	0.182	0.008	0.916

All values are mean ± standard deviation (SD). *v*: speed; tc: contact time; ta: aerial time; *f*: step frequency; F_{max}: maximal vertical ground reaction force; BM: body mass; Δz: downward displacement of centre of mass during contact; ΔL: displacement of the leg spring; k_{vert}: vertical stiffness; k_{leg}: leg stiffness.

P: significance by GLM Repeated Measures with two factors of the main effects of Group (G), Distance (D) and Interaction (G x D).

TABLE 4. Body mass, maximal mechanical power (MMP) of the lower limbs and tensiomyographic parameters in the *vastus lateralis* muscle, measured before and immediately after the race in the 9 fastest and 9 slowest runners of the group.

	9 fastest runners		9 slowest runners		p		
	Before	After	Before	After	Group	Time	GxT
Body mass (kg)	65.6 ± 5.7	63.2 ± 6.1	74.8 ± 5.8	72.3 ± 5.8	0.004	0.001	0.001
MMP ($W \cdot kg^{-1}$)	31.2 ± 8.2	23.8 ± 9.3	24.1 ± 5.5	18.5 ± 4.2	0.048	0.008	0.379
$T_{contraction}$ (ms)	25.8 ± 5.4	22.0 ± 3.5	25.3 ± 4.1	22.6 ± 2.4	0.972	0.004	0.382
Ts (ms)	139.8 ± 60.0	93.8 ± 69.1	134.2 ± 63.2	72.6 ± 47.9	0.611	0.012	0.447
Tr (ms)	92.3 ± 45.4	50.2 ± 45.8	89.6 ± 45.6	47.6 ± 46.5	0.883	0.007	0.995
Td (ms)	23.9 ± 1.9	21.4 ± 2.1	25.3 ± 3.9	22.3 ± 1.9	0.172	0.003	0.697
Dm (mm)	6.6 ± 1.7	8.1 ± 3.0	6.2 ± 2.3	7.9 ± 2.8	0.785	0.010	0.874

All values are mean ± standard deviation (SD). $T_{contraction}$: contraction time; Ts: sustain time; Tr: relaxation time; Td: delay time; Dm: maximal radial displacement; p: Significance by GLM Repeated Measures with two factors of the main effects of Group (G), Time (T) and Interaction (G x T).

FIGURE 1

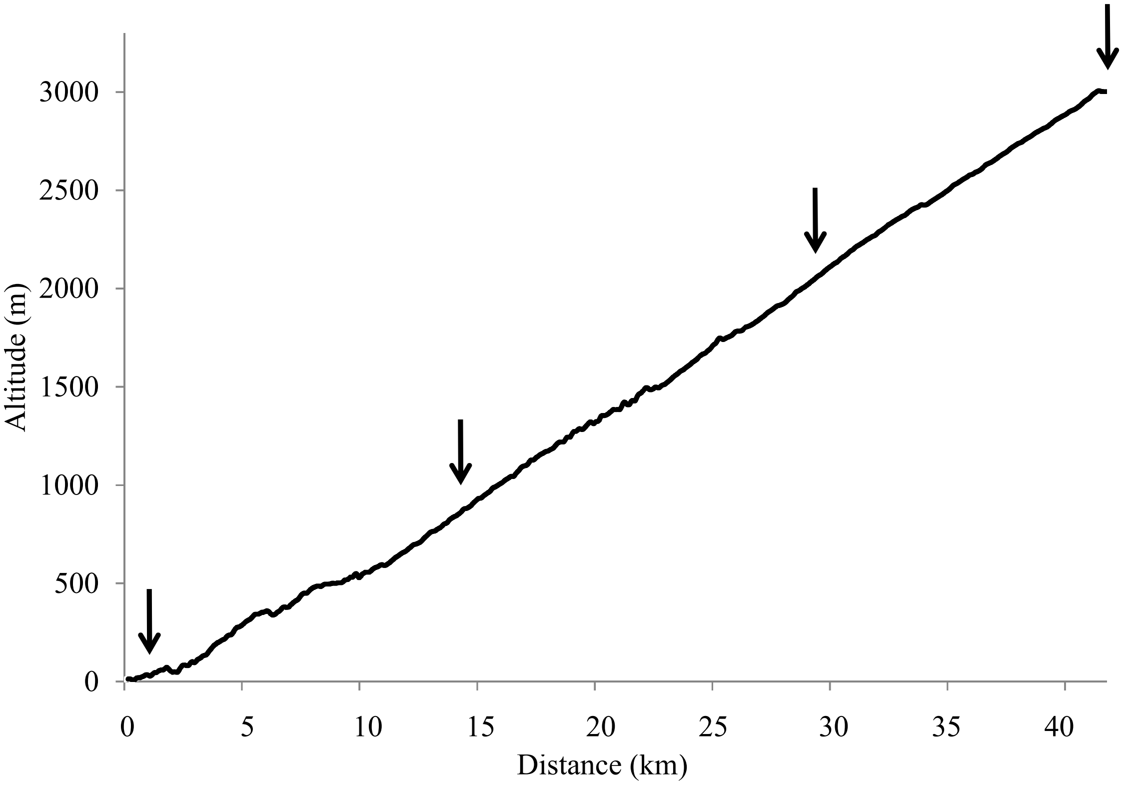

3000
2500
2000
1500
1000
500
0
Altitude (m)
0
5
10
15
20
25
30
35
40
Distance (km)

FIGURE 2

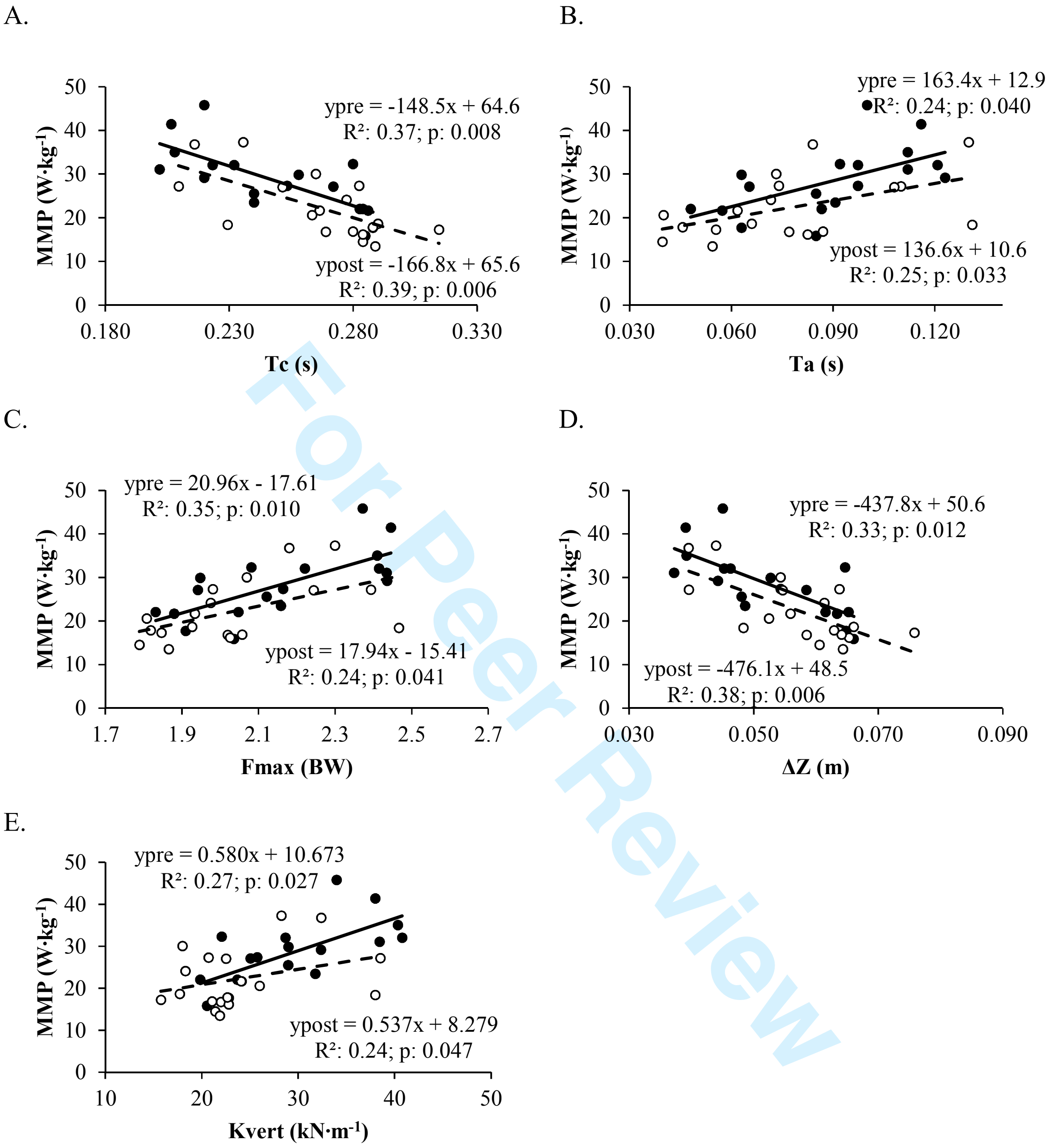
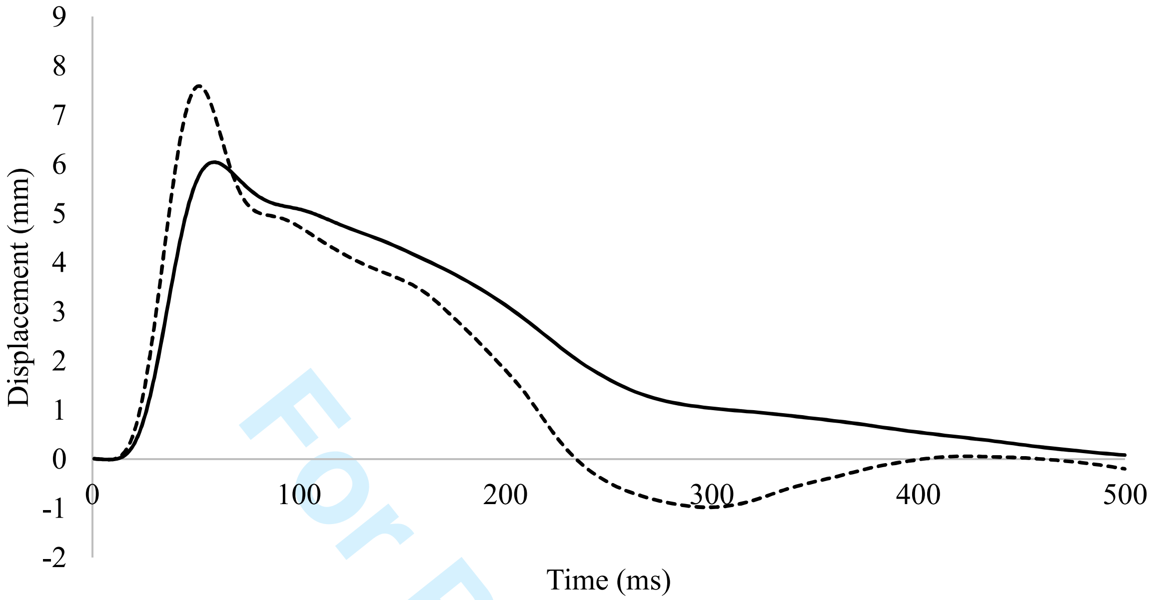

A.
MMP (W·kg-1)
Tc (s)
ypre = -148.5x + 64.6
R²: 0.37; p: 0.008
ypost = -166.8x + 65.6
R²: 0.39; p: 0.006
B.
MMP (W·kg-1)
Ta (s)
ypre = 163.4x + 12.9
R²: 0.24; p: 0.040
ypost = 136.6x + 10.6
R²: 0.25; p: 0.033
C.
MMP (W·kg-1)
Fmax (BW)
ypre = 20.96x - 17.61
R²: 0.35; p: 0.010
ypost = 17.94x - 15.41
R²: 0.24; p: 0.041
D.
MMP (W·kg-1)
ΔZ (m)
ypre = -437.8x + 50.6
R²: 0.33; p: 0.012
ypost = -476.1x + 48.5
R²: 0.38; p: 0.006
E.
MMP (W·kg-1)
Kvert (kN·m-1)
ypre = 0.580x + 10.673
R²: 0.27; p: 0.027
ypost = 0.537x + 8.279
R²: 0.24; p: 0.047

FIGURE 3



Energetics of vertical kilometer foot races; is steeper cheaper?

Nicola Giovanelli[1,2,3], Amanda Louise Ryan Ortiz[3], Keely Henninger[3] and Rodger Kram[3]

1. Department of Biomedical Sciences and Technology, University of Udine, Italy
2. School of Sport Sciences, University of Udine, Italy
3. Locomotion Lab, Integrative Physiology Department, University of Colorado, Boulder CO, 80309-0354, USA

RUNNING HEAD: energetics of uphill running

Corresponding author:
Nicola Giovanelli
University of Udine
Department of Medical and Biological Sciences
P.le Kolbe 4
33100 Udine, Italy
Phone: +39 0432 494330 - Fax: +39 0432 494301
e-mail: nicola.giovanelli@uniud.it

Author contributions: N.G., A.L.R.O. and R.K. conceived and designed the research. N.G., A.L.R.O., and K.H. performed the experiments. N.G and R.K. analyzed data. N.G., A.L.R.O. and R.K. interpreted the results. N.G. and A.L.R.O. prepared the figures and drafted the manuscript. N.G., A.L.R.O., K.H. and R.K all edited and revised the manuscript. N.G., A.L.R.O., K.H. and R.K all approved the final version of the manuscript.

ABSTRACT

Vertical kilometer foot races consist of a 1,000 m elevation gain in less than 5,000 m of overall distance and the inclines of the fastest courses are ~30°. Previous uphill locomotion studies have focused on much shallower angles. We aimed to quantify the metabolic costs of walking and running on very steep angles and to biomechanically distinguish walking from running. Fifteen runners (10 M, 5 F, 32.9±7.5 years, 1.75±0.09 m, 64.3±9.1 kg) walked and ran for 5 minutes at 7 different angles (9.4°, 15.8°, 20.4°, 24.8°, 30.0°, 35.0° and 39.2°) all at a fixed vertical velocity (0.35 m/s). We measured the metabolic rates and calculated the vertical costs of walking (Cw_{vert}) and running (Cr_{vert}). Using video analysis, we determined stride frequency, stride length and duty factor (fraction of stride that each foot is in ground contact). At all angles other than 9.4°, Cw_{vert} was cheaper than Cr_{vert} (average -8.45%±1.05%; $p<0.001$). Further, broad minima for both Cw_{vert} and Cr_{vert} existed between 20.4° and 35° (average Cw_{vert} 44.17±0.41 $J \cdot kg^{-1} \cdot m^{-1}$ and average Cr_{vert} 48.46±0.35 $J \cdot kg^{-1} \cdot m^{-1}$). At all angles and speeds tested, both walking and running involved having at least one foot on the ground at all times. But, in walking, stride frequency and stride length were ~28% slower and longer, respectively than in running. In conclusion, we found that there is a range of angles for which energy expenditure is minimized. At the vertical velocity tested, on inclines steeper than 15.8°, athletes can reduce their energy expenditure by walking rather than running.

Keywords: walking, running, uphill, cost of transport

INTRODUCTION

In vertical kilometer foot races (VK), athletes complete a course with 1,000 m vertical elevation increase in less than 5,000 m of total race length (International Skyrunning Federation rules http://www.skyrunning.com). Terrain, slope and length vary between racecourses. To date, the world record for men in the VK is 29 minutes and 42 seconds, set on a course with a length of 1,920 m, an average inclination of 27.5° (Km vertical de Fully, Switzerland). That equates to an average vertical velocity of ~0.56 m/s and an average velocity parallel to the ground of 1.21 m/s. A VK course with only a slight incline would require an unreasonably fast parallel velocity. For instance, a racecourse with an incline of only 1° would require the impossible running speed of 31.84 m/s to rise 1,000 m in 30 minutes. Conversely, a course with a gradient of 40° would require a speed of only 1.03 m/s to gain 1,000 m in 30 minutes. But, if the course is too steep, the rock-climbing techniques required would likely be slower than walking/running at more moderate slopes. Analysis of the best performances in different VK races suggests that there may be an optimal angle for achieving the best time (Figure 1). Since there are no VK races with an average incline steeper than 28.9° (La Verticale du Grand Serre, France), it is unknown if the optimal gradient is actually steeper.

Another factor to consider is that in VK races, some athletes walk, some run and some alternate gaits. It is not clear which gait or combination is optimal. On level ground or treadmills, at matched speeds slower than ~2.0 m/s, walking requires less metabolic energy than running (3, 15, 17, 25). This is generally attributed to the more effective inverted pendulum-like exchange of mechanical energy at slower walking speeds and the superior elastic energy storage and recovery of running at faster speeds (6). However, on uphill grades both of those mechanisms are disabled (8, 24). On the level (17) as well as moderate inclines and declines (18, 19) the preferred walk-run transition speed occurs near but not exactly at the metabolically optimal transition speed. As speed is increased, people typically first adopt a running gait at a speed slightly slower than the metabolic crossover point.

The metabolic cost of uphill walking and running has long been of interest to exercise physiologists (3, 14, 15, 18) but almost all studies have examined uphill walking or running on angles less than 9°. One highly relevant exception is the innovative study by Minetti et al. (21). They measured the metabolic cost ($J \cdot kg^{-1} \cdot m^{-1}$) of walking (Cw) and running (Cr) on a range of slopes up to 24.2°. Note, for Cw and Cr, the calculated distance is parallel to the surface or treadmill. They concluded that at a given treadmill belt speed, Cw and Cr are directly proportional to the slope above +15% (8.5°) and that Cw and Cr converge at steeper angles. Minetti et al. (21) also defined the vertical costs of walking (Cw_{vert}) and running (Cr_{vert}), as the energy expended to ascend one meter vertically. Cw_{vert} and Cr_{vert} both decreased at steeper angles reaching minimum values at slopes ranging from 20% (11.3°) to 40% (21.8°). However, we are reluctant to extrapolate from the data of Minetti et al. to the steeper slopes at which VK races are often contested. Further, VK competitors often alternate between walking and running at the same speed and Minetti et al. did not directly compare the energetics of the two gaits at matched speeds. Finally, it is not clear if the traditional biomechanical distinction between walking and running on level ground (i.e. in running, the center of mass trajectory reaches its lowest point at mid-stance and there is an aerial phase when no feet are in contact with the ground) applies on very steep slopes. Previous investigators have used the terms “Groucho running” (16) and “grounded running” (23) to describe a bouncing gait that does not involve an aerial phase.

To the best of our knowledge, there are no prior scientific studies of human walking or running at the steep angles that are encountered in the fastest VK races. Minetti et al. (20) analyzed stair running races but such “skyscraper races” are much shorter duration than VK (from 50 s to about 14 min compared with ~30 min) and they did not measure the metabolic cost. Intriguingly, Kay’s mathematical analysis of uphill mountain running races (12) concluded that if an optimum gradient for ascent exists, it is steeper than the range of gradients studied so far.

The primary purpose of this study was to quantify the metabolic costs of walking and running across a wide range of inclines up to and beyond those used in VK races. We aimed to determine if walking or running is more economical and if there are energetically optimal angles for the two gaits. Specifically, we compared walking and running at a fixed vertical velocity (0.35 m/s) at angles ranging from ~10 to ~40°. Based on the findings of Minetti et al. (21), and because the treadmill belt speeds we studied are < 2.0 m/s, we hypothesized that: 1. walking would require less metabolic energy than running. We further hypothesized that: 2. for both walking and running, there would be distinct intermediate angles (~30°) that minimize the energetic cost of ascending at a fixed vertical velocity.

Our secondary purpose was to distinguish the biomechanics of walking vs. running on steep inclines. We hypothesized that: 3. at steep angles and slow treadmill belt speeds, running would not involve an aerial phase. However, a greater stride frequency during running would distinguish it from walking.

MATERIALS AND METHODS

Subjects. Fifteen healthy, competitive mountain runners (10 males, 5 females, 32.9±7.5 years, 1.75±0.09 m, 64.3±9.1 kg) volunteered and provided informed consent as per the University of Colorado Institutional Review Board.

Experimental design.

We modified a custom treadmill so that it was inclinable from 0 to 45° (Figure 2). To provide adequate traction, we adhered a wide swath of skateboard grip tape (i.e. sandpaper) to the treadmill belt (Vicious Tape, Vancouver, BC Canada). To protect the electronic motor controller, we mounted three v-belt pulleys on the treadmill drive roller, hung ropes over the pulleys and attached moderate weights to the ropes (~8 kg). We chose the minimum amount of weight such that when the subject stood on the belt with the motor turned off, the belt did not move. Providing a mechanical resistance to the motor allowed it to produce power and maintain a nearly constant treadmill belt speed.

The study consisted of three sessions. During the first session (familiarization), each athlete walked and ran for 2 to 3 minutes on the treadmill at 4 angles (9.4, 30.0, 35.0 and 39.2°). During the second and third visits, subjects either walked (e.g. Day 2) or ran (e.g. Day 3) for 5 minutes at 7 different angles (9.4°, 15.8°, 20.4°, 24.8°, 30.0°, 35.0° and 39.2°) and corresponding treadmill belt speeds (2.14, 1.29, 1.00, 0.83, 0.70, 0.61, 0.51 m/s). Subjects had five minutes rest between trials. Half of the subjects walked on Day 2 and ran on Day 3; the other half did the opposite. These angle and speed combinations fixed the vertical velocity at 0.35 m/s. We chose this vertical velocity knowing the VK records for men (29:42 = 0.56 m/s vertical velocity) and women (36:04 = 0.46 m/s vertical velocity) and recognizing the need for submaximal intensities so that we could record steady-state metabolic rates. Pilot testing indicated that faster vertical velocities would elicit non-oxidative metabolism. For each subject, we randomized the order of the angles used on both Days 2 and 3.

Metabolic data. To determine the metabolic rates during walking and running, we used an open-circuit expired gas analysis system (TrueOne 2400, ParvoMedic, Sandy, UT, USA). Subjects wore a mouthpiece and a nose clip allowing us to collect the expired air determine measure the rates of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$). We averaged the data of the last 2 minutes of each trial. We then calculated metabolic rate in W/kg using the Brockway equation (2). We only included trials with respiratory exchange ratios (RER) less than 1.0. We calculated the vertical costs ($J \cdot kg^{-1} \cdot m^{-1}$) of walking ($Cw_{vert}$) and running ($Cr_{vert}$) by dividing the gross metabolic power by the vertical velocity.

Biomechanical parameters. To measure stride parameters, we recorded each trial using a high-speed video camera (Casio EX-FH20) at 210 fps. We extracted contact and stride times for 10 strides using Kinovea 0.8.15 software (www.kinovea.org) and then calculated stride frequency (=1/stride time) and stride length (=velocity/stride frequency). To determine duty factor, we divided contact time for one foot by the total stride period.

Statistical analysis

We analyzed the data using SPSS with significance set at $p \leq 0.05$. We analyzed the vertical cost of walking (Cw_{vert}), vertical cost of running (Cr_{vert}) and biomechanical parameters with a general linear model repeated measures considering two factors (slope and gait: walking versus running). We followed up with a Bonferroni post-hoc test when significant differences were detected. At 9.4° the treadmill belt speed was faster than the walk-run transition speed, thus only 9 subjects were able to complete the entire 5-minute trial using a walking gait. Therefore, when making statistical comparisons of the 9.4° trials, we calculated the variables for just those 9 subjects.

RESULTS

Vertical cost of walking vs. running. At 9.4°, the vertical cost of walking (Cw_{vert}) was numerically slightly greater than vertical cost of running (Cr_{vert}) but they were not statistically different (n=9; +1.54%; p=0.545). However, Cw_{vert} was significantly less than Cr_{vert} at 15.8° (-6.35%; p=0.001), 20.4° (-8.45%; p=0.001), 24.8° (-8.73%; p=0.001), 30.0° (-9.23%; p=0.001), 35.0° (-8.99%; p=0.001) and 39.2° (-8.93%; p=0.001) (Table 1).

Cw_{vert} was numerically least at 30° (43.86±2.02 $J \cdot kg^{-1} \cdot m^{-1}$), but was not statistically distinguishable from 20.4° (44.23±1.69 $J \cdot kg^{-1} \cdot m^{-1}$), 24.8° (44.10±2.10 $J \cdot kg^{-1} \cdot m^{-1}$) or 35.0° (44.57±2.14 $J \cdot kg^{-1} \cdot m^{-1}$) (Table 1, Figure 3). Cw_{vert} at 15.8° was less than Cw_{vert} at 9.4° (n=9; -18.2%; p=0.001). Further, Cw_{vert} at 20.4°, 24.8°, 30.0° and 35.0° was less than Cw_{vert} at 15.8° (average -5.47%; p<0.001). Additionally, Cw_{vert} at 39.2° was significantly greater than Cw_{vert} at 20.4°, 24.8°, 30.0° and 35.0° (average +4.31%; p<0.001).

Cr_{vert} was numerically least at 24.8° (48.22±2.57 $J \cdot kg^{-1} \cdot m^{-1}$), but was not statistically distinguishable from at 20.4° (48.31±2.54 $J \cdot kg^{-1} \cdot m^{-1}$), 30.0° (48.32±3.07 $J \cdot kg^{-1} \cdot m^{-1}$) or 35.0° (48.97±3.01 $J \cdot kg^{-1} \cdot m^{-1}$) (Table 1, Figure 3). Cr_{vert} at 15.8° was less than Cr_{vert} at 9.4° (-7.88%; p=0.001). As was true for walking, Cr_{vert} at 20.4°, 24.8°, 30.0° and 35.0° was less than Cr_{vert} at 15.8° (average -2.90%; p<0.001). Finally, Cr_{vert} at 39.2° was greater than Cr_{vert} at 20.4°, 24.8°, 30.0° and 35.0° (average +4.42%; p<0.001).

Biomechanical parameters. Walking stride frequency was slower than running stride frequency at every incline (average -27.99%±7.75%; p<0.001) (Figure 4A). Thus, walking stride length was longer than running stride length at every incline (Figure 4B). In both walking and running, stride frequency and stride length decreased on steeper inclines at the correspondingly slower treadmill belt speeds (Figure 4A and 4B). Duty factor was greater than 50% for both walking and running conditions at all speed/incline combinations tested, indicating non-aerial gaits. Walking duty factor was greater than the running duty factor at every incline (average 10.29±5.92%; p<0.001) except at 40°.

DISCUSSION

Our major findings are: 1) across the range of angles and speeds tested, which fixed the vertical velocity, walking is less expensive than running, 2) there is a broad range of angles for which the vertical costs of walking and running are minimized, 3) at the angle/speed combinations we studied, in both walking and running, at least one foot is always in contact with the ground.

Our results support the hypothesis that at a fixed vertical velocity of 0.35 m/s, walking would be less expensive than running at steep inclines, though at 9.4° there was not a significant difference between gaits. Explaining the energetic difference between walking and running is not straightforward. We know that the inverted pendulum and spring mechanisms that conserve mechanical energy during level walking and running respectively are disabled during uphill locomotion (8, 24), but it is not yet possible to quantify those effects. Minetti et al. (18) showed that during uphill locomotion the "internal work" for reciprocating the limbs is actually greater in walking than in running despite the slower stride frequencies in walking. Kram and Taylor (13) established that metabolic rate is inversely proportional to contact time during level running. At the inclines and speeds in the present study, the contact times for running averaged 34.4±3.2% less than for walking and that may at least partially explain the metabolic cost difference between the two gaits. Further, because of how the legs are positioned differently in the two gaits, the mechanical advantages of the extensor muscles at the knee are larger in level walking vs. running (1). Smaller muscle forces require a smaller active muscle volume which is energetically cheaper. However, we are not aware of any mechanical advantage measurements for steep uphill locomotion.

At 9.4°, the treadmill belt speed (2.14 m/s) was much faster than during the other trials, and is equal to the spontaneous walk-run transition speed on level ground, ~2 m/s (3, 11, 15). Previous studies (4, 10, 11) have demonstrated that the preferred transition speed is slower on moderate inclines and that humans generally choose the gait that minimizes their metabolic cost (17). In the present study, at 9.4° and 2.14 m/s, all of the subjects informally expressed that they would prefer to run. At 15.8° and 1.29 m/s, walking was significantly cheaper but most of the subjects expressed that they would prefer to run. Between 20.4° and 1.00 m/s and 30.0° and 0.70 m/s subjects mentioned that walking felt better. But, if there were no constraints, they thought that they would prefer to alternate between the two gaits every one or two minutes. At 35.0° and 0.61 m/s and 39.2° and only 0.51 m/s, gait preference was ambiguous. Subjects expressed that they did not strongly prefer walking (the less expensive gait) because they felt running involved less musculoskeletal "stress" and also balance was more challenging when walking. A future study focused on gait preference, metabolic cost and perceived effort during both walking and running on steep inclines is needed to better understand this topic.

We reject our second hypothesis. Rather than there being a distinct optimum, we found that there is a range of angles for which Cw_{vert} and Cr_{vert} are minimized. For both walking and running, the minimum values were reached between 20.4° and 35°. A second order polynomial regression suggests that the minimum values for Cw_{vert} and Cr_{vert} would be attained at 28.4° (R^2=0.64) and 27.0° (R^2=0.33), respectively. At angles shallower than 20°, both Cw_{vert} and Cr_{vert} are significantly greater. This could be due in part to the greater metabolic power required to support body weight at faster treadmill belt speeds (9). Further, at our extreme angle, 39.2° there was an increase in Cw_{vert} and Cr_{vert} which we believe is caused by the difficulty of maintaining balance at such steep angles. Part of the balance challenge was due to the fact that at 39.2°, the treadmill belt speed was only 0.55 m/s and involved exaggerated contact times (0.924±0.09 s for walking and 0.588±0.11 s for running). In a pilot study, two subjects tried to walk and run with the treadmill inclined to 45° and the Cw_{vert} and Cr_{vert} both increased dramatically compared to ~40°. Balance was quite difficult for those pilot subjects and they frequently grabbed the handrails. Moreover, at that extreme slope, both subjects reported discomfort in their calves and feet because of excessive stretch. For that reason, we "only" studied up to 39.2° in the actual experiment. For Cw and Cr at angles between 10° and 24.8°, our results are congruent with the 5th order polynomial regression formula given by Minetti et al. (21). However, extrapolating beyond 24.8°, that formula leads to large overestimates of the Cw and Cr (Figure 5).

A recent paper from our lab, Hoogkamer et al. (9), proposed a new explanation for the metabolic cost of running up relatively shallow inclines < 9°. In that model, the cost of running (Cr) is determined by three factors: the cost of perpendicular bouncing, the cost of parallel braking and propulsion and the cost of lifting the center of mass. They assumed a constant efficiency for performing the center of mass lifting work, their results supported that assumption and they derived a value of ~29% efficiency. In the present study, the vertical work rate was held constant between the different inclines and thus with the same efficiency the vertical cost would be the same between running conditions. In the Hoogkamer et al. study, as the incline approached 9°, the cost of parallel braking and propulsion approached zero. At the even steeper angles used in the present study, the cost of parallel braking and propulsion (the "wasted impulse") presumably is nil. Finally, Hoogkamer et al. reasoned that the cost of perpendicular bouncing would not change over the moderate inclines they studied. At the steeper inclines used in the present study, just based on trigonometry, the perpendicular forces would be less than during level running (e.g. ~13% reduced on a 30° incline, cosine = 0.866). However, the running speeds on the inclines studied here were much slower than typical level running speeds and involved prolonged contact times. Prolonged contact times presumably would allow recruitment of slower (and more economical) muscle fibers to generate the perpendicular forces, but long contact times impair the spring-like bouncing motion and therefore might be less economical (5). Overall, from the Hoogkamer et al. perspective, the broad plateau of Cr_{vert} observed for running at angles from 20.4° to 35° probably results from counteracting savings vs. costs for perpendicular bouncing at the different speed and angle combinations. A similar model for uphill walking has not yet been put forth.

As we hypothesized, there was no aerial phase in steep uphill running, i.e. the duty factor (average 62.7±0.80%) was greater than 50% at every incline tested. This suggests that other parameters should be considered to distinguish between walking and running uphill. McMahon et al. (16) defined “Groucho running” as a non-aerial gait that still involved a bouncing center of mass trajectory, i.e. the center of mass was lowest at mid-stance. Rubenson et al. (23) used the term “grounded running” for the same phenomenon in running birds. Because our subjects were running uphill, the center of mass-based definition probably does not apply (8). Nonetheless, when we asked our subjects to either “walk” or “run”, they all subjects immediately and intuitively distinguished the two gaits. Previous studies reported that when treadmill speed is fixed, on steeper inclines, stride length and aerial time decrease and stride frequency increases (7, 22). We observed decreases in both stride frequency and stride length at steeper angles (figure 4 and 5) because treadmill speed was slower at the steeper angles we tested. Thus, with our experimental design, we could not determine how speed and incline independently affect stride frequency and stride length.

Limitations and future research

One limitation of our study is that it was conducted on a treadmill whereas VK races are performed on uneven terrain (ski slopes, trails) with the presence of stones, stairs, gravel etc. Voloshina and Ferris report that the energy expenditure of running on an uneven terrain treadmill was only 5% higher than on a smooth treadmill (26). But, Zamparo et al. showed that running on a sandy terrain requires 20% more energy than on firm terrain (27). Thus, the cost of transport during a real VK race is surely somewhat greater than what we measured on our treadmill. Another limitation was that our treadmill did not permit the use of poles. The VK world record as well as most of the fastest performances outdoors were achieved using poles.

Future studies should compare uphill walking and running with and without poles in order to determine if using poles is advantageous. Further studies involving different combinations of vertical velocity, treadmill speed and angle are also needed. Finally, a more thorough biomechanical comparison of walking vs. running is in order since on steep inclines the defining characteristic(s) of these two gaits are not yet clear.

In conclusion, we studied the cost of walking and running at angles substantially steeper than any previous study. We found that for both walking and running there is a range of angles (20.4 degrees to 35.0 degrees) for which energy expenditure is minimized. Our data suggest that, to achieve the best results, VK races should be contested within this range of angles. Although other factors may be important, on very steep slopes, athletes can reduce their energy expenditure by walking rather than running.

ACKNOWLEDGMENTS

We thank Dr. Wouter Hoogkamer for his valuable suggestions all of our participants for their time and effort.

GRANTS

We are grateful to the University of Colorado Boulder Undergraduate Research Science Training (BURST) and Undergraduate Research Opportunity Program (UROP) for supporting Ms. Amanda Ortiz.

REFERENCES

1. **Biewener AA, Farley CT, Roberts TJ, and Temaner M**. Muscle mechanical advantage of human walking and running: implications for energy cost. *J Appl Physiol* 97: 2266-2274, 2004.

2. **Brockway JM**. Derivation of formulae used to calculate energy expenditure in man. *Hum Nutr Clin Nutr* 41: 463-471, 1987.

3. **di Prampero PE**. The energy cost of human locomotion on land and in water. *Int J Sports Med* 7: 55-72., 1986.

4. **Diedrich FJ, and Warren WH**. The dynamics of gait transitions: effects of grade and load. *J Mot Behav* 30: 60-78, 1998.

5. **Farley CT, Blickhan R, Saito J, and Taylor CR**. Hopping frequency in humans: a test of how springs set stride frequency in bouncing gaits. *J Appl Physiol* 71: 2127-2132, 1991.

6. **Farley CT, and Ferris DP**. Biomechanics of walking and running: center of mass movements to muscle action. *Exerc Sport Sci Rev* 26: 253-285, 1998.

7. **Gottschall JS, and Kram R**. Ground reaction forces during downhill and uphill running. *J Biomech* 38: 445-452, 2005.

8. **Gottschall JS, and Kram R**. Mechanical energy fluctuations during hill walking: the effects of slope on inverted pendulum exchange. *J Exp Biol* 209: 4895-4900, 2006.

9. **Hoogkamer W, Taboga P, and Kram R**. Applying the cost of generating force hypothesis to uphill running. *PeerJ* 2: e482, 2014.

10. **Hreljac A**. Determinants of the gait transition speed during human locomotion: kinematic factors. *J Biomech* 28: 669-677, 1995.

11. **Hreljac A, Imamura R, Escamilla RF, and Edwards WB**. Effects of changing protocol, grade, and direction on the preferred gait transition speed during human locomotion. *Gait Posture* 25: 419-424, 2007.

12. **Kay A**. Pace and critical gradient for hill runners: an analysis of race records. *Journal of Quantitative Analysis in Sports* 8: 1-17, 2012.

13. **Kram R, and Taylor CR**. Energetics of running: a new perspective. *Nature* 346: 265-267, 1990.

14. **Margaria R**. Sulla fisiologia e specialmente sul consumo energetico della marcia e della corsa a varia velocita` ed inclinazione del terreno. In: *Atti Accad Naz Lince*1938, p. 299-368.

15. **Margaria R, Cerretelli P, Aghemo P, and Sassi G**. Energy cost of running. *J Appl Physiol* 18: 367-370, 1963.

16. **McMahon TA, Valiant G, and Frederick EC**. Groucho running. *J Appl Physiol* 62: 2326-2337, 1987.

17. **Mercier J, Le Gallais D, Durand M, Goudal C, Micallef JP, and Prefaut C**. Energy expenditure and cardiorespiratory responses at the transition between walking and running. *Eur J Appl Physiol Occup Physiol* 69: 525-529, 1994.

18. **Minetti AE, Ardigo LP, and Saibene F**. Mechanical determinants of the minimum energy cost of gradient running in humans. *J Exp Biol* 195: 211-225, 1994.

19. **Minetti AE, Ardigo LP, and Saibene F**. The transition between walking and running in humans: metabolic and mechanical aspects at different gradients. *Acta Physiol Scand* 150: 315-323, 1994.

20. **Minetti AE, Cazzola D, Seminati E, Giacometti M, and Roi GS**. Skyscraper running: physiological and biomechanical profile of a novel sport activity. *Scand J Med Sci Sports* 21: 293-301, 2011.

21. **Minetti AE, Moia C, Roi GS, Susta D, and Ferretti G**. Energy cost of walking and running at extreme uphill and downhill slopes. *J Appl Physiol* 93: 1039-1046, 2002.

22. **Padulo J, Annino G, Migliaccio GM, D'Ottavio S, and Tihanyi J**. Kinematics of running at different slopes and speeds. *J Strength Cond Res* 26: 1331-1339, 2012.

23. **Rubenson J, Heliams DB, Lloyd DG, and Fournier PA**. Gait selection in the ostrich: mechanical and metabolic characteristics of walking and running with and without an aerial phase. *Proc Biol Sci* 271: 1091-1099, 2004.

24. **Snyder KL, Kram R, and Gottschall JS**. The role of elastic energy storage and recovery in downhill and uphill running. *J Exp Biol* 215: 2283-2287, 2012.

25. **Thorstensson A, and Roberthson H**. Adaptations to changing speed in human locomotion: speed of transition between walking and running. *Acta Physiol Scand* 131: 211-214, 1987.

26. **Voloshina AS, and Ferris DP**. Biomechanics and energetics of running on uneven terrain. *J Exp Biol* 218: 711-719, 2015.

27. **Zamparo P, Perini R, Orizio C, Sacher M, and Ferretti G**. The energy cost of walking or running on sand. *Eur J Appl Physiol Occup Physiol* 65: 183-187, 1992.

FIGURE AND TABLE CAPTIONS

Figure 1. The average of the five best performances for ten different VK races in the year that the each course record was set. 1: The Rut VK (USA); 2: Val Resia VK (I); 3: Mont Blanc VK (F); 4: Limone Vertical Extreme (I); 5: Latemar VK (I); 6: VK Lagunc (I); 7: VK face de Bellevarde (F); 8: Dolomites VK (I); 9: VK Col de Lana (I); 10: VK de Foully (CH); 11: La Verticale du Grand Serre (F); USA: United State of America; I: Italy; F: France; CH: Switzerland.

Figure 2. Customized treadmill mounted at 30°.

Figure 3. Metabolic power (W/kg) and vertical cost of transport (CoT_{vert}, J/kg·m) of walking (black circles) and running (white circles) plotted as a function of angle (degrees) and treadmill speed (m/s) for 15 subjects. At 9.4° only 9 subjects were able to walk at the required speed (2.14 m/s). Except for 9.4°, walking was less metabolically expensive than running. See text for more details.

Figure 4. Stride frequency (strides/s, 4A) and stride length (m, 4B) for walking (black circles) and running (white circles) as a function of angle (degrees) and treadmill speed (m/s) for 15 subjects. At 9.4° only 9 subjects were able to walk at the required speed (2.14 m/s).

Figure 5. Mean cost of running (Cr, in J/kg·m) measured in the present study (white circles) and computed with the formula of Minetti et al. (21) (black line). The dashed line extrapolates to angles steeper than 24.2° (45%). The relationship between Cr and the slope for our data is described by the formula Cr = 1.3614 + 0.7686 (angle in degrees) (R^2=0.97).

TABLE 1. Vertical cost of walking and running (mean±SD, in J/kg·m) as a function of the slope angle (°) and treadmill belt speed (m/s). Vertical velocity was fixed at 0.35 m/s. At 9.4° only 9 subjects were able to walk at the required speed (2.14 m/s). For all other angles n=15.

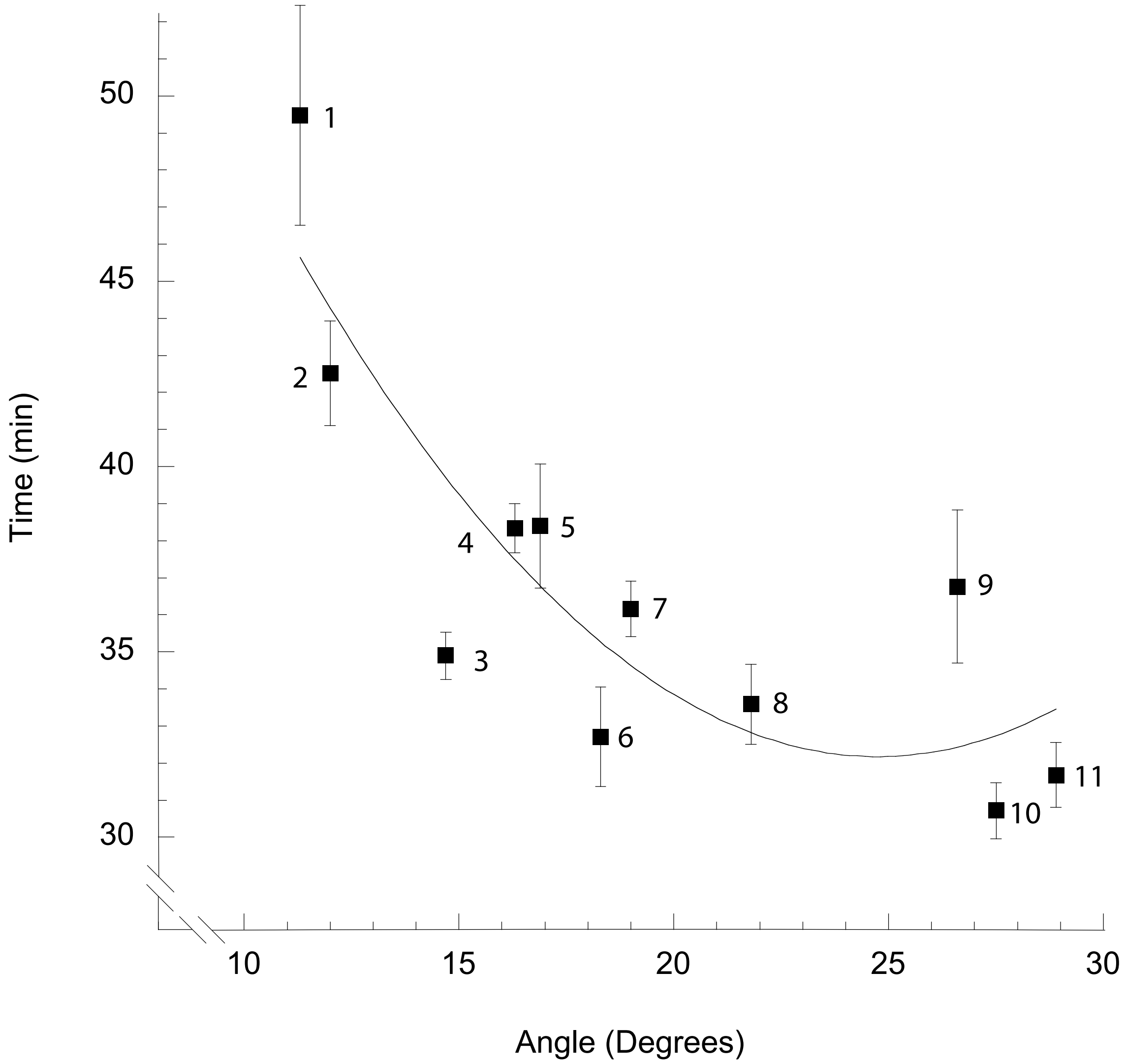

Time (min)
50
45
40
35
30
10
15
20
25
30
Angle (Degrees)
1
2
3
4
5
6
7
8
9
10
11

Figure 2

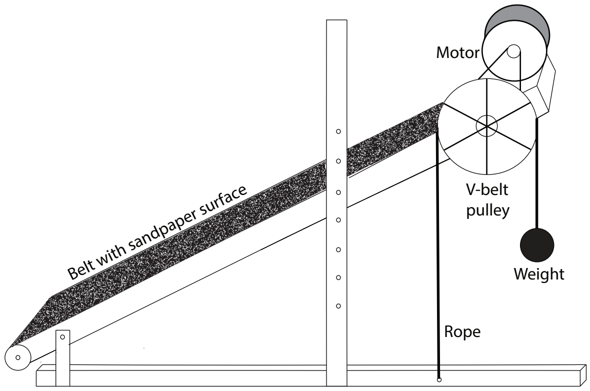

Motor
Belt with sandpaper surface
V-belt pulley
Weight
Rope

Figure 3

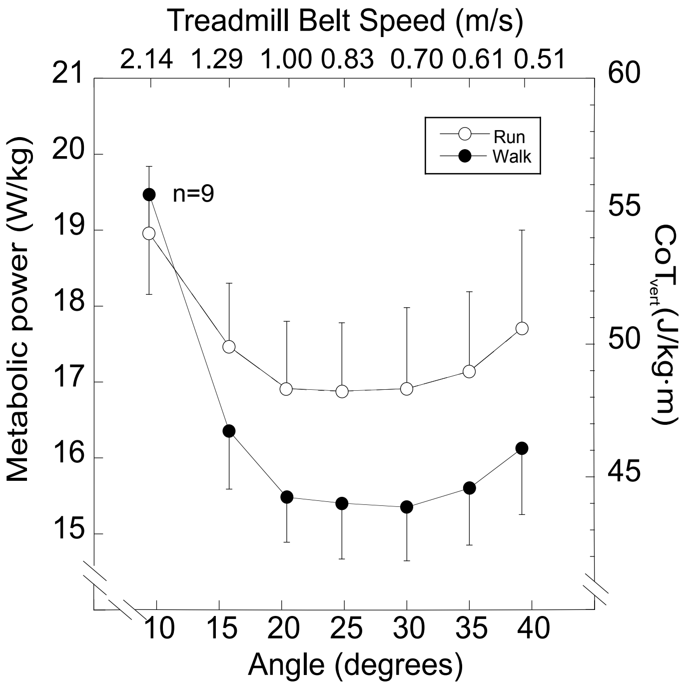

Treadmill Belt Speed (m/s)
2.14
1.29
1.00
0.83
0.70
0.61
0.51
Metabolic power (W/kg)
21
20
19
18
17
16
15
60
55
50
45
CoT_{vert} (J/kg·m)
Run
Walk
n=9
10
15
20
25
30
35
40
Angle (degrees)

Figure 4

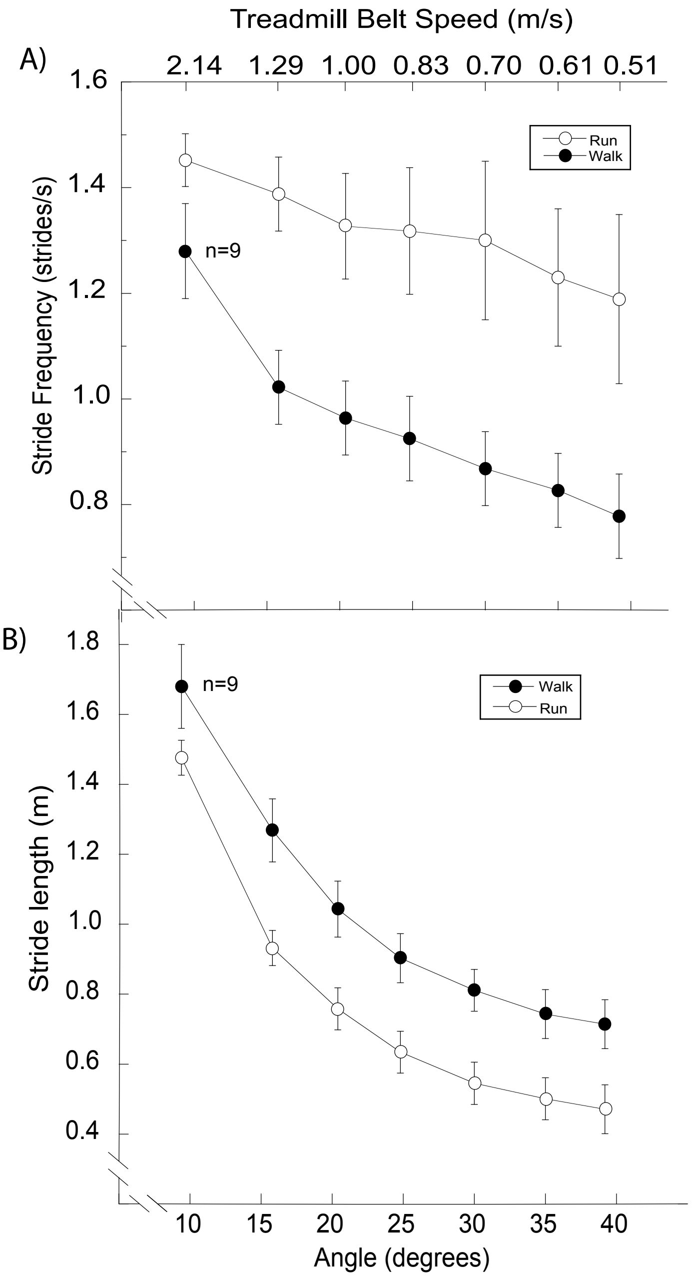

Treadmill Belt Speed (m/s)
A)
2.14
1.29
1.00
0.83
0.70
0.61
0.51
1.6
1.4
1.2
1.0
0.8
Stride Frequency (strides/s)
Run
Walk
n=9
B)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
Stride length (m)
Walk
Run
n=9
10
15
20
25
30
35
40
Angle (degrees)

Figure 5

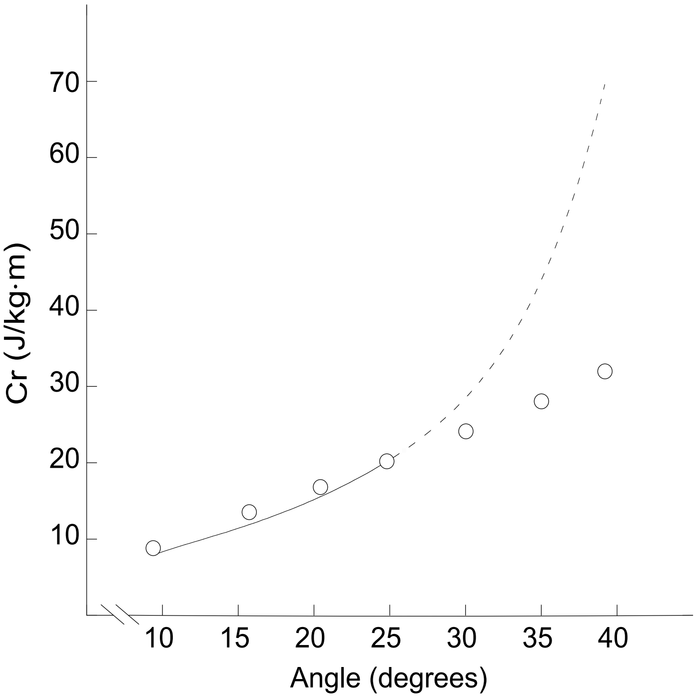


Table 1. The vertical cost of walking and running as function of the slope angle.

Angle (degrees)	Treadmill belt speed (m/s)	Walk ($J \cdot kg^{-1} \cdot m^{-1}$)	Run ($J \cdot kg^{-1} \cdot m^{-1}$)	Difference (%)	p
9.4	2.14	55.67 ± 3.80	54.83 ± 2.29	1.53	0.545
15.8	1.29	46.73 ± 2.19	49.90 ± 2.37	-6.35	0.001
20.4	1.00	44.23 ± 1.69	48.31 ± 2.54	-8.45	0.001
24.8	0.83	44.01 ± 2.10	48.22 ± 2.57	-8.73	0.001
30.0	0.70	43.86 ± 2.02	48.32 ± 3.07	-9.23	0.001
35.0	0.61	44.57 ± 2.14	48.97 ± 3.01	-8.99	0.001
39.2	0.51	46.07 ± 2.49	50.59 ± 3.70	-8.93	0.001

A Mechatronic System Mounted on Insole for Analyzing Human Gait

Davide Giovanelli[1], Nicola Giovanelli[2], Paolo Taboga[2], Erfan Shojaei Barjuei[3], Paolo Boscariol[3], Renato Vidoni[4],
Alessandro Gasparetto[3] and Stefano Lazzer[2]

[1] Dipartimento di Elettronica e Informatica, University of Padova, PD 35122, ITALY, e-mail:giovanel@dei.unipd.it.
[2] Dipartimento di Scienze Mediche e Biologiche, University of Udine, UD 33100, ITALY, e-mail: nicola.giovanelli@uniud.it, paolo.taboga@uniud.it, stefano.lazzer@uniud.it.
[3] Dipartimento di Ingegneria Elettrica, Gestionale e Meccanica, University of Udine, UD 33100, ITALY, e-mail: erfan.shojaei@uniud.it, paolo.boscariol@uniud.it, gasparetto@uniud.it.
[4] Facoltà di Scienze e Tecnologie, Free University of Bolzano/Bozen, BZ 39100, ITALY, e-mail: renato.vidoni@unibz.it

***Abstract*—In this paper, designing and fabricating a mechatronic system for analyzing exerted forces by human gait has been described. Force sensitive resistors (FSRs) sensors as well as Arduino Due (Microcontroller) have been utilized in the system which is mounted on a shoe insole. Furthermore, the applied Interrupt Service Routine (ISR) programming technique in microcontroller and signal conditioning circuit design has been explained. The mechatronic system has been tuned and calibrated through the experimental tests and some of the important results have been presented and discussed.**

***Keywords*— Force sensitive resistors (FSRs), Arduino Due, insole, force platform, gait**

I. INTRODUCTION

It might be indeed true to say that human body motion has been under investigation since about fifty years ago. Several researchers focused on analyzing human body motion from different points of view; in fact, there are some motivating reasons for them. From scientific perspective, realizing and comprehending details of human motion is an important problem. Walking and running efficiently; that is, moving with minimum energy consumption is an interesting issue for the sportive researchers. From the medical point of view, diagnosing and preventing some injuries and diseases such as diabetes can be done by analyzing body motion. Ankle moment has a remarkable effect on trunk acceleration propulsion, and balance while walking [1].

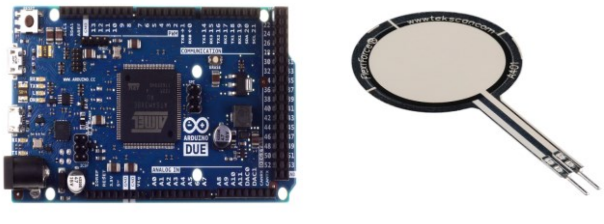
Some types of systems are using air coils in the shoes for measuring the pressure in order to monitor human gait, as in the work by Kyoungchul in [4]. Furthermore, different kind of instruments have been presented so far for gait event detections or fault diagnostician [5]–[9]. Generally speaking, recognition and analysis of the human gait can be subdivided in three different approaches: image processing, floor sensors and sensors placed on the body [10].

Precise result from motion Kinect requires analyzing the steps during walking on a surface of the force plates; otherwise, may lead to inaccuracies. Many methods have been developed in order to analyze human walking. Infrared cameras and force plates have been used in some laboratories because of their accurate measurement and also availability of standards for them. The high price is the main disadvantage of these type of instruments. Some other types of instruments such as treadmills are available but walking in a normal way is different from walking on the treadmills. Moreover other types of instruments are commercially available in market but they are expensive products so they are not easily available for general usage [2], [3].

A simple cheap footswitch system has been presented in [11] in order to measure accurately the initial and end foot contact time. The idea of this footswitch not only has been widely used in the systems for mobile gait analysis, but also is used in our work for the purpose of building an accurate and inexpensive mechatronic system mounted on a shoe insole with use of force sensitive resistors (FSRs). Applying FSRs sensors result in high nonlinear-response which leads to difficulty in parameters calculating that is a challenging problem for designing a measurement system for gait analysis [12].

In this paper, the design and the test of a mechatronic system mounted on insole platform in order to measure and analyze force reaction during walking is discussed and presented. The mechatronic system is based on two main parts: the sensorial insole and the data acquisition device. The shoe insole includes five force sensitive resistors (FSRs) with separated signal channels. The main part of data acquisition block is a microcontroller (Arduino) for ADC conversion and data logging on a SD card. For calibrating the system and finding the most optimized coefficient of conversions (volt-newton) and also reducing the value of RMS of error signals, a force platform has been used.

Although a number of works and products has been done in this area and some of them are similar to our work such as newest one in [13], there are some remarkable difference



(a) (b)

Fig. 1. a) Front view of Arduino Due, b) FSR sensor (Flexiforce A401)

978-1-4799-6743-8/14/$31.00 ©2014 IEEE

between our work and the others. Using low-cost instruments, average error of 5% and the minimum invasiveness at the end of the athletic gesture are the main advantages and differences between our work and the others.

This paper is organized as follows: Section II briefly explains the data acquisition technique. The description of the sensors is give in Section III. Section IV provides a description on the system realization. In Section V the methodology of calibration and tuning has been discussed.

II. DATA ACQUISTION

Data acquisition and logging is performed by a custom microcontroller system that has the ability of recording the measured data on a SD memory card. In our system the Arduino Due (Fig. 1.a) which is a microcontroller board based on the Atmel SAM3X8E ARM Cortex-M3 CPU [14] has been used for force data logging on a SD card. In our system the Arduino Due (Fig. 1.a) which is a microcontroller board based on the Atmel SAM3X8E ARM Cortex-M3 CPU [14] has been used for force data logging on a SD card.

First, the available data values will be copied in the first cell of the circular buffer, then they will be transferred from the buffer to the memory card. The last step will be done during the pause time between two sequence acquisition times. Fig. 2 illustrates the flowchart of the main program of microcontroller (Arduino Due). The Fig. 2.a shows the main program flowchart for data acquisition of the received signals and saving data on a memory card. The flowchart of the program for Interrupt Service Routine (ISR) of data sampling is illustrated in Fig. 2.b.

According to Fig. 2, once the sampling ISR has been completed, the microcontroller goes back to the main () loop, in which the acquired samples are transferred to the SD card. When the timer generates another interrupts (so the sampling Routine is started again), the transfer is stopped and can be restricted once the ISR is completed.

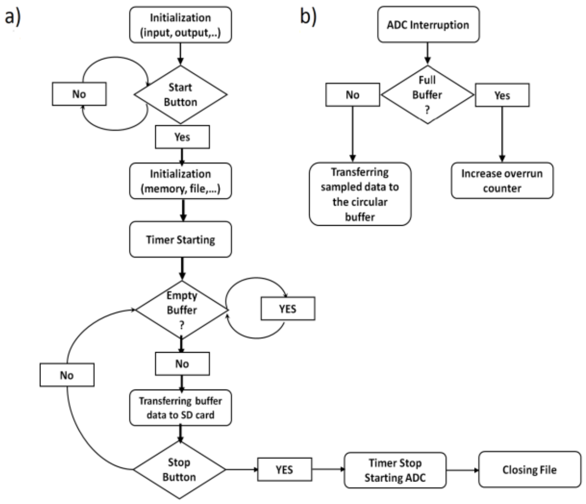


Fig. 2. Flowchart of Arduino program, a) the flowchart of the main () program, b) the flowchart of the interruption service routine (ISR) program for data sampling

TABLE I
TYPICAL PERFORMANNCE OF SENSOR FLEXIFORCE A401

Typical Performance	Evaluation Condition
Linearity (Error) < ± 3%	Line drawn from 0 to 50% load
Repeatability < +2.5% of full scale	Conditioned sensor, 80% of full force applied
Hysteresis < 4.5% of full scale	Conditioned sensor, 80% of full force applied
Drift < 5% per logarithmic time scale	Constant load of 25 lb (111N)
Response Time < 5 µsec	Impact load, output recorded on oscilloscope

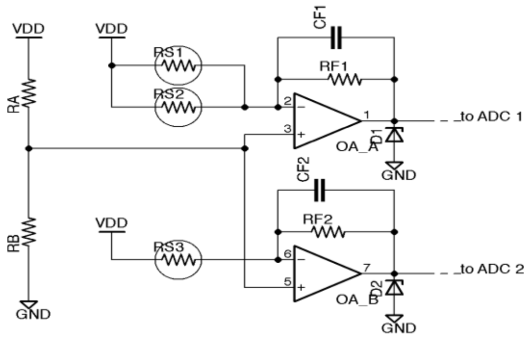


Fig. 3. Signal Conditioning Circuit (In the figure, only two channels of six channels have been demonstrated)

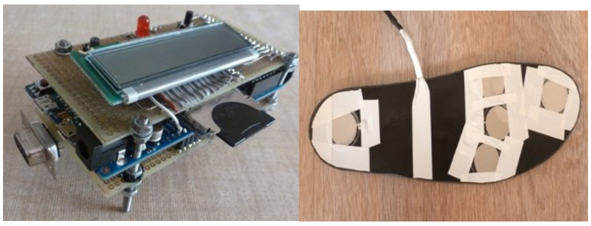

(a) (b)

Fig. 4. a) The mounted boards of the acquisition system. In order from the bottom: Signal conditioning circuit (on the left side, the arrived signals connector from the sensors is visible), the board of Arduino Due, the control board with the SD slot for memory and the display, b) Sensorial insole, the five Flexiforce A401 sensors are visible

III. SENSORS

A. FSR Sensors

The measurement of the ground reaction force (GRF) is done by force sensing resistors (FSR). This kind of the sensors are frequently used in similar works [13], [15], [16]. Teksan Flexiforce A401 (Fig.1.b) is the type of the sensor that has been chosen in our system. The typical performance of this sensor is reported in Table I.

B. Signal Conditioning Circuit

Signal conditioning circuit which is a conductance-voltage convertor, has been used to convert the received signals from sensors in readable mode for ADC (Analog Digital Converter). Fig. 3 shows the signal conditioning circuit; R_{S1}, R_{S2} and R_{S3} are the resistive sensors. The DC gain of the first channel can be calculated by considering the superposition effect as follows:

$$V_{out2} = -V_{DD}\frac{R_F}{R_{S1}} - V_{DD}\frac{R_F}{R_{S2}} + V + \left(1 + \frac{R_F}{R_{S1}||R_{S2}}\right) \qquad (1)$$

By reorganizing the equation 1, the following equation can be obtained:

$$V_{out2} = -\Delta V\frac{R_F}{R_{S1}} - \Delta V\frac{R_F}{R_{S2}} + V_+ \qquad (2)$$

Where $\Delta V = V_{DD} - V_+$ and $V_+ = 3.2\,Volt$ for Arduino Due.

As it can be seen in Fig. 3, two 3.6 Volt Zener diodes (D_1 and D_2) are mounted in parallel to the output. These diodes act as a voltage protection for the inputs of the ADC channels. In fact if the voltage of one of the input pins surpass the breakdown voltage of the Zener diode, the diode acts and prevents the probable damages on microcontroller.

The values of R_A and R_B resistors are chosen 1.8 kΩ and 3.3 kΩ respectively. The value of the feedback resistor has been chosen to fully exploit the ADC range. Knowing that during running the reaction of vertical forces to the ground reaches up to twice the body weight [17] and estimating the maximum weight of a person on the test is 100 kg so the scale measuring system is based on 200 kg (less than 2000 N). Regarding the information on the data sheet of the Teksan Flexiforce A401 sensor, it is possible to estimate about the conductance of the sensor under 200 kg (440 lb) pressure.

The conductance / weight ratio can be calculates as:

$$m = \frac{G_S(120lb) - G_S(20lb)}{120lb - 20lb} = 1.6 \times 10^{-7}\left[\frac{S}{lb}\right] \qquad (3)$$

With knowing the value of slope of the conductance/weight, the conductance can be evaluated as:

$$G_s(440lb) = m.f = m.440\ lb = 70.4\ \mu S \qquad (4)$$

And the resistance of the sensor can be obtained as:

$$R_S(440lb) = \frac{1}{G_S(440lb)} = 14.2\ k\Omega \qquad (5)$$

Now by considering the equation 2, the value of R_F (neglecting the second sensor) is:

$$R_{F2} = \frac{V_+ - V_{out}}{\Delta V}R_S = \frac{3.2V - 0.2V}{1.8V} \times 14.2\ k\Omega = 23.5\ k\Omega \qquad (6)$$

The nearest commercial resistor to $RF_{\_DUE}$ is $22k\Omega$. The capacitor is also used in signal conditioning circuit in order to have a cutoff filter for the frequency about F_C=250 Hz and can be obtained as follows:

$$C_{F\_DUE} = \frac{1}{2\pi F_C R_{F\_DUE}} = 29nF \qquad (7)$$

The nearest commercial capacitor to C_{F2} is 27nF. A filter capacitor has been used to reduce filter noise at high frequencies.

IV. SYSTEM REALIZATION

The final version of our system includes two main blocks: Data Acquisition Block and Sensorial Insole. The data acquisition block is mounted on a mounting box that is kept trough a belt on the person's body during the running; while, the sensorial insole is located in the shoe. The two blocks are connected with heavy duty multipolar cables.

A. Data Acquisition Block

The data acquisition block is based on three boards: The Arduino Due microcontroller board, an expansion board that mounts the slot for the memory card and some circuits for the control of the acquisition and the signal conditioning circuit for six separated channels of data acquisition.

These boards are positioned one above another and interconnected with the connector (header) so they can easily be disassembled for any changes or tune-ups. The final structure of data acquisition block is shown in Fig. 4.a.

B. Sensorial Insole

Fig. 4.b illustrates the last version of the sensorial insole that was placed in a shoe. As it can be seen, the sensors are located on a nominally flat surface. Several arrangements of force sensors have been investigated to experimentally determine the optimal placement, as shown in Fig. 5. The main advantages of this type of sensor location is that sensors can work in an optimal condition. The wiring was done with the normal copper wires at the bottom of the slab, cables and connectors are protected by sheaths shrink and held in place by adhesive tape.

V. TUNING AND CALIBRATION

Similar to other measuring systems also our system needs to be calibrated. In fact, our mechatronic system records and registers the behavior of the output voltage of the op-amp.

The gain of the signal conditioning circuit depends on the resistance of the circuit and can change the coefficient of the conversion.

By neglecting the fixed-terms, following equation turns up:

$$V_{out} = -\Delta V R_F G_S \qquad (8)$$

Where the value of the conductance G_S is chosen approximately from the sensor data sheet. Regarding the presented material in section III:

$$G_S = m.F_{lb} \qquad (9)$$

Where F_{lb} is the applied force on the sensor measured in pound. By substation the equation 9 in 8 and rearranging the expression for explication the fore, we can obtain:

$$F_{lb} = -\frac{V_{out}}{m\Delta V R_F} \qquad (10)$$

For converting the force in pound to newton, multiplication to 4.45 is needed:

$$F_N = -\frac{V_{out}}{m\Delta V R_F} \times 4.45 \qquad (11)$$

The value of ΔV is 1.8 V for signal the conditioning circuit of Arduino Due. Therefore, a conversion constant can be obtained:

$$k_{v\to N} = -\frac{1}{m\Delta v R_F} \times 4.45 = 702\left[\frac{N}{V}\right] \qquad (12)$$

Noting that multiplication of this value by assuming the output swing of the op-amp causes in $702\ \times 3 = 2180[N]$ which is the maximum force that the system measures.

In fact the reading carried out from the acquisition is not measuring of voltage but is direct reading of the digital conversion of the signal. Consequently, a range number from 0 to 4095 will present the voltage between 0 and full scale voltage of ADC ($V_{FS\_ADC} = 3.3\,Volt$). Thus, it is possible to find directly the conversion between the numerical value converted by the ADC and the force:

$$k_{ADC\rightarrow N} = -\frac{1}{m\Delta V R_F} \times 4.45 \times \frac{V_{FS_{ADC}}}{2^N} = 5.66 \times 10^{-3} \left[\frac{N}{LSB}\right] \qquad (13)$$

A. Characteristic Limitation of the System

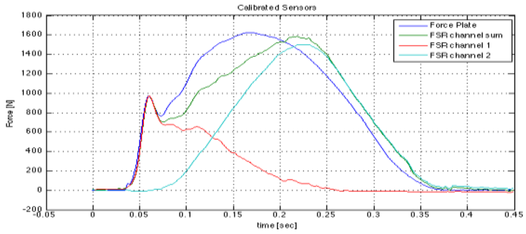


Fig. 6. Initial calibration of the sensors for comparison with the force platform

The technical limitations imposed by the choice of sensors and the variability of each individual body structure require the use of a proper calibration procedure. Also the insole mounted on the shoe can be in different forms that results in different force distributions during the walking. Moreover, the quality of the sensors, their positions and also conditions of their performance (humidity and temperature) can affect the performance of the system.

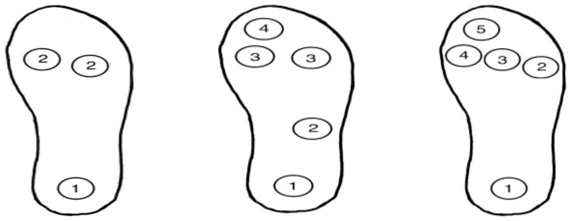
B. Calibration Methodology

We started from a configuration with 3 sensors on 2 separated channels and then a configuration with 5 sensors on 5 separated channels, as it can be seen in Fig. 5. The methodology used is based on linear regression, the method has been used in similar works where it seems to give good results, leading to an error close to 5% [13]. We can choose among two different strategies for calibration: 1) minimizing the RMS value of the error signal between the readings of the force platform and those of the sensors: this leads to have a behavior of the force which should approximate the overall performance of the platform with some margin of errors. 2) Minimizing the RMS error on the features: this should lead to a trace of the force that deviates the most from the force platform, but it will be less uncertainty about the value of the extracted features.

C. First Configuration

The first sensor configuration of the insole with three sensors and two separated channels is shown in Fig. 5.a. The position of the sensors are chosen in order to consider maximum pressure during the motion [18] . Some tests have been done in the laboratory with the force platform, in Fig. 6 the behavior of the acquired signals of sensors and the force platform has been used. The coefficient for the calibration have been found in a different way for the two channels. For the first channel (where the sensor is located under the heel) a multiplicative constant (K_1) has been found in order to match the amplitude of the first peak recorded by the sensors and the amplitude recorded from the platform. For the second channel the term (K_2) is to minimize the RMS value of the error signal given by:

$$e = f_{ForcePlate} - k_1.s_{channel\_1} - k_2.s_{channel\_2} \qquad (14)$$



(a) (b) (c)

Fig. 5. Arrangement of the sensors on the insole in the a) first version

In which e is the error signal between the acquisition of the force platform and the sensors, $f_{ForcePlate}$ is the signal acquired from the force platform of strength and used as a reference, $K_{1.schannel}$ represents the first channel signal acquisition (k_1 is found by comparison of peaks), k_2 is the factor that multiplies the signal of the second channel and $s_{\text{channel\_2}}$ minimizes the RMS value of e.

Accurate force estimation, correct recording contact time and superimposed of initial part of curves could be concluded from Fig. 6.

D. Second Configuration

The aim of the second configuration is to achieve the greater accuracy of the curve and to obtain this goal, two additional sensors are mounted (four channels and five sensors in total). This arrangement of the sensors is shown in Fig. 5.b. The additional central sensor has the purpose of providing the missing information in the central phase of the step while the sensor located on the tip is used to obtain the information on the toe force.

TABLE I II

CALIBRATION COEFFICIENT K WHICH IS CALCULATED WITH USE OF MATLAB. THE VALUES OF EVERY ROW REPRESENTS THE MULTIPLICATIVE COEFFICIENT FOR ACQUIRED DATA BY THE SENSORS AND THEY ARE EXPRESSED IN $\left[\frac{N}{LSB}\right]$

CHANNEL	TEST 1	TEST 2	TEST 3	TEST 4
1	3.37	1.65	2.66	4.49
2	4.45	3.13	6.42	2.90
3	0.79	0	0	0
4	0	1.25	1.22	0.86
5	5.40	0.84	0	3.34

TABLE I III

RMS ERROR IN THE VALUE OF FORCE PEAKS REACHED AT THE MOMENT OF CONTACT WITH THE GROUND (PEAK RELATIVE TO THE HEEL) AND IN THE MOMENT OF MAXIMUM THRUST UPWARDS (PEAK RELATIVE TO THE METATARSALS). THE VALUES WERE NORMALIZED TO THE MAXIMUM VALUE OF THE PEAKS RECORDED FOR EACH TEST.

PEAK	TEST 1	TEST 2	TEST 3	TEST 4
Heel	6.60%	10.7%	15.5%	11.6%
Metatarsal	2.79%	8.84%	7.78%	4.31%

Adding channels definitely increases the availability of the information; however, it increases both system complexity and calibration procedure. The results of two experimental tests are shown in Fig 7. As it can be seen, the acquired signal by channel 2 is different (green curve in Fig 7.a and 7.b). This is because of force platform position, runner style and the higher travel speed achieved during the second test. Another observation made always on the channel 2 is that the signal shape is irregular and noisy by increasing the speed. The results are more accurate in comparison to Fig. 6 (first configuration) due to the more accurate positioning of the sensors.

E. Third Configuration

For the third force sensors configuration, which is depicted in Fig. 5.c, it was decided to follow the approach used in [16], and to remove the central sensor in order to have a better coverage forefoot. The aim is to detect more accurately the impulsive force peaks.

The applied changes in the third configuration are modification of the position of sensor 2 (see the Fig. 5.c) and placement of all the sensors on the independent channels (totally 5 channels).

F. Procedure

The tests for data acquisition have been done based on third configuration in this way: force platform was placed in a running track (in a proper position compared to the level of the ground) and the reaction force exerted by four different tests (subjects) have been recorded and registered. Every test has registered totally fifteen steps, this is to understand how the coefficient may be varied depending on the work condition.

TABLE I V

COEFFICIENT CALCULATED TO MINIMIZE THE RMS VALUE OF THE ERROR BETWEEN THE FORCE PEAKS REGISTERED AND RECORDED WITH FSR SENSORS AND THE FORCE PLATFORM K_{PEAKS}. THE MEASUREMENT UNIT IS $\left[\frac{N}{LSB}\right]$.

CHANNEL (Heel)	TEST 1	TEST 2	TEST 3	TEST 4
1	2.85	0	0.4	2
2	7.88	44.1	54.5	11.7
CHANNEL (Metatarsal)	TEST 1	TEST 2	TEST 3	TEST 4
2	1.60	0	1.08	0.35
3	3.18	2.26	1.06	1.90
4	2.39	0.24	2.17	0
5	4.47	0	1.49	7.45

Two types of coefficients were calculated with the linear regression. The first set of coefficients is chosen in order to minimize the error between the extracted curves from the force platform and the sensors, and have been calculated by appending the various tests carried out by the same person and also by applying linear regression to the whole performance of all the steps. The second set of coefficients is targeted instead of minimizing the error on the two characteristics peaks of GRF in a way similar to what was done for the first set of coefficients.

For the first peak, which shows the pressure at the moment of heel contact with the ground, channels 1 and 2 (see Fig. 5.c) is considered and for the second peak, which realizes the exerted force of metatarsals the channels 2, 3, 4 and 5 is taken into account.

This procedure is performed independently for each test. Fig. 7 shows some test results. In particular, the figure refers to the obtained performance with the coefficient that minimize the RMS value of them error signal between the registered force by the force platform and the sensors (this set of coefficient will be K_{graph}).

G. Results

Although these results seems be worse respect to the second configuration it should be taken into account that these results are extracted from 15 steps and not the two first steps of the tests. The calculated coefficient are given in Table II. From these data. We can definitely say that the coefficient vary greatly among each singular test.

Another important observation is that some coefficients are zero, this means that the corresponding channel does not bring any additional information with referring to other channels.

The results are obtained using the second set of coefficient (K_{peaks}), those that minimize the error relating only to the peaks of the curve, are summarized in the Fig. 8 and 9.

The graphs represent the force peaks (on the heel and metatarsals) recorded by the FSR sensors and each point is related to a test. In all cases, we see the trend monotonically increasing (as was expected) showing a certain linearity between the measured force and the exerted force, however, we see that the dispersion of the points is very wide between the tests. The RMS value of the error of these measurement was normalized to the maximum force peak recorded and registered by the force platform from the heel and metatarsals for every test. The results for every test are reported in Table III.

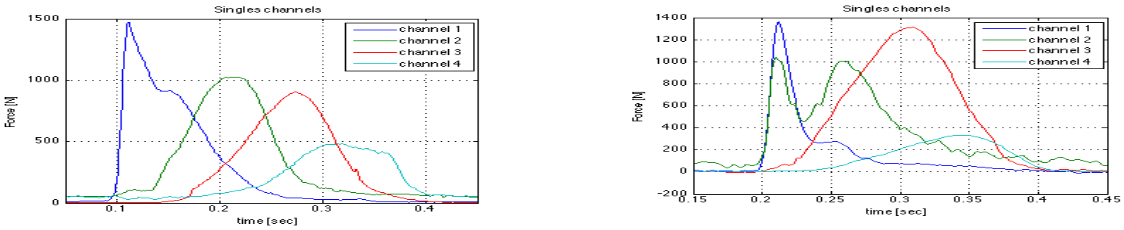
The average value of the RMS error (considering both peaks) is about 8.5%, which is a result not far from the 5% accuracy which was the target of the system. The set of coefficient calculated on the basis of the force peaks are reported in Table IV.

VI. CONCLUSION

In this work an economic mechatronic system mounted on insole in order to measure accurately the vertical forces for the human gait analysis has been realized and presented. While almost the similar systems has dealt with analysis of the walk, in the presented system race forces which conclude higher and more impulsive nature, are also involved.

Laminar FSR sensors have been placed on the insole of the shoe, it was seen that the sensor positions is an important factor in final results. Moreover, it was concluded that high number of sensors will not necessarily increase the accuracy of the system.

The main aspects that have characterized in this work are: Choices of the sensors, physical realization of the prototype in a reliable compact, arrangement of the sensors on the insole in order to obtain the maximum amount of information as possible and Sensor calibration.

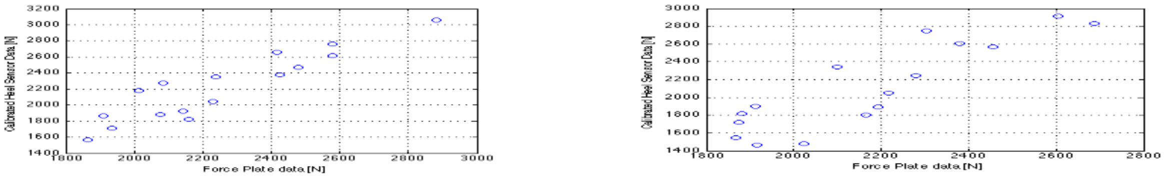


(a) (b)

Fig. 7. Data acquisition with the second configuration of two different tests. Total number of channels weighted to reduce the RMS value of the error between two curves. (a-b) performance of individual channels

(a) (b)

Fig. 8. Acquired signals from two tests. Recorded track process by the force platform (in blue) and that of the calibrated sensors for reducing the RMS value of the error signal between two curves. Here only two steps are shown but totally 15 steps exists for every test.



(a) (b)

Fig. 9. Peak forces on the heel which are measured by the FSR sensors and actual measured with the force platform. Monotonically trend increasing is clear.

Particularly, the most important features of our system are the parameterization of the force curves and possibility of reconstructing the overall trend described by the parameterized curve.

Reference

[1] C. R. Nott, F. E. Zajac, R. R. Neptune, and S. A. Kautz, “All joint moments significantly contribute to trunk angular acceleration,” J. Biomech., vol. 43, no. 13, pp. 2648–2652, Sep. 2010.

[2] “Tekscan.” [Online]. Available: http://www.tekscan.com/.

[3] “novel.de.”[Online].Available: http://novel.de/novelcontent/pedar.

[4] K. Kong and M. Tomizuka, “A Gait Monitoring System Based on Air Pressure Sensors Embedded in a Shoe,” Mechatronics, IEEE/ASME Transactions on, vol. 14, no. 3. pp. 358–370, 2009.

[5] S. J. M. Bamberg, A. Y. Benbasat, D. M. Scarborough, D. E. Krebs, and J. A. Paradiso, “Gait Analysis Using a Shoe-Integrated Wireless Sensor System,” Information Technology in Biomedicine, IEEE Transactions on, vol. 12, no. 4. pp. 413–423, 2008.

[6] M. Hanlon and R. Anderson, “Real-time gait event detection using wearable sensors,” Gait Posture, vol. 30, no. 4, pp. 523–527, Nov. 2009.

[7] M. N. Nyan, F. E. H. Tay, and E. Murugasu, “A wearable system for pre-impact fall detection,” J. Biomech., vol. 41, no. 16, pp. 3475–3481, Dec. 2008.

[8] S. Patel, K. Lorincz, R. Hughes, N. Huggins, J. Growdon, D. Standaert, M. Akay, J. Dy, M. Welsh, and P. Bonato, “Monitoring Motor Fluctuations in Patients With Parkinson's Disease Using Wearable Sensors,” Information Technology in Biomedicine, IEEE Transactions on, vol. 13, no. 6. pp. 864–873, 2009.

[9] M. Benocci, L. Rocchi, E. Farella, L. Chiari, and L. Benini, “A wireless system for gait and posture analysis based on pressure insoles and Inertial Measurement Units,” Pervasive Computing Technologies for Healthcare, 2009. PervasiveHealth 2009. 3rd International Conference on. pp. 1–6, 2009.

[10] A. Muro-de-la-Herran, B. Garcia-Zapirain, and A. Mendez-Zorrilla, “Gait Analysis Methods: An Overview of Wearable and Non-Wearable Systems, Highlighting Clinical Applications,” Sensors, vol. 14, no. 2, pp. 3362–3394, 2014.

[11] J. M. Hausdorff, Z. Ladin, and J. Y. Wei, “Footswitch system for measurement of the temporal parameters of gait,” J. Biomech., vol. 28, no. 3, pp. 347–351, Mar. 1995.

[12] N. Maalej and J. G. Webster, “A miniature electrooptical force transducer,” Biomedical Engineering, IEEE Transactions on, vol. 35, no. 2. pp. 93–98, 1988.

[13] A. Howell, T. Kobayashi, H. Hayes, K. Foreman, and S. Bamberg, “Kinetic Gait Analysis Using a Low-Cost Insole.,” IEEE Trans. Biomed. Eng., Mar. 2013.

[14] “Arduino Due.” [Online]. Available: http://arduino.cc/en/Main/ArduinoBoardDue.

[15] Simon Cooke, “The Bip Buffer - The Circular Buffer with a Twist,” 2003.

[16] E. S. Sazonov, G. Fulk, J. Hill, Y. Schutz, and R. Browning, “Monitoring of posture allocations and activities by a shoe-based wearable sensor,” IEEE Trans. Biomed. Eng., April, 2011.

[17] S. J. M. Bamberg, A. Y. Benbasat, D. M. Scarborough, D. E. Krebs, and J. A. Paradiso, “Gait analysis using a shoe-integrated wireless sensor system,” in the IEEE Transactions on Information Technology in Biomedicine, 2006.

[18] P. Taboga, “Energetics and Mechanics of Running: The Influence of Body Mass and the Use of Running Specific Prostheses,” Università degli Studi di Udine, 2013.

[19] L. Shu, T. Hua, Y. Wang, Q. Li, D. D. Feng, and X. Tao, “In-Shoe Plantar Pressure Measurement and Analysis System Based on Fabric Pressure Sensing Array,” Information Technology in Biomedicine, IEEE Transactions on, vol. 14, no. 3. pp. 767–775, 2010.

Acknowledgments

First of all, thanks to my supervisor Stefano Lazzer, who believed in me and led me during my PhD studies and taught me the many secrets of the awesome world of science. Also, I acknowledge Alessandro Gasparetto and the Department of Electrical, Managerial and Mechanical Engineering of the University of Udine for the assistance during these three years. Thanks to Jean Benoit Morin, a great scientist and enthusiast sportman, for the suggestions along these three years.

Special thanks to my American supervisor Rodger Kram for letting me play with our “steepest treadmill in the world”, pushing me to discover new metabolic frontiers. Also, thanks to all my new friends at the Colorado University, all of them are great scientists and athletes. Such an environment was very inspiring. Thanks to all Rocky Mountain Runners in Boulder, for their kindly effort in involving a (new) European runner and for showing me so many places in US. Also, I want to thank the best roommate ever, Jeff. He helped me in improving my English, teaching me some slang, discovering the best American beers and in writing my papers.

Thanks to my friends Mirco and Alberto for the valuable and copious ideas they gave me. The best friends and a good beverage always bring great ideas.

Thanks to Paolo, for teaching me lots of running mechanics and for firstly introducing me to Colorado University. Thanks to Enrico, for helping me with the complicated world of EMG.

Thanks to Martina, for the assistance in writing papers and thesis. Thanks to all my colleagues Desy, Lea, Alessandro, Alex, Stefano for the kind discussions.

Thanks to all my family, my mom Anna, my dad Tomaso, who supported my efforts through my endless studies. Also, thanks to my brothers Davide and Matteo and their girlfriends Mariaclara and Elena, but above all thanks to my little nephews Daniel and Eva for the inspiration.

Thanks to Alessandro da Ponte for the assistance and the suggestions during dozens of maximal tests in our lab. We were a great team in getting the athletes so exhausted!

Last, but probably the most important person, thanks to my girlfriend Monica, who supported me and gave me valuable help in writing papers and the thesis from Stockholm. Lots of work was done in flight, waiting to meet her again.

And thanks to my Westy, I spent so many nights up in the mountains looking for the best way of life. I lived my dream!

Thanks to all athletes, subjects, patients who participated in our studies. Science is possible because there are both scientists and “guinea pigs”. Finally, thanks to all people, friends, colleagues, institutions who allowed me to pursue my PhD.