© 2014. Published by The Company of Biologists Ltd | The Journal of Experimental Biology (2014) 217, 787-795 doi:10.1242/jeb.091645

RESEARCH ARTICLE

Factors affecting metabolic cost of transport during a multi-stage running race

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ABSTRACT

The aim of this study was to investigate: (1) the role of $\dot{V}_{O2,max}$, the fraction of $\dot{V}_{O2,max}$ (F) and the 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. Eleven runners (aged 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 $\dot{V}_{O2,max}$ were determined before the competition. In addition, biomechanics of running and CoT were determined, before and immediately after each running stage. Performance was directly proportional to $\dot{V}_{O2,max}$ (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) and vertical stiffness (r=–0.65) and low footprint 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 is associated with the myotendinous characteristics of the lower limb. Thus, 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 an ultra-endurance running competition.

KEY WORDS: Maximal oxygen uptake, Ultra-marathon, Kinematics, Stiffness, Energy cost of running

INTRODUCTION

Middle- and long-distance running performance depends 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 performance are: (1) a large value of maximal oxygen uptake ($\dot{V}_{O2,max}$, ml O$_2$ kg^{-1} min^{-1}), (2) a large fraction (F, %) of $\dot{V}_{O2,max}$ that can be sustained throughout the competition and (3) a small value of metabolic cost of transport (CoT, ml O$_2$ kg^{-1} m^{-1}). As shown elsewhere (di Prampero et al., 1986), the endurance speed (v_{end}, m min^{-1}) in long-distance running can be predicted, for any given runner, provided that their values of CoT, $\dot{V}_{O2,max}$ and F are known:

$$v_{end} = F \times \dot{V}_{O2,max} \times \mathrm{CoT}^{-1}\,. \qquad (1)$$

Indeed, strong correlations were found between $\dot{V}_{O2,max}$ 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 $F\times\dot{V}_{O2,max}$, 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 (M_b) over a distance of 1 m. CoT is independent of speed, at least for speeds ranging from 2.2 m s^{-1} (8 km h^{-1}) to about 5 m s^{-1} (18 km h^{-1}) wherein the air resistance is negligible (Jones and Doust, 1996). When normalized per unit of M_b, CoT above resting, on flat compact terrain, shows a variability among subjects of 10–20%; its average reported value (di Prampero et al., 1986) amounts to 0.182±0.014 ml O$_2$ kg^{-1} 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).

A 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 $\dot{V}_{O2,max}$ and F (Lazzer et al., 2012; Scrimgeour et al., 1986). Indeed, Brueckner and colleagues (Brueckner et al., 1991) reported an increased CoT throughout a marathon, although to a relatively minor extent (0.142% km^{-1} 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 $\dot{V}_{O2,max}$, 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 (Davies and Thompson, 1986) observed a linear increase of $\dot{V}_{O2,max}$ with time from the 50th to

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Received 27 May 2013; Accepted 28 October 2013

List of symbols and abbreviations

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 analysed steps
EMG	electromyography
$\mathrm{Ext}_{\mathrm{max}}$	maximal extension joint angle of the knee
f	step frequency
F	fraction of $\dot{V}_{\mathrm{O}_2,\mathrm{max}}$
f_{H}	heart rate
$f_{\mathrm{H,max}}$	maximum heart rate
$\mathrm{Flex}_{\mathrm{max}}$	maximal flexion joint angle of the knee
FPI	footprint index
$\mathbf{F}_{\mathrm{tendon}}$	tendon force
GM	gastrocnemius medialis
GRF	ground reaction force
k_{tendon}	tendon stiffness
k_{vert}	vertical stiffness
L	fibre fascicle length
$\mathrm{Load}_{\mathrm{max}}$	maximal load joint angle
M_{b}	body mass
MVC	maximal voluntary contraction
MVT	maximal voluntary torque
P_{max}	maximal explosive muscle power of one leg
RER	respiratory exchange ratio
t_{a}	aerial time
t_{c}	contact time
v	speed
$\dot{V}_{\mathrm{CO}_2}$	CO_2 uptake
$\dot{V}_{\mathrm{O}_2,\mathrm{max}}$	maximal O_2 intake
v_{end}	endurance speed
$v_{\mathrm{end,mean}}$	mean endurance speed
W_{ext}	mass-specific external mechanical work per unit distance
$W_{\mathrm{ext,tot}}$	total external mechanical work
X	variable

240th minute during a 4 h race on a treadmill at constant speed, the rise becoming significant ($P<0.01$) after 110 min of exercise. In addition, the ability to maintain a high F over a 24 h treadmill run was found to be mainly related to a low CoT (Gimenez et al., 2013; Millet et al., 2011), and in the same study participants a significant change in running biomechanics, including higher oscillation frequency, lower vertical stiffness and lower ground reaction force (GRF), was observed (Morin et al., 2011).

Indeed, interventions to reduce CoT are constantly sought by athletes, coaches and sport scientists. Strength (Støren 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 with less economical ones (Arampatzis et al., 2006), thus suggesting that the functionality of the muscle–tendon unit at submaximal running speeds is dependent not only 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 $\dot{V}_{\mathrm{O}_2,\mathrm{max}}$, 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 race fatigue on several biomechanical parameters that are likely to affect CoT.

Table 1. Physiological characteristics of subjects (N=11) before the race

	Mean ± s.d.	Range
Age (years)	40.5±8.4	29.5–54.0
Body mass (kg)	68.6±8.2	57.0–81.0
Height (m)	1.72±0.06	1.62–1.80
BMI ($\mathrm{kg\,m^{-2}}$)	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
$\dot{V}_{\mathrm{O}_2,\mathrm{max}}$ ($\mathrm{ml\,min^{-1}}$)	3755±467	2969–4387
$\dot{V}_{\mathrm{O}_2,\mathrm{max}}$ ($\mathrm{ml\,min^{-1}\,kg^{-1}}$ M_{b})	55.2±6.7	40.0–62.7
RER	1.08±0.04	1.02–1.13
$f_{\mathrm{H,max}}$ ($\mathrm{beats\,min^{-1}}$)	175.5±13.7	147.2–194.9
v_{max} ($\mathrm{km\,h^{-1}}$)	17.8±1.6	14.2–19.8
CoT ($\mathrm{ml\,O_2\,kg^{-1}\,m^{-1}}$)	0.190±0.008	0.182–0.197
Gas exchange threshold		
$\dot{V}_{\mathrm{O}_2}$ ($\mathrm{ml\,min^{-1}}$)	3251±488	2741–4091
$\dot{V}_{\mathrm{O}_2}$ (% $\dot{V}_{\mathrm{O}_2,\mathrm{max}}$)	86.6±6.5	75.9–90.0
RER	0.93±0.05	0.83–1.02
RER (% $\mathrm{RER}_{\mathrm{max}}$)	86.4±4.0	81.2–93.2
f_{H} ($\mathrm{beats\,min^{-1}}$)	154.1±11.9	122.4–168.2
f_{H} (% $f_{\mathrm{H,max}}$)	87.9±5.1	81.1–92.2
v ($\mathrm{km\,h^{-1}}$)	14.1±1.7	10.7–16.3
v (% v_{max})	79.2±4.6	71.2–85.3

BMI, body mass index; P_{max}, maximal explosive muscle power of one leg; $\dot{V}_{\mathrm{O}_2,\mathrm{max}}$, maximum oxygen uptake; RER, respiratory exchange ratio; $f_{\mathrm{H,max}}$, maximum heart rate; v_{max}, maximum velocity; CoT, metabolic cost of transport.

RESULTS

Characteristics of the subjects

The anthropometric characteristics of the 11 subjects who completed the race are reported in Table 1. The average $\dot{V}_{\mathrm{O}_2,\mathrm{max}}$ and maximal explosive muscle power of the lower limb (P_{max}) were 55.2±6.7 $\mathrm{ml\,min^{-1}\,kg^{-1}}$ M_{b} and 1759±202 W, respectively. The characteristics of the triceps surae muscle–tendon complex are reported in Table 2.

Factors determining performance

The role of the three factors of Eqn 8 (see Materials and methods) individually evaluated with a simple linear regression, showed that $\dot{V}_{\mathrm{O}_2,\mathrm{max}}$ ($\mathrm{ml\,min^{-1}\,kg^{-1}}$ M_{b}) had the largest role in determining mean speed (r=0.79), followed by the mean value of CoT throughout the race ($\mathrm{CoT}_{\mathrm{mean}}$, $r=-0.64$) and F (r=0.58).

When the multiple regression between them and $v_{\mathrm{end,mean}}$ (Eqn 8) was considered, the overall r increased to 0.91, whereas r values for $\dot{V}_{\mathrm{O}_2,\mathrm{max}}$, F and $\mathrm{CoT}_{\mathrm{mean}}$ were 0.77, 0.36 and −0.30, respectively, the multiple regression being described by:

$$\log v_{\mathrm{end,mean}} = 0.708 \times \log \dot{V}_{\mathrm{O}_2,\mathrm{max}} + 0.979 \times \log F - 1.559 \times \log \mathrm{CoT}_{\mathrm{mean}} - 3.067 \quad (2)$$

(R^2=0.83, s.e.=0.035 $\mathrm{km\,h^{-1}}$).

One of the main aims of this study was to investigate the role of several biomechanical factors in determining CoT. Analysis of CoT, measured before the first running stage, and the biomechanical variables revealed an inverse relationship between CoT and (1) the P_{max} ($r=-0.74$, $P<0.001$) and (2) the vertical stiffness (k_{vert}, $r=-0.65$, $P<0.05$). In addition, direct relationships between CoT and footprint index (FPI, r=0.70, $P<0.05$), step frequency (f, r=0.62, $P<0.05$) and external work per unit distance (W_{ext}, r=0.60, $P<0.05$) were found.

In view of these data, a multiple linear regression among these five biomechanical parameters and CoT was performed. Only three

Table 2. Physiological characteristics of the triceps surae tendon and gastrocnemius medialis of subjects (*N*=11) before the race

	Mean ± s.d.	Range
Triceps surae tendon		
Cross-sectional area (mm^2)	92±14	79–130
Resting length (L_0, mm)	212±21	190–250
Strain ($\Delta L \times L_0^{-1}$, %)	7.9±0.8	7.0–9.2
Force (N)	4758±828	3240–5421
Stiffness (N mm^{-1})	463±85	357–612
Young's modulus (GPa)	1.07±0.13	0.90–1.30
Gastrocnemius medialis		
Fibre length (mm)	61.0±6.5	49.0–68.9
Pennation angle (deg)	19.3±2.6	15.0–23.0
Thickness (mm)	18.2±2.9	14.6–25.0

retained a significant role in affecting CoT: (1) P_{max} (r=–0.74, $P<0.001$) followed by (2) W_{ext} (r=0.42, $P<0.05$) and (3) f (r=0.38, $P<0.05$). The resulting overall relationship is described by Eqn 3:

$$\mathrm{CoT} = -0.000004 \times P_{\mathrm{max}} + 0.195193 \times W_{\mathrm{ext}} + 0.022361 \times f + 0.115432 \quad (3)$$

(R^2=0.86, s.e.=0.003 ml kg^{-1} M_{b} m^{-1}).

Physiological and biomechanical responses to the race

Running time, mean speed (v_{mean}) and mean heart rate ($f_{\mathrm{H,mean}}$) of the three stages are reported in Table 3. Mean cumulative running time was 8:15:08±1:36:49 h:min:s, v_{mean} was 12.8±2.0 km h^{-1} and $f_{\mathrm{H,mean}}$ (as percentage maximum heart rate, $\%f_{\mathrm{H,max}}$) was 85.0±3.2% (corresponding to 76.2±4.6% of $\dot{V}_{\mathrm{O_2,max}}$).

As shown in Table 4, there was not a chronic stage effect (i.e. P=0.124) on M_{b}; conversely, an acute stage effect (i.e. $P<0.001$) on M_{b} was observed after the first and second stage (by −1.3±1.1 and −3.9±3.0 kg, respectively, $P<0.001$). 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 (Table 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 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 $[(X_{\mathrm{b}} - X_{\mathrm{a}})X_{\mathrm{c}}^{-1}]\times 100$ where X denotes any given variable before (b) or after (a) the stage considered divided by the corresponding X measured before the competition (X_{c}). To this aim, only the biomechanical parameters that were significantly correlated with CoT before the race (k_{vert}, FPI, f and W_{ext}) 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 changes in CoT (ΔCoT) followed by changes in f (Δf, r=0.39, $P<0.001$) and changes in k_{vert} (Δk_{vert}, r=–0.35, $P<0.05$). The resulting overall relationship was described by the following equation:

$$\Delta\mathrm{CoT} = 0.09 \times \Delta\mathrm{FPI} + 0.69 \times \Delta f - 0.09 \times \Delta k_{\mathrm{vert}} + 33.97 \quad (4)$$

(R^2=0.62, s.e.=4.73%).

It is worth noting 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 change in FPI (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:

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

(R^2=0.79, s.e.=3.35%).

As 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 P_{max} (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 ($\mathbf{F}_{\mathrm{tendon}}$, r=–0.69, $P<0.05$). Conversely, $\mathbf{F}_{\mathrm{tendon}}$ was directly related to morphological properties of the GM as pennation angle (r=0.73, $P<0.001$), fibre length (r=0.74, $P<0.001$) and muscle thickness (r=0.70, $P<0.001$).

DISCUSSION

The main results of the present study were that: (1) high level performance in long-distance running depends on high $\dot{V}_{\mathrm{O_2,max}}$ (r=0.77), high F (r=0.36) and low $\mathrm{CoT_{mean}}$ (r=–0.30); (2) low CoT values before the race are related to high P_{max} 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 (di Prampero et al., 1986), high level performance in long-distance running depends on: (1) a large value

Table 3. Running time, mean velocity and mean heart rate of the three stages (*N*=11)

	Stage 1 (25 km)	Stage 2 (55 km)	Stage 3 (13 km)	Total
Running time (h:min:s)	1:45:40±0:14:00 [1:29:12 to 2:09:31]	5:30:43±1:12:15 [4:09:31 to 7:44:33]	0:58:49±0:12:28 [0:48:29 to 1:30:43]	8:15:08±1:36:49 [6:27:00 to 11:24:41]
v_{mean} (km 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]
$f_{\mathrm{H,mean}}$ (% of $f_{\mathrm{H,max}}$)	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 means ± s.d. with range given in brackets.
v_{mean}, mean velocity; $f_{\mathrm{H,mean}}$, mean heart rate; $f_{\mathrm{H,max}}$, maximum heart rate.

Table 4. Body mass, metabolic cost of transport, gas exchange ratio 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	Stage×time
M_{b} (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 (ml $\mathrm{O_2}$ $\mathrm{kg^{-1}}$ $\mathrm{m^{-1}}$)	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
t_{c} (s)	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
t_{a} (s)	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 (steps^{-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
FPI (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} (ml $\mathrm{O_2}$ $\mathrm{kg^{-1}}$ $\mathrm{m^{-1}}$)	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 m^{-1})	28,360±5734	27,315±5001	29,120±5868	29,644±5492	30,771±6324	30,848±5836	0.083	0.726	0.517
Ext$_{\mathrm{max}}$ (deg)	169±5	169±3	170±4	170±6	170±4	171±4	0.362	0.899	0.558
Load$_{\mathrm{max}}$ (deg)	143±5	143±5	145±5	146±6	145±5	145±5	0.221	0.461	0.304
Flex$_{\mathrm{max}}$ (deg)	89±8	88±7	93±7	91±8	94±9	90±7	0.073	0.142	0.884

All values are means ± s.d.

M_{b}, body mass; CoT, cost of transport; RER, respiratory exchange ratio; t_{c}, contact time; t_{a}, aerial time; FPI, footprint index, CoM: centre of mass; GRF, maximal vertical ground reaction force; W_{ext}, external mechanical work; k_{vert}, vertical stiffness; Ext$_{\mathrm{max}}$, maximal extension joint angle of the knee; Load$_{\mathrm{max}}$, maximal load joint angle; Flex$_{\mathrm{max}}$, maximal flexion joint angle of the knee.

Significance by GLM repeated measures with two factors of the main effects of stage, time (before versus after) and their interaction.

of $\dot{V}_{\mathrm{O2,max}}$; (2) a large fraction ($F$) of $\dot{V}_{\mathrm{O2,max}}$ that can be sustained throughout the competition; and (3) a small value of CoT. Indeed, high correlations have been demonstrated between $\dot{V}_{\mathrm{O2,max}}$ and running performance in groups of runners of quite different abilities (Maughan and Leiper, 1983). Also, in the present study, $\dot{V}_{\mathrm{O2,max}}$ 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 $\dot{V}_{\mathrm{O2,max}}$ are studied, $\dot{V}_{\mathrm{O2,max}}$ becomes a less sensitive predictor of performance, with F and CoT becoming crucial for performance in distance running (Maughan and Leiper, 1983).

Saunders and colleagues (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 P_{max} (r=−0.74), high k_{vert} (r=−0.65) and low FPI (r=0.70), confirming previous studies that underline the role of muscle–tendon complex stiffness in storing and releasing elastic energy (Spurrs et al., 2003). In particular, a low FPI indicates that the trajectory of the foot centre of pressure (CoP) 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 (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 as 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 (Saunders et al., 2004) and also shown 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 also found by Bourdin and colleagues (Bourdin et al., 1995), who showed that W_{ext} could explain a large part of the variation of CoT among subjects at a given velocity.

In addition, Cavanagh and Williams (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 follow are therefore devoted to a discussion of the factors associated with the increase in 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 average, only two biomechanical parameters changed significantly at the end of each stage: FPI and maximal GRF. These results are in line with those of a previous study (Morin et al., 2011) that considered running biomechanics over a 24 h treadmill run and found changes in biomechanics parameters only after 4 h. Kyröläinen and colleagues (Kyröläinen 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, as they observed significant interindividual variations inside the group. This suggests that other parameters, such as the differences of internal work 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 may 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 of 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) have the greatest role (r=0.74) in determining ΔCoT, followed by changes in k_{vert} (Δk_{vert}, r=–0.38) and f (Δf, r=0.32).

A significant increase in FPI observed at the end of each stage underlines a reduction in ankle control and then an increase in ankle instability as shown previously (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 is responsible for about 30% of the overall elastic energy recovery (Ker et al., 1987). This hypothesis is coherent with our results showing that lower FPI was related to higher P_{max}, k_{vert}, k_{tendon} and $\mathbf{F}_{\mathrm{tendon}}$.

In relation to this, it is interesting to note that the k_{tendon} observed in our runners (463 N mm^{-1}, Table 2) is greater than that observed in sedentary subjects (319 N 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 (Table 2) as compared with 73 mm^2 in sedentary subjects (Rosager et al., 2002). Thus, when normalizing k_{tendon} for the corresponding tendon length and CSA, the obtained results (i.e. Young's modulus, 1.07 GPa, Table 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 hypertrophy of the tendon (i.e. to an increased CSA) without any change of its material properties, as shown by the unchanged Young's 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, something that probably did not occur in our subjects.

In addition, the greater $\mathbf{F}_{\mathrm{tendon}}$ was related to greater pennation angle (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) and hence an increased number of sarcomeres in parallel (Abe et al., 1997). Moreover, the observed increase in fibre length, probably enabling the sarcomers to operate closer to optimal length (see Narici and Maganaris, 2007), may be an additional factor contributing to the greater $\mathbf{F}_{\mathrm{tendon}}$. As noted previously (Fletcher et al., 2010), these observations emphasize the importance of the lower limb muscle characteristics in 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 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 $\dot{V}_{\mathrm{O_2,max}}$ and F, and inversely proportional to CoT$_{\mathrm{mean}}$. In particular, we have shown that low CoT values before the race are related to high P_{max} and k_{vert}, and low FPI, f and W_{ext}. Finally, for the first time to our knowledge, we have shown that the increase in CoT during the stages of the competition can be predicted by the change in FPI, which is responsible for about 50% of the change in CoT, which in turn is 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.

MATERIALS AND METHODS

Subjects

Fifteen healthy Caucasian male runners (age range 29–54 years) participated in the ultra-endurance competition '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 with 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 in 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 ± s.d.) 12±5 years, of which 6±3 years involved ultra-endurance running. They reported running on average 75.8±16.8 km week^{-1}. Fifteen athletes who were eligible for the study began the race, and the 11 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 recorded. 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 2 days preceding the test and on a preliminary testing session they were thoroughly familiarized with all the different measurements.

The Magraid competition took place in summer. It consisted of three stages of 25, 55 and 13 km on three consecutive days in the northeast of Italy. The geological texture of the terrain is an unusual soil in comparison with the vast majority of ultra-endurance competitions; it is characterized by gravel (locally named 'Magredi') from the braided river Cellina-Meduna. Stage 1, on the first day, began at 06:00 h with temperature and relative humidity of 26°C and 77%. Stages 2 and 3, on the second and third days, began at 10:00 h 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, M_{b}, CoT, RER, running biomechanics and mechanical work were measured. In addition, f_{H} and GPS coordinates were continuously recorded throughout the three stages (Garmin Forerunner 305 GPS, Kansas City, MO, USA).

Physiological measurements before the race

Anthropometric characteristics and body composition

M_{b} was measured to the nearest 0.1 kg with a manual weighing scale (Seca 709, Hamburg, Germany). Height was measured to the nearest 0.001 m on a standardized wall-mounted height board. Body mass index (BMI) was calculated as M_{b} (kg) × height^{-2} (m). Body composition was measured by bioelectrical impedance (BIA, Human IM Plus, DS Dietosystem, Milan, Italy) according to a previous method (Lukaski et al., 1986). Body composition (fat-free mass and fat mass) 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 lying prone. His right foot was tightened around the adapter of an isokinetic dynamometer (Cybex Norm, CSMi, Stoughton, MA, USA). Straps were also tightened around the hips to prevent forward displacement of the body during maximal plantarflexion. Participants were positioned with the knee fully extended and an ankle angle of −20 deg, 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 plantarflexor torque was obtained by 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 the subject performed maximal isometric plantarflexions and dorsiflexions by pregelled surface EMG electrodes (circular contact area of 1 cm diameter; Biopac Systems Inc., Santa Barbara, CA, 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 a sampling frequency of 2000 Hz and processed with a multichannel analog-to-digital converter (Biopac Systems). 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 centre 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 centre of rotation of the ankle joint to the distal head of the first metatarsal bone. Then, the triceps surae $\mathbf{F}_{\mathrm{tendon}}$ 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 are given elsewhere (Maganaris and Paul, 2000). First, consecutive axial-plane scans were taken along the belly of the GM 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 mid-longitudinal 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, http://www.kinovea.org/).

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 millimetre and considered to be the tendon's original length. The triceps surae tendon CSA 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 versus 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 CSA.

Morphological properties of the GM

The participants lay prone, with the foot secured at −20 deg dorsiflexion. Fibre fascicle length (L) and pennation angle (deg) were measured using a B-mode ultrasound probe (Esaote Biomedica). Images were obtained along the mid-sagittal 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 analysed offline with digitizing software (ImageJ 1.44p, National Institutes of Health, Bethesda, MD, 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 limbs

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 deg to the horizontal. The subject, sitting on a seat that is fixed to a carriage which is free to move on the rail, accelerates himself and the carriage seat backward by pushing on two force platforms (PA 300, Laumas, Parma, Italy). The velocity along the direction of motion is continuously recorded by a wire tachometer (SGI, Lika Electronic, Vicenza, Italy). The analog outputs of the force and velocity transducers are digitized and recorded by a data acquisition system (MP 100, Biopac). The subjects were asked to perform four all-out efforts with the right leg and four with the left leg, starting from the same knee angle (110 deg). The requested starting knee angle was obtained by adjusting the position of the mechanical blocks, which also prevented the carriage seat from moving 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 ($\mathbf{F}$, N) multiplied by the backward speed (v, m s^{-1}):

$$P(t) = \mathbf{F}(t) \times v(t)\,. \tag{6}$$

Analysis of the time course of P allowed us to assess its peak (P_{max}, W).

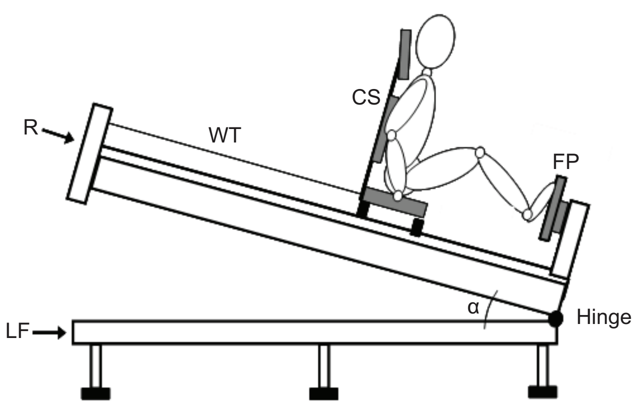

Fig. 1. Schematic view of the explosive ergometer. WT, wire tachometer; CS, carriage seat; FP, force platform. The rail system (R) and lower frame (LF) are hinged.

Graded exercise test to exhaustion

$\dot{V}_{O_2,max}$ and $f_{H,max}$ were determined by a graded exercise test on a treadmill (Saturn, HP Cosmos, Nußdorf, Germany) under medical supervision. During the experiment, ventilatory and gas exchange responses were measured continuously with a metabolic unit (Quark-b^2, Cosmed, Rome, Italy). The volume and gas analysers were calibrated using a 3 l calibration syringe and calibration gas (16.0% O_2: 4.0% CO_2), respectively. During the tests, electrocardiogram was continuously recorded and displayed online for visual monitoring, and f_H was measured with a dedicated device (Polar, Kempele, Finland). The tests were performed 1 week before the race and comprised a 5 min rest period followed by running at 10 km h^{-1} for 5 min (on a slope of 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 1 or 2 min of the exercise test indicating that $\dot{V}_{O_2,max}$ had been attained. $\dot{V}_{O_2,max}$ and $f_{H,max}$ were calculated as the average oxygen uptake and f_H of the last 20 s of the test. RER was calculated as $\dot{V}_{O_2,max}\times\dot{V}_{O_2}{}^{-1}$. The gas exchange threshold was determined by the V-slope method (Beaver et al., 1986).

Metabolic CoT 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, Isny, Germany) at a constant speed of 10 km 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 running surface. The treadmill was connected to a personal computer integrated with running analysis software (Win FDM-T, v 2.1.1. Zebris Medical) yielding contact (t_c, s) and aerial (t_a, s) times at a sampling rate of 100 Hz; duty factor (%), obtained by dividing t_c 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 CoP with respect to the foot axis as a function of time during the stance phase used elsewhere (Willems et al., 2005; Huang et al., 2011); it was obtained from the area between the foot axis (a line connecting the centre of the heel to the midpoint of the second and third 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 indicates that the trajectory of the CoP remains close to the foot axis; higher values indicate 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 analysed and FPI was calculated by means of a custom-written Matlab program.

CoT and RER were measured continuously with a metabolic unit (Quark-b^2, Cosmed), as follows. The analyser, 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 $\dot{V}_{O_2}$, f_H 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 (ml O_2 kg^{-1} s^{-1}) by speed (m s^{-1}). As mentioned in the Introduction, CoT in running, in the range of speeds where air resistance is negligible (i.e. <18 km h^{-1}), is independent of speed. Hence, the 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 frames s^{-1} (Pilot, Basler, Ahrensburg, Germany). The video sequences were recorded between the fourth and fifth minute because 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 from each other, and were calibrated using a square frame (1×1 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 (Simi Reality Motion System, Max-Planck-Straße, Unterschleißheim, Germany) and the three-dimensional position of each marker was reconstructed.

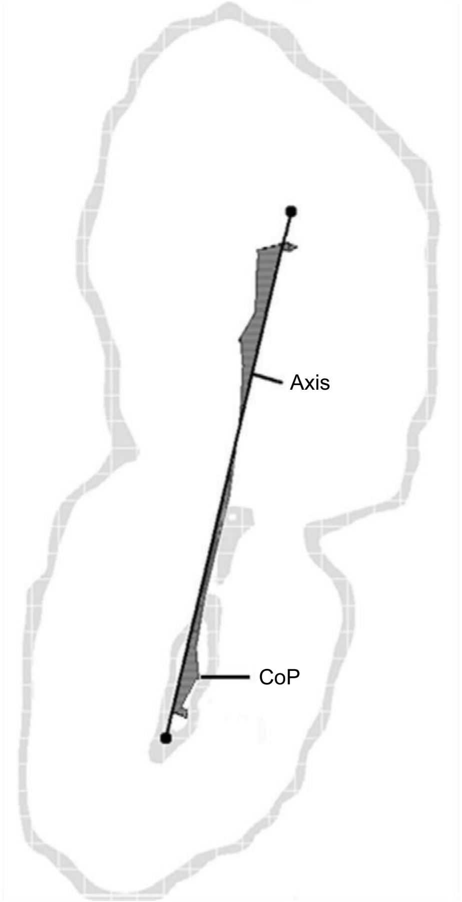

Fig. 2. Footprint index (FPI). The thick line is the foot axis; the thin line is the trajectory of the centre of pressure (CoP) during stance phase. The shaded area between the CoP and foot axis (cm^2) is the FPI.

The data were smoothed through a moving-average filter (radius=1) and the position of the 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 analysed by means of a custom-written Matlab program. External mechanical work was calculated from the positive variations of total mechanical energy (potential and kinetic) of CoM, as described previously (Cavagna et al., 1976). Total external mechanical work per unit of distance was then calculated as $W_{ext,tot}/d$ (ml O_2 kg^{-1} m^{-1}), where d is the distance travelled during the analysed steps. Mass-specific external mechanical work per unit distance W_{ext} (ml O_2 kg^{-1} m^{-1}) was then calculated by dividing $W_{ext,tot}/d$ by the M_b 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 10 steps, stride cycle (between two consecutive heel strikes of the left foot) was analysed. The joint angles of the knee were also measured at maximal extension (Ext$_{max}$, deg), maximal load (Load$_{max}$, deg) and maximal flexion (Flex$_{max}$, deg).

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 s.d. Changes of M_b, CoT, RER and biomechanical parameters during the race were studied with general linear model (GLM) repeated measures with two factors considering chronic stage effect (called 'Stage': stage 1 versus stage 2 versus stage 3) and the acute stage effect (called 'Time': before versus after). When significant differences were found, a Bonferroni *post hoc* test was used to determine the exact location of the difference.

Eqn 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 ($\mathrm{CoT_{mean}}$) was estimated as follows:

$$\begin{aligned}\mathrm{CoT_{mean}} = \{&[(\mathrm{CoT_{Ib}} + \mathrm{CoT_{Ia}}) \times 0.5 \times d_{\mathrm{I}}] \\ &+ [(\mathrm{CoT_{IIb}} + \mathrm{CoT_{IIa}}) \times 0.5 \times d_{\mathrm{II}}] \\ &+ [(\mathrm{CoT_{IIIb}} + \mathrm{CoT_{IIIa}}) \times 0.5 \times d_{\mathrm{III}}]\} \times (d_{\mathrm{I}} + d_{\mathrm{II}} + d_{\mathrm{III}})^{-1}\ , \quad (7)\end{aligned}$$

where the suffixes 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, and the distances of the stages are indicate by *d* (d_{I}=25,000 m; $\mathrm{d_{II}}$=50,000 m; $\mathrm{d_{III}}$=13,000 m). Therefore, applying Eqn 1 to the overall competition and taking into account the average value of $\mathrm{CoT_{mean}}$ from Eqn 7, one obtains:

$$v_{\mathrm{end,mean}} = \mathbf{F} \times \dot{V}_{\mathrm{O_2,max}} \times \mathrm{CoT_{mean}}^{-1}\ . \quad (8)$$

In turn, **F** was estimated from the mean f_{H} determined throughout each individual stage and expressed as a fraction of the corresponding maximal f_{H}. It should be noted that **F** may be better predicted by considering the ratio of the mean f_{H} increase above resting, throughout each stage, to the f_{H} reserve (f_{H} reserve $= f_{\mathrm{max}} - f_{\mathrm{rest}}$). However, in view of the fact that resting f_{H} 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 Eqn 8 was evaluated with a simple linear regression determined between each individual variable and endurance speed; Pearson's correlation coefficients were used to analyse the association between variables. Subsequently, in order to calculate multiple regression coefficients of all three factors combined as in Eqn 8, it is convenient to use the logarithmic transformation:

$$\begin{aligned}\log v_{\mathrm{end,mean}} &= \log(F \times \dot{V}_{\mathrm{O_2,max}} \times \mathrm{CoT_{mean}}^{-1}) \\ &= \log F + \log \dot{V}_{\mathrm{O_2,max}} - \log \mathrm{CoT_{mean}}\ . \quad (9)\end{aligned}$$

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.

Acknowledgements

We are grateful to the athletes for their kind collaboration, to A. Iossa, P. Tedeschi and the A.s.d. Triathlon Team – Pordenone, Italy, for their valuable contribution in carrying out measurements during the race. We are grateful to Dr S. Poser, M. Viotto and M. Conforto for their kind assistance during the study.

Competing interests

The authors declare no competing financial interests.

Author contributions

All authors participated in the design of the experimental protocol and in the discussion of results. S.L., P.T., D.S., E.R., B.S., M.V.N., A.B. and N.G. performed most of the measurements and data analyses. S.L. also drafted and revised the manuscript. G.A., B.G. and R.P. recruited volunteers and carried out medical supervision. P.E.d.P. was responsible for all stages of the study, participated in measurements and interpretation of results, and revised the manuscript.

Funding

The financial support of the Lions Club, Udine-Duomo, Italy, is gratefully acknowledged.

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-S14.

Arampatzis, A., De Monte, G., Karamanidis, K., Morey-Klapsing, G., Stafilidis, S. and Brüggemann, G. P. (2006). Influence of the muscle–tendon unit's mechanical and morphological properties on running economy. *J. Exp. Biol.* **209**, 3345-3357.

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**, 1291-1295.

Beaver, W. L., Wasserman, K. and Whipp, B. J. (1986). A new method for detecting anaerobic threshold by gas exchange. *J. Appl. Physiol.* **60**, 2020-2027.

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 305-306.

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**, 2078-2085.

Bramble, D. M. and Lieberman, D. E. (2004). Endurance running and the evolution of *Homo*. *Nature* **432**, 345-352.

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**, 385-389.

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

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**, 30-35.

Daniels, J. T. (1985). A physiologist's view of running economy. *Med. Sci. Sports Exerc.* **17**, 332-338.

Davies, C. T. and Thompson, M. W. (1986). Physiological responses to prolonged exercise in ultramarathon athletes. *J. Appl. Physiol.* **61**, 611-617.

di Prampero, P. E. (2003). Factors limiting maximal performance in humans. *Eur. J. Appl. Physiol.* **90**, 420-429.

di Prampero, P. E., Atchou, G., Brückner, J. C. and Moia, C. (1986). The energetics of endurance running. *Eur. J. Appl. Physiol. Occup. Physiol.* **55**, 259-266.

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**, 1037-1046.

Gimenez, P., Kerhervé, H., Messonnier, L. A., Féasson, L. and Millet, G. Y. (2013). Changes in the energy cost of running during a 24-h treadmill exercise. *Med. Sci. Sports Exerc.* **45**, 1807-1813.

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**, 831-838.

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-e467.

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**, 321-327.

Karamanidis, K., Arampatzis, A. and Brüggemann, G. P. (2003). Symmetry and reproducibility of kinematic parameters during various running techniques. *Med. Sci. Sports Exerc.* **35**, 1009-1016.

Kawakami, Y., Abe, T. and Fukunaga, T. (1993). Muscle-fiber pennation angles are greater in hypertrophied than in normal muscles. *J. Appl. Physiol.* **74**, 2740-2744.

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**, 147-149.

Kyröläinen, 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**, 224-228.

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**, 1709-1715.

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**, 1768-1775.

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**, 662-673.

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**, 1327-1332.

Maganaris, C. N. (2002). Tensile properties of *in vivo* human tendinous tissue. *J. Biomech.* **35**, 1019-1027.

Maganaris, C. N. (2003). Force-length characteristics of the *in vivo* human gastrocnemius muscle. *Clin. Anat.* **16**, 215-223.

Maganaris, C. N. and Paul, J. P. (2000). In vivo human tendinous tissue stretch upon maximum muscle force generation. *J. Biomech.* **33**, 1453-1459.

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**, 277-288.

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**, 211-223.

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**, 80-87.

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**, 507-509.

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**, 829-836.

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**, 469-474.

Myers, M. J. and Steudel, K. (1985). Effect of limb mass and its distribution on the energetic cost of running. *J. Exp. Biol.* **116**, 363-373.

Narici, M. V. and Maganaris, C. N. (2007). Plasticity of the muscle-tendon complex with disuse and aging. *Exerc. Sport Sci. Rev.* **35**, 126-134.

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**, 287-297.

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**, 90-98.

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**, 465-485.

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**, 202-209.

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.

Støren, O., Helgerud, J., Støa, E. M. and Hoff, J. (2008). Maximal strength training improves running economy in distance runners. *Med. Sci. Sports Exerc.* **40**, 1087-1092.

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**, 4027-4033.

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**, 379-387.