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Physiological and biological factors associated with a 24 h treadmill ultra-marathon performance

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The purpose of this study was to examine the physiological and biological factors associated with ultra-endurance performance. Fourteen male runners volunteered to run on a treadmill as many kilometers as possible over a 24-h period (24TR). Maximal oxygen uptake ($\dot{V}O_{2max}$), velocity associated with $\dot{V}O_{2max}$ ($V_{\dot{V}O_{2max}}$) and running economy (RE) at 8 km/h were measured. A muscle biopsy was also performed in the *vastus lateralis* muscle. The subjects ran 149.2 ± 15.7 km in 18 h 39 ± 41 min of effective attendance on the treadmill, corresponding to 39.4 ± 4.2% of $V_{\dot{V}O_{2max}}$. Standard multiple-regression analysis showed that performance was significantly ($R^2 = 0.82$; $P = 0.005$) related to $\dot{V}O_{2max}$ and specific endurance, i.e. the average speed sustained over the 24TR expressed in $\%V_{\dot{V}O_{2max}}$. $\dot{V}O_{2max}$ was associated with a high capillary tortuosity ($R^2 = 0.66$; $P = 0.01$). Specific endurance was significantly related to RE and citrate synthase activity. It is concluded that a high $\dot{V}O_{2max}$ and an associated developed capillary network are essential for ultra-endurance running performance. The ability to maintain a high $\%\dot{V}O_{2max}$ over a 24TR is another factor associated with performance and is mainly related to RE and high mitochondrial oxidative capacity in the *vastus lateralis*.

Ultra-endurance exercise can be performed in different activities such as cycling, running or multi-sports events (e.g. Ironman triathlon, adventure races). However, a consensus on the definition of an ultra-endurance exercise does not exist. For some authors, it applies to events that exceed 4 h (Knez et al., 2006) or 6 h in duration (Zaryski & Smith, 2005) and can last up to 40 h or even several days (e.g. 6-day races). In running, it is sometimes considered that it is relevant for a distance greater than a marathon and there are basically two types of ultra-marathon events: those performed on a mostly flat road (24 h, 100 km) and those run on various terrain trails (e.g. Western States 100 miles, Trail de Tiranges or Ultra-trail du Mont-Blanc). The present study focused on the first type of ultra-marathon events.

In marathon running, it is understood that maximal oxygen uptake ($\dot{V}O_{2max}$), running economy (RE), biomechanical parameters and fractional utilization of maximal oxygen uptake ($\%\dot{V}O_{2max}$) are important factors of performance (Sjodin & Svedenhag, 1985; Joyner & Coyle, 2008). Despite the fact that the number of athletes competing in ultra-endurance events is continually increasing as evidenced by the number of new races being established each year (Knez et al., 2006), the determining factors of ultra-marathon performance are poorly identified. In fact, most studies dedicated to such events have focused on injuries and muscle damage (Rehrer et al., 1992; Holtzhausen et al., 1994; Kim et al., 2007), biochemical changes following a race (Noakes & Carter, 1982; Overgaard et al., 2002) or neuromuscular fatigue (Millet et al., 2002; Place et al., 2004).

Only two studies have been dedicated to the characterization of physiological parameters linked to ultra-endurance performance. Davies and Thompson (1979a) suggested that success in an 84-km ultra-marathon was crucially and (possibly) solely dependent on the development and utilization of a large $\dot{V}O_{2max}$. Since then, only Noakes et al. (1990) have compared the factors related to marathon and ultra-marathon performance. They found that peak treadmill running velocity reached during an incremental test was the best laboratory-measured predictor of performance in ultra-marathon specialists but significant relationships were also found with running velocity at the lactate inflection point $\%\dot{V}O_{2max}$ at 16 km/h and $\dot{V}O_{2max}$.

These two studies have tested aerobic parameters as potential factors of ultra-marathon performance; however, the relationships of muscle characteristics with ultra-marathon performance are not known. In addition, factors associated with performance on distances >100 km have never been investigated. While it has been argued that a small improvement in RE could have a large impact on distance-running performance, particularly in events such as ultra-marathon events (Jung, 2003), only Noakes et al. (1990) have tested this hypothesis in ultra-marathon runners. They found that RE, determined as $\dot{V}O_2$ at 16 km/h, was not correlated with performance. The objective of the present study was to determine which physiological and biological factors were related to ultra-endurance performance in a heterogeneous group of runners during a 24-h treadmill run (24TR).

Materials and methods

Subjects

Fourteen male volunteers (mean ± SD: age 41.1 ± 8.9 years; weight 73.6 ± 8.2 kg, height 176.9 ± 5.8 cm, body mass index (BMI) 23.5 ± 1.9 kg/m^2 and body fat: 17.7 ± 4.3%) participated in this experiment. The details of the skin-fold thickness are as follows: 7.1 ± 2.6 mm for tricipital fold, 11.3 ± 4.8 mm for the inter scapulo-vertebral fold, 3.9 ± 0.9 mm for the bicipital fold and 12.7 ± 6.9 mm for the supra-iliac fold. Further individual characteristics of the subjects are presented in Table 1. They were recruited among experienced ultra-endurance runners and all of them had run at least a race longer than 24 h or >100 km. On average, they had 15.3 ± 7.1 years of training history in running and 7.1 ± 4.4 years of ultra-endurance experience. They reported to run an average of 80.5 ± 11.7 km/week. Written informed consent was obtained from the subjects. The study was conducted according to the Declaration of Helsinki. The protocol has been approved by the local ethics committee (Comité de Protection des Personnes Sud-Est 1, France) and registered in http://clinicaltrial.gov (# NCT 00428779). Among the 14 subjects, 12 were able to complete the 24TR. One subject was excluded by the physician because of a hematoma due to the initial muscle biopsy procedure and the other one was excluded because of low blood pressure.

Procedures

The subjects visited the laboratory twice, with each session separated by 3–4 weeks. All subjects had run at least three times on a motorized treadmill before taking part in the experiment. In the first session, body mass (BM), height and percentage of body fat (%BF, measurements of skin-fold thickness) were measured. The subjects then performed a maximal test on a motorized treadmill (Gymrol S2500, HEF Tecmachine, Andrezieux-Boutheon, France), which aimed at determining the anaerobic threshold, $\dot{V}O_{2max}$ and the velocity associated with $\dot{V}O_{2max}$ ($V_{\dot{V}O_{2max}}$). The initial velocity was set at 10 km/h and the first stage lasted 6 min. Then, the test consisted of a maximal discontinuous incremental test (slope = 0%), where the speed was progressively increased by 1.5 km/h every 3 min, followed by 1 min of rest for the collection of blood samples from the finger tips. The last stage entirely completed was considered as $V_{\dot{V}O_{2max}}$. The highest $\dot{V}O_2$ value was considered as $\dot{V}O_{2max}$.

The second session consisted of the 24TR. About 2 h before starting, a muscle biopsy was performed. Muscle biopsy samples (~ 120 mg) were collected under local anesthesia from the superficial portion of the left *vastus lateralis* muscle

Table 1. Individual performances and the main physiological characteristics of the subjects

Subject #	Performance 24TR (km)	$\dot{V}O_{2max}$ (mL/kg/min)	$V_{\dot{V}O_{2max}}$ (km/h)	$\%V_{\dot{V}O_{2max}}$ (velocity during effective running time)	$\%V_{\dot{V}O_{2max}}$ (absolute mean velocity)	$V_{4\,mmol}$ ($\%V_{\dot{V}O_{2max}}$)	RE (mL/kg/min)
4	172.6	54.1	19	44	38	79	28.7
12	171	60.9	20.5	40	35	87	28.5
5	164.5	58	19	39	36	89	26.4
1	157.7	–	–	–	–	–	29.8
10	156.4	53.1	19	38	34	86	29.3
6	153.8	56.2	19	37	34	86	29.4
14	143.1	54.8	19	40	31	92	29.5
7	142.4	41.7	16	49	37	86	24
3	136	54.7	19	33	30	91	30
11	135.2	50.9	16	38	35	94	28.1
13	129.9	46.8	17.5	38	31	87	30.8
2	127.6	41.4	16	37	33	82	29.1
8	DNF	50.8	17.5	/	/	85	30.8
9	DNF	57.8	20.5	/	/	79	26.6
X	149.2	52.4	18.3	39.4	34.0	86	28.6
SD	15.7	6.0	1.6	4.2	2.6	4	1.9

24TR, 24 h treadmill running; DNF, did not finish; $V_{\dot{V}O_{2max}}$, velocity associated with $\dot{V}O_{2max}$; $\%V_{\dot{V}O_{2max}}$, velocity over the 24TR in percentage of velocity associated with $\dot{V}O_{2max}$, the velocity being expressed either as the mean velocity during the time spent running or walking on the treadmill or in absolute values (i.e. distance/24 h); $V_{4\,mmol}$, velocity at blood lactate concentration equal to 4 mmol/L (in $\%V_{\dot{V}O_{2max}}$); RE, running economy = $\dot{V}O_2$ at 8 km/h.

using a percutaneous technique (Henriksson, 1979). A large well-organized fascicle of fibers was oriented and included in an embedding medium (Cryomount; Histolab, Göteborg, Sweden), frozen in isopentane, cooled to its freezing point in liquid nitrogen and stored in liquid nitrogen until further cryostat sectioning. The remaining pieces of the sample were rapidly frozen and stored in liquid nitrogen until enzyme activity and protein analyses were performed.

After the muscle biopsy procedure, the subjects rested for approximately 2 h and then they were asked to start the 24TR. Ten minutes after the start of the 24TR, subjects were asked to run 4 min at 8 km/h for measurements of RE. A slow speed was chosen for RE because the average speed over the 24TR was 7.9 ± 1.0 km/h, and so 8 km/h is representative of the pace over such an extreme exercise. The food and water intakes during the 24TR were managed by the subjects as in a normal race.

Gas exchange measurements

Oxygen uptake ($\dot{V}O_2$) and related gas exchange measures were determined during the last minute of each 3-min (maximal test, first session) or 4-min (submaximal run, 24TR) period as presented elsewhere (Foissac et al., 2008).

Lactate concentration

During the first session, the blood lactate concentration ($[La^-]$) was determined at rest and after each stage. A blood sample was taken from the fingertip to analyze $[La^-]$ using an enzymatic method (YSI 1500 Sport, Yellow Springs Instruments, Yellow Springs, Ohio, USA). The blood lactate analyzer was calibrated before each test using standard solutions of 5 and 15 mmol/L. The speeds at a $[La^-]$ of 2 and 4 mmol/L ($V_{2\,mmol}$ and $V_{4\,mmol}$) and the speed that corresponds to the first increase in $[La^-]$ of 1 mmol/L ($V_{\Delta 1\,mmol}$) above the resting level were considered and expressed in km/h and in $\%V_{\dot{V}O_{2max}}$.

Muscle biology

Enzyme activity assays

Muscle biopsy samples (approximately 15 mg) were freeze-dried (Lyovac GT2, Leybold-Heraeus, Köln, Germany), dissected free from the connective tissue and blood and powdered in a chamber of controlled humidity (<40% RH). The muscle powder was weighted in the same chamber and homogenized at 4 °C in an appropriate extraction buffer. This tissue suspension was used to measure the maximal activity of phosphofructokinase (PFK), citrate synthase (CS) and β-hydroxy-acyl-CoA-deshydrogenase (HAD) fluorometrically (Mansour et al., 1966). All enzyme activities were measured at 25 °C and expressed as μmol/min/g dry weight.

Immunocytochemical and histochemical assays

Serial transverse muscle biopsy sections, 10 μm thick, were cut using a microtome at −20 °C (HM 560, Microm, Walldorf, Germany). Muscle sections were viewed under a light microscope (Eclipse E400, Nikon, Badhoevedorp, the Netherlands) connected to a digital camera (Coolpix 990, Nikon). All the subsequent measurements were performed under blinding procedures. Photographs were taken at a ×400 magnification and analyzed using an image analysis software (SigmaScan Pro 5.0, SPSS Science, Chicago, Illinois, USA). An average of eight fields was examined in each section, yielding an average of 70 fibers per individual sample.

Muscle capillarization

The capillaries were visualized using the monoclonal antibody (mAb) CD31 (Dako, Glostrup, Denmark) that recognizes vascular endothelial cells (for details, see Charifi et al., 2004). Transverse or longitudinal vascular profiles with a diameter >15 μm were not considered. Capillary density (CD) was expressed as the number of capillaries counted per square millimeter (cap/mm^2). For each fiber, the capillary-to-fiber ratio (C/F) was determined as the sum of the fractional contribution of each capillary around a fiber, based on the number of fibers sharing each capillary (sharing factor). On transverse sections, the length of the contact (LC) between the capillaries and the muscle fibers can be measured. The index of tortuosity was calculated as LC/PF (perimeter of fiber). LC/PF, originally called "length of capillary-fiber contact", is expressed as a percent of muscle fiber perimeter in contact with the capillary wall (Charifi et al., 2004).

Muscle fiber distribution

Fiber-type distribution was studied on immunohistochemical serial preparations using anti-fast IIa myosin heavy-chain N2.261 and anti-slow myosin heavy-chain A4.951 monoclonal antibodies (Alexis Biochemicals, San Diego, California, USA). The fibers were designated as I, I–IIa, IIa, IIa–IIx and IIx types.

Statistical analysis

Values are presented as mean ± SD throughout the manuscript. The coefficient of variation (CV = SD/mean) was calculated for the 24TR performance. Data were screened for normality of distribution and homogeneity of variances. Standard multiple-regression analyses were conducted to evaluate (i) the contribution of $\dot{V}O_{2max}$, $\%V_{\dot{V}O_{2max}}$ sustained over 24TR and RE to the performance and (ii) the contribution of anaerobic threshold, RE and percentage of type I fibers to the $\%V_{\dot{V}O_{2max}}$ sustained over 24TR. A predictor variable was considered as a significant contributor to performance or $\%V_{\dot{V}O_{2max}}$ if its P value from the regression was <0.05. Pearson's correlation coefficients (R) or Spearman's rank correlation coefficient (R_s) were also used to analyze the association between various other selected variables. A P value of 0.05 was also accepted as the level of statistical significance.

Results

Performance

The performance of the 12 subjects who finished the 24TR is reported in Table 1. The average speed was 39 ± 4% of $V_{\dot{V}O_{2max}}$ when considering the effective running time, i.e. the real time spent running or walking on the treadmill of each subject (18 h 39 min 41 min, Table 1). The average speed was 34 ± 3% of $V_{\dot{V}O_{2max}}$ when considering the mean velocity over the 24TR, i.e. the number of kilometers divided by 24 h (Table 1). The coefficient of variation for the 24TR performance was 10%.

Physiological variables

Means ± SD, as well as individual values of $\dot{V}O_{2max}$, $V_{\dot{V}O_{2max}}$, $V_{4\,mmol}$ and RE at 8 km/h are presented in Table 1. For clarity, the velocities associated with the lactate inflection point and $V_{\Delta 1\,mmol}$ were not presented in this table but these two parameters were highly correlated with $V_{4\,mmol}$ ($R = 0.89$ and 0.82, respectively; $P < 0.001$). Blood glucose concentration, hematocrit and body mass over the 24TR are presented in Table 2. Standard multiple-regression analyses ($R^2 = 0.82$; $P = 0.005$) revealed that performances were predicted by $\dot{V}O_{2max}$ ($P = 0.001$) and specific endurance, i.e. the average speed sustained over the 24TR expressed in $\%V_{\dot{V}O_{2max}}$ ($P < 0.05$). The $\%V_{\dot{V}O_{2max}}$ sustained over 24TR was predicted by RE ($P < 0.05$) but not by the percentage of type I fibers or by the anaerobic threshold. No correlation existed between the average speed sustained over the 24TR (in $\%V_{\dot{V}O_{2max}}$) and the $V_{2\,mmol}$, $V_{4\,mmol}$ and $V_{\Delta 1\,mmol}$, expressed either in absolute (km/h) or relative ($\%V_{\dot{V}O_{2max}}$) values. There was a tendency toward a relationship between $\dot{V}O_{2max}$ expressed in $mL/min/kg^{fat\text{-}free\ \ mass-1}$ ($R = 0.58$; $P = 0.06$) and performance. The correlation between $\dot{V}O_{2max}$ and %BF was significant ($R_s = -0.65$; $P = 0.015$).

Biological variables

The CD, C/F and LC/PF were 308 ± 74 cap/mm^2, 2.14 ± 0.33 capillaries per fiber and 0.17 ± 0.02, respectively. C/F and LC/PF were significantly correlated with 24TR performance ($R = 0.62$, $P < 0.05$ and $R = 0.58$; $P < 0.05$). There was a significant correlation between $\dot{V}O_{2max}$ and LC/PF ($R = 0.82$; $P = 0.01$; Fig. 1). No correlation was found between the percentage of type I fibers and performance (Fig. 2) or $\dot{V}O_{2max}$.

CS and HAD activities were significantly correlated with 24TR performance ($R = 0.70$; $P = 0.015$ and $R = 0.72$; $P = 0.012$, respectively; Fig. 3). $\%V_{\dot{V}O_{2max}}$ sustained over 24 h was negatively correlated with PFK activity ($R = -0.68$; $P < 0.05$) and positively correlated with CS activity ($R = 0.65$; $P < 0.05$). While CS and HAD were highly correlated ($R = 0.80$; $P = 0.001$), $\%V_{\dot{V}O_{2max}}$ sustained over 24TR was not related to HAD activity ($R = 0.34$, *NS*).

Discussion

The main results of the present study were that (i) a high maximal aerobic power and its associated developed capillary network and (ii) a high percent $\dot{V}O_{2max}$ maintained over a 24-hour run are related to ultra-endurance running performance. These results suggest that performance in ultra-marathon running is similar to that in shorter distances like 10 km or marathon in that maximal aerobic power and the ability to maintain a high $\%\dot{V}O_{2max}$ throughout the event are major variables associated with performance. The latter factor appears to be at least partly related to the running efficiency and a high CS activity but not to the anaerobic threshold.

Performance

The subjects in the current experiment ran on average ~19 h as compared with the expected 24 h; this was mainly due to the number of mandatory measurements, such as neuromuscular measurements, blood samples or cognitive function assessment, requested every 4 h (data not shown). As a result, it can be estimated that the distance (approximately 149 km) is comparable with previous field studies where participants ran mean distances of 159–163 km (Wu et al., 2004; Ohta et al., 2005) over a 24-h race. The mandatory stops during the experiment were comparable among subjects so that it can be

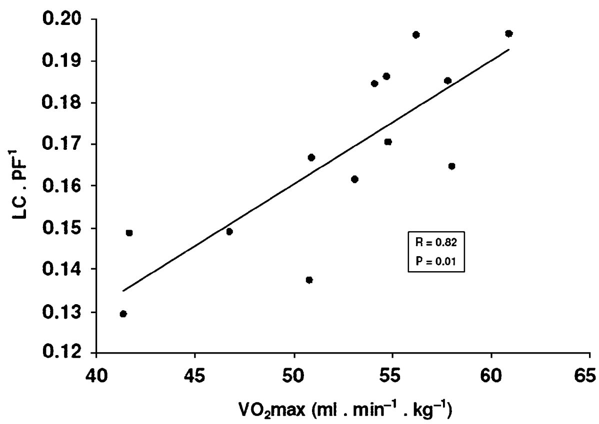

Fig. 1. Relationship between the maximal oxygen uptake ($\dot{V}O_{2max}$) and the length of capillary-fiber contact (LC/PF).

Table 2. Hematocrit, blood glucose and body mass over the 24TR

	PRE	4 h	8 h	12 h	16 h	20 h	Post
Hematocrit (%)	46.5 ± 4.9	45.5 ± 3.3	45.5 ± 4.2	44.9 ± 4.1	44.6 ± 4.7	43.9 ± 4.3	43.8 ± 4.8
Blood glucose (mmol/L)	5.2 ± 0.6	6.1 ± 1.2	6.0 ± 0.9	5.5 ± 1.1	5.9 ± 1.2	5.5 ± 1.0	5. 7 ± 0.5
Body mass (kg)	76.0 ± 7.0	74.8 ± 6.9	74.6 ± 6.7	74.6 ± 6.6	74.4 ± 6.6	74.3 ± 6.6	74.2 ± 6.6

Values are mean ± SD.
24TR, 24-h treadmill run.

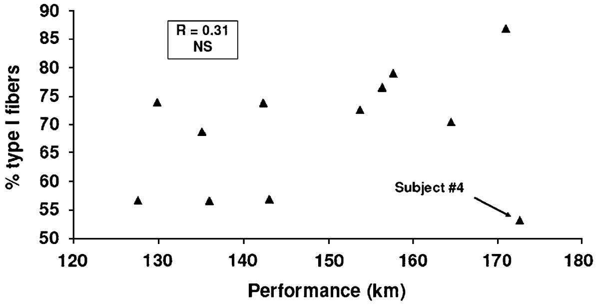

Fig. 2. Relationship between the percentage of type I fibers and the performance over the 24-h treadmill run (24TR).

considered that the difference in running time among subjects was primarily due to the subjects' willingness to stay on the treadmill as much as they could (e.g. some subjects were able to eat while walking on the treadmill, and some preferred to stop to eat). The subjects were allowed to stop at their convenience but were requested to perform their best. Thus, even if field studies that examine performance during a competition are needed, because motivation is probably higher during a real race, the subjects of the present study can be considered highly motivated (half of the subjects traveled twice 500–1300 km to participate) and prepared for the 24TR as if it was their first race of the season.

Physiological variables

The importance of a high $\dot{V}O_{2max}$ for performance in long-distance events has been recognized for decades. Previous research has shown that in heterogeneous groups, coefficients of correlation between performance in marathon running and $\dot{V}O_{2max}$ ranged between 0.63 and 0.91 (Sjodin & Svedenhag, 1985; Loftin et al., 2007), which is comparable with the value found in the present study. Such relationships are more contradictory when focusing on a more homogenous group of elite athletes (Billat et al., 2001; Legaz Arrese et al., 2006). Only two studies have focused on the relationship between the physiological characteristics and ultra-marathon performance (Davies & Thompson, 1979a; Noakes et al., 1990), but both studies focused on ultra-marathon events shorter than or equal to 90 km. In these two experiments, maximal aerobic power (expressed either as $\dot{V}O_{2max}$ or $V_{\dot{V}O_{2max}}$) was the best laboratory-measured predictor of performance in ultra-marathon specialists. While it has been suggested that $\dot{V}O_{2max}$ is of less importance when the running distance increases (Davies & Thompson, 1979b; Sjodin & Svedenhag, 1985), the present results are thus in agreement with the experiments of Davies and Thompson (1979a) and Noakes et al. (1990), since a correlation as high as 0.72 has been found

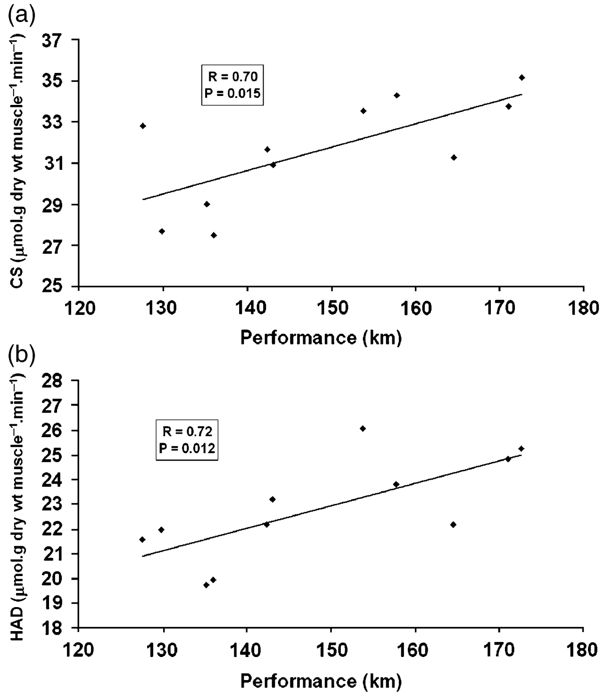

Fig. 3. Relationship between (i) the performance over the 24-h treadmill run (24TR) and (ii) the citrate synthase (CS, a) or the maximal activity of β-hydroxy-acyl-CoA-deshydrogenase (HAD, b).

between $V_{\dot{V}O_{2max}}$ and performance. This demonstrates that maximal aerobic power is still associated with performance in ultra-endurance events, even in events lasting as long as 24 h. It can be suggested that an elevated $\dot{V}O_{2max}$ is interesting for performance in ultra-marathon because the metabolic state of the runner (e.g. balance of lipid and carbohydrate utilization, muscle metabolism by-products) is determined by the relative intensity, i.e. the $\%\dot{V}O_{2max}$. Thus, the higher the $\dot{V}O_{2max}$, the easier it is to run at a given submaximal speed. $\dot{V}O_{2max}$ is also negatively affected by body fat and this may also explain the relationship between $\dot{V}O_{2max}$ and performance because a high %BF affects both $\dot{V}O_{2max}$ (when expressed in mL/min/kg) and performance. Both factors probably explained the $\dot{V}O_{2max}$–performance relationship in the present study because (i) there was a strong tendency ($P = 0.06$) toward a relationship between performance and $\dot{V}O_{2max}$ expressed in mL/min/kg$^{\text{fat-free mass}-1}$ and (ii) there was a correlation between $\dot{V}O_{2max}$ and %BF.

Endurance, defined as the reduction in peak aerobic power with the natural logarithm of race duration (Peronnet & Thibault, 1989), was not determined in the present study. However, the specific endurance to ultra-marathon can be appreciated throughout the average speed sustained over the 24TR when expressed in $\%V_{\dot{V}O_{2max}}$. The average value reached 40% of $V_{\dot{V}O_{2max}}$ (range: 33–49%). It has been found that trained athletes can maintain an average of about

45% of $V_{\dot{V}O_{2max}}$ for a 24-h race (Davies & Thompson, 1979a). As a comparison, Davies and Thompson (1979a) reported that the ultra-marathon runners tested in their study were able to sustain 67% $\dot{V}O_{2max}$ (range: 53–76%) over 84 km. Also, the average intensity sustained over a flat course marathon has been found to range between 60% in slow runners and 86% in elite runners (Sjodin & Svedenhag, 1985).

The average speed sustained over the 24TR (in $\%V_{\dot{V}O_{2max}}$) was not correlated to any of the indexes of aerobic and anaerobic threshold, either expressed in absolute values or relative to $V_{\dot{V}O_{2max}}$. As expected, $V_{\dot{V}O_{2max}}$ correlated with $V_{2\,mmol}$, $V_{4\,mmol}$ and $V_{\Delta 1\,mmol}$ when expressed in km/h (all $R \geq 0.81$ and $P \leq 0.001$) but 24TR performance was not correlated with Vmmol, $V_{4\,mmol}$ or $V_{\Delta 1\,mmol}$ when expressed in $\%V_{\dot{V}O_{2max}}$ (all $R<0.30$, NS). More importantly, 24TR performance was not correlated with $V_{4\,mmol}$ and $V_{\Delta 1\,mmol}$ even when expressed in km/h. This result is very different from experiments that have investigated marathon running because correlations as high as 0.94–0.98 have been consistently reported between the velocity at anaerobic threshold and performance (Sjodin & Svedenhag, 1985). Altogether, these results show that indexes of anaerobic threshold are not as important for ultra-endurance performance as they are for events ranging from 10 to 42 km. This result appears to be contradictory to the one observed by Noakes et al. (1990), where a significant relationship was found between performance and running velocity at the lactate inflection point, but it was observed for a much shorter (90 km) ultra-marathon to compare with the present study.

$\dot{V}O_{2max}$ and endurance are only two of the factors that determine success in long-distance running performance. In particular, RE has been found to be an important part of the success in subjects with comparable $\dot{V}O_{2max}$ and $\%\dot{V}O_{2max}$ sustained during competition (Scrimgeour et al., 1986) but conflicting results have also been reported (Sjodin & Svedenhag, 1985). Even if RE can explain performance differences in subjects with similar $\dot{V}O_{2max}$, this relationship does not exist (Davies & Thompson, 1979a) or is weaker than the correlation between performance and $\dot{V}O_{2max}$ in marathon runners (Sjodin & Svedenhag, 1985). In line with previous studies (Davies & Thompson, 1979a; Noakes et al., 1990), no direct relationship was observed between performance and RE in the present study. However, the velocity sustained during the 24TR when expressed in $\%V_{\dot{V}O_{2max}}$ was predicted by RE. This suggests that rather than the "aerobic or anaerobic threshold" indexes, a good RE is essential for endurance in "low-intensity" ultra-endurance events. RE is probably not the only factor implicated in this specific endurance.

Biological variables

The average percentage of type I fibers in the *vastus lateralis* of the subjects who took part in the present experiment almost reached 70%. This value is slightly lower than elite marathon runners but higher than good and slower marathon runners (Sjodin & Svedenhag, 1985), which is a level of performance more comparable with the level of performance of our subjects. However, no correlation was found between the percentage of type I fibers and performance. Surprisingly, the best subject over the 24TR (subject #4) had the lowest % type I fibers (see Fig. 2). It has to be noted that this subject was quite atypical compared with the other participants, and removing him from our analyses would have made the % type I fibers correlate with performance ($R=0.68$; $P<0.05$). In marathon running, significant correlations between % type I fibers and performance have been observed, the advantage of such a typology being attributed to a greater ability to oxidize fat and lactate in this type of fiber (Sjodin & Svedenhag, 1985). While the first factor probably plays a role in ultra-marathon running, as shown by the correlation found in the present study between HAD activity and performance, lactate oxidation is not implied because the intensity is too low. In contrast, it may be hypothesized that a high percentage of slow twitch fibers is an advantage in ultra-marathon running because of the higher ability of these fibers to resist muscle damage (Friden et al., 1983).

Despite his low % type I fibers, subject #4 had a reasonable capillary network so that a significant correlation existed between performance and indexes of capillarization. Contrary to previous studies in marathon runners (Sjodin & Jacobs, 1981), no correlation was found in the present study between CD and 24TR performance ($R=0.46$; NS) but significant relationships were found between 24TR performance and C/F or LC/PF. The latter factor, which was also related to $\dot{V}O_{2max}$ (Fig. 1), represents the percent of muscle fiber perimeter in contact with the wall of the microvessel and takes into account the tortuosity of the vessel. It has been suggested that LC/PF is the best indicator representative of the blood–muscle exchange surface (Charifi et al., 2004). Based on the present results that (i) $\dot{V}O_{2max}$ or performance were not related to the percentage of type I fibers and (ii) relationships were found between $\dot{V}O_{2max}$ (or performance) and LC/PF with no significant relationships between $\dot{V}O_{2max}$ and CD, it can be speculated that ultra-endurance training does not modify the knee extensor muscle typology but improves the blood–muscle exchange surface by increasing capillary tortuosity. Interestingly, CS activity was significantly correlated with performance and $\%V_{\dot{V}O_{2max}}$ sustained over 24 h but not with $\dot{V}O_{2max}$. This suggests that increased CS activity observed with training (Bylund et al., 1977) affects ultra-endurance performance by improvement of low-intensity endurance (i.e. the "specific endurance").

Limits of the study

The main limitation of the present study is that the design was mainly built to study the kinetics of fatigue so that mandatory stops were requested from the subjects. It is unlikely that the "recovery" periods or the neuromuscular or cognitive tests changed the results of the study because they were comparable among subjects but the fact that some subjects took advantage of these short "recovery" periods more than others cannot be ruled out. Nevertheless, because the subjects really did their best, just as in a real race, this experiment represented an excellent opportunity to present some new data relative to factors of performance that are lacking in the literature for that type of extreme exercise. One should keep in mind that some of the measurements performed here would be hard to perform on the field during an actual race. Another limit of the study is the fact that the precision level of the $\dot{V}O_{2max}$ determination was poor because the increment was 1.5 km/h. Finally, it is possible to explain from a physiological point of view why a high $\dot{V}O_{2max}$ is important for ultra-marathon performance but the fact that $\dot{V}O_{2max}$ is statistically associated with performance does not mean that these two variables are causally related.

Conclusion

While central (i.e. cardiac) factors of $\dot{V}O_{2max}$ still have to be investigated in this subject population, this study shows that a high maximal aerobic power, together with its connected capillary network and a high mitochondrial oxidative potential, are associated with ultra-endurance running performance. The present experiment also shows that RE is not directly related to performance but may nevertheless be important to be able to maintain a high $\%\dot{V}O_{2max}$ over an ultra-marathon event. Finally, the anaerobic threshold values do not seem to be associated with performance or specific endurance for this type of exercise.

Perspectives

These results suggest that high-intensity interval training should be conducted to improve ultra-marathon performance, i.e. to develop maximal aerobic power, even for events as long as 24 h. The present data deal with a heterogeneous group of trained ($V_{\dot{V}O_{2max}} = 18.3 \pm 1.6$ km/h) but non-elite runners. It must be understood that the present results can be influenced by (i) whether the investigated group is homogeneous or heterogeneous and (ii) the performance level of subjects. Thus, it still has to be determined whether physiological and biological determinants of performance would be similar in a more homogenous group, particularly in elite ultra-endurance athletes. Also, because it has been suggested that females perform better in ultra-marathon races, further studies are needed to verify whether the results of the present study also apply to females.

Key words: maximal oxygen uptake, muscle typology, capillary network, enzymatic activity, ultra-endurance, anaerobic threshold.

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