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EXERCISE, TEMPERATURE REGULATION

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Training effect on performance, substrate balance and blood lactate concentration at maximal lactate steady state in master endurance-runners

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Abstract Training effects on time-to-exhaustion, substrate and blood lactate balances at the maximal lactate steady state velocity (MLSSv) were examined. Eleven male, veteran, long-distance runners performed three tests before and after 6 weeks of training at MLSSv: an incremental test to determine maximum O_2 uptake ($\dot{V}O_{2,max}$) and the velocity at the lactate threshold (vLT), a sub-maximal test of two stages of 20 min at 95 and 105% of vLT separated by 40 min rest to determine the MLSSv and the corresponding lactate concentration (MLSSc) and a time-to-exhaustion run at MLSSv for which the substrate balance was calculated. Duration and distance run at MLSSv increased dramatically respectively from 44±10 to 63±12 min and from 10.4 to 15.7 km respectively (P<0.01). MLSSv increased significantly with training but the relative fraction of $\dot{V}O_{2,max}$ remained the same (85.2±4.5 vs. 85.3±5.2%, P=0.93). MLSSc was unaffected by training as determined from the percentage of energy yielded by carbohydrates (80%) during the exhaustive run at MLSSv. These findings show that training at MLSS elicits small increases in MLSSv and $\dot{V}O_{2,max}$, but enhances time-to-exhaustion (endurance) at MLSSv substantially (+50%). Training does not change the proportion of carbohydrate oxidized, which is the major substrate used during an exhaustive run at MLSS lasting 1 h.

Keywords Blood lactate · Running · Training · Carbohydrates · Exercise · Cross-over point

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Introduction

The "maximal lactate steady state" is defined as the highest blood lactate concentration (MLSSc) and work load (MLSSw) or velocity (MLSSv) that can be maintained over time without continual blood lactate accumulation [4, 10]. Furthermore, MLSSw is determined by the ability to maintain ATP supply and by the products of glycolysis that may limit oxidative phosphorylation [14]. MLSSw is reportedly sensitive to endurance training [6] in terms of both the absolute and relative maximum O_2 uptake ($\dot{V}O_{2,max}$), since it can switch from 60 and 90% of $\dot{V}O_{2,max}$ [10]. MLSSv is slightly faster than the speed of the marathon run [10] which lasts more than 2 h, but the exact time-to-exhaustion at MLSSv and the effect of training on this time-to-exhaustion has not been examined to date and the time limit for work at this intensity remains unclear.

The limiting factors of exhaustion at MLSSv are also still unknown. Indeed, substrate utilization at this exercise intensity is not well investigated. It is also not clear whether MLSSv corresponds to the speed at which the respiratory exchange ratio (RER) is about 1 [6], and above which energy output comes only from carbohydrates (CHO). Indeed, MLSSv does not indicate a given work load but rather an exercise intensity above which metabolism changes qualitatively [6]. This is a relevant question if we consider that glycogen availability could be modified by training and could affect endurance at MLSSv. Indeed, the corresponding glycogen cost for a 45-min exercise at an MLSSw of 3.4 W kg^{-1} and a working efficiency of 20% depletes muscle and liver glycogen by about 50% [6]. The pattern of substrate utilisation of an individual at any

time depends on the cross-over between the exercise intensity-induced responses that increase CHO utilisation and the endurance training-induced responses that promote lipid oxidation [13]. This "cross-over point" is defined as the power output at which energy derived from oxidation of CHO-based fuels predominates over that derived from lipids [13] and is well below MLSSv, which had also previously been identified as a "cross-over point" but in reference to the accumulation of lactate [29].

MLSSc reportedly varies widely between subjects (2–8 mM in capillary blood) and is independent of performance [6]. However, few studies have focused on the value of the MLSSc and the effect of training thereon. Previous studies have only shown that if the average value is 4 mM, the variability is large (2–7 mM) and that this is linked to the power output per unit muscle mass involved in exercise. To date there are no studies describing the influence of training on MLSSc in individual subjects.

Most reports have shown that endurance training results in muscular biochemical adaptations that enhance lipid oxidation and decrease sympathetic nervous system activity in response to a given absolute exercise intensity [13]. The increasing lactate accumulation represents increased CHO oxidation and, thus, the cross-over to CHO dependency. However, it remains to be determined whether blood lactate concentration or change in blood lactate concentration with training correlates with any modification of the crossover point [13]. Most studies have reported an increased fat combustion compared with before training when exercising at the same absolute intensity. This may be due to the concurrent decrease in relative exercise intensity allowing a higher fat oxidation at the lower relative exercise intensity [13]. At the same relative exercise intensity, the presence of a shift in substrate use towards a higher fat utilisation is less clear [24]. This absence of clear data concerning the effects of training on substrate use at the same relative work rate could be due to the fact that the same relative work load is expressed usually as a percentage of $\dot{V}O_{2,max}$ and not as a percentage of the cross-over point velocity (CROv), which is more representative of the substrate balance than an exercise intensity expressed as a percentage of $\dot{V}O_{2,max}$.

The purpose of this study was thus to clarify the effect of training at MLSSv on the time-to-exhaustion at MLSSv in relation to the possible modifications of substrate balance in master long-distance runners.

Materials and methods

Subjects and Methods

Subjects

Eleven highly experienced, male, veteran, endurance-trained subjects aged 43–51 (48±2.9 years) commenced the study but two withdrew during the study for job-related reasons. The subjects were specialist long-distance runners (best performance for the half-marathon 83±5 min). All the subjects belonged to a running club and had similar training habits. They had trained 11 months a year for 10±3 years, 5 times weekly, only undertaking slow, long-distance runs lasting 65±17 min at 12.4±2.7 km h^{-1}. These runners had never used MLSSv for training and had only used slow, long-distance training that had become inefficient with respect to further improvement in performance. They hoped to improve their aerobic capacity and performance through this new training schedule. There was no control group since the purpose of this study was not to compare two forms of training but to choose an efficient training method to ensure that the endurance time at the MLSSv improved. The study objectives, procedures, and possible risks were described in detail to the subjects and signed informed consent was obtained. The study was approved by the Ethics Committee of the University of Paris. No remuneration was offered. The experiments were carried out at sea-level in March and May over a period of 6 weeks.

Experimental design

All the tests were performed on a synthetic 400-m track at 17–23°C without wind (<2 m/s, anemometer, Windwatch, Alba, Silva, Sweden). For each runner the tests were performed over a 3-day interval at the same hour each day. The outdoor conditions for the tests were preferred to exercising indoors on a treadmill as we wished to measure the improvement of performance under conditions similar to those for normal outdoor training sessions. Before and after training, the runners performed the three following tests: First, an incremental test to exhaustion, second, a sub-maximal test of two stages below and above the lactate threshold velocity to determine MLSSv and MLSSc and, third, a time-to-exhaustion test at MLSSv to determine the time limit (endurance) at MLSSv. Before exercise on the 1st day, height and weight were recorded and fat mass estimated using skin-fold thickness. For the 2 days before each test, subjects were asked to train lightly, i.e. 30 min at a pace that could be managed easily.

Nutritional controls

The subjects were asked to eat a standard snack containing 3,000 kJ (57% CHO, 28% fat, 15% protein) for their evening meal (8.00 p.m.). This was repeated the night before each trial. The subjects were asked to eat a standardized breakfast containing 2,300 kJ (85% CHO, 5% fat, 10% protein) 3 h before the start of the exercise trials. A rest day preceding the exercise trials was allowed to normalize muscle glycogen concentration.

Procedures

For the all three tests, running speed was controlled by a cyclist acting as a pace-maker moving at the required velocity. The cyclist received audio cues via a "Walkman", the cue rhythm determined the speed needed to cover 50 m. Visual marks were set at 50-m intervals along the track inside the first lane. The velocity was checked using a GPS system (Cosmed, Rome, ITALY). Blood lactate concentration was analysed using a Lactate pro LT device (Arkray, Kyoto, Japan) [34]. $\dot{V}O_2$ was measured throughout each test using a telemetric system weighing 0.7 kg, which was worn on the back and abdomen (K4b^2, Cosmed). Expired gases were measured, breath-by-breath and averaged every 30 s in the incremental test and every 5 s during the constant velocity run at MLSSv (Data Management Software, Cosmed). The response times of the O_2 and CO_2 analysers are less than 120 ms to reach 90% of the flow sample. The ventilation range of the flow meter is from 0 to 300 l min^{-1}. The time delay of the gas analyser (time necessary for the gas to transit through the sampling line before being analysed) is about 500 ms. This time delay is measured automatically and is taken into account in the calculations when a delay calibration procedure is performed according to the manufacturer's specifications. Before each test, the O_2 analysis system was calibrated using

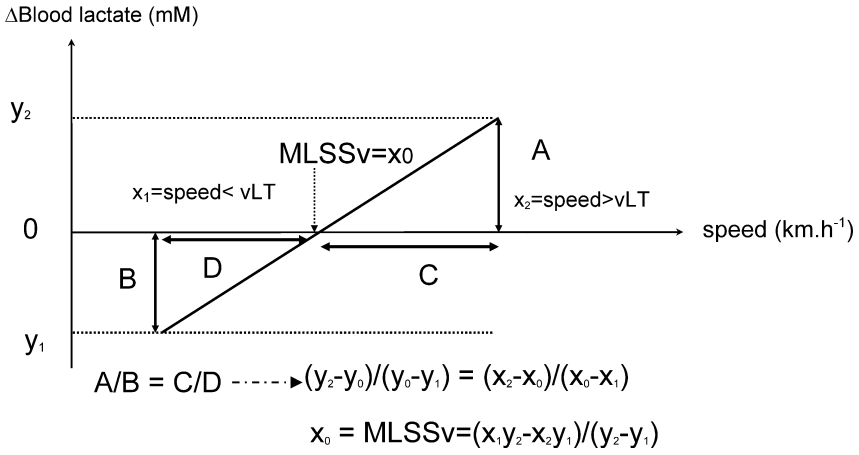

Fig. 1 Graphical representation of the determination of the velocity at maximal lactate steady state (MLSSv) from the change in blood lactate concentration (*ordinate*, $\Delta[la^-]_b$) and running speed (*axis*), from two 20-min runs, one 5% below (x_1 at velocity v_1), one 5% above (x_2 at velocity v_2) the velocity at which blood lactate accumulation begins to accumulate (velocity at the lactate threshold, v*LT*). The latter was determined in a previous incremental test which also included the determination of maximum O_2 uptake ($\dot{V}O_{2,max}$) and the velocity at $\dot{V}O_{2,max}$ (v$\dot{V}O_{2,max}$) [8]. $\Delta[la^-]_b$ is the difference in $[la^-]_b$ from the 5th to the 20th min of the runs (20th−5th). The algebraic calculation of MLSSv is shown. For example, the second subject had a vLT of 14.5 km h^{-1}. In the run at 95%vLT i.e. 13.8 km h^{-1}) $[la^-]_b$ fell from 4 mM at 5 min to 3 mM at 20 min ($\Delta[la^-]_b$ −1 mM). In the run at 105%vLT i.e. 15.2 km h^{-1}); $[la^-]_b$ rose from 4.0 mM at 5 min to 6.5 mM at 20 min ($\Delta[la^-]_b$ +1.5 mM). Inserting these values into the algebraic function yields MLSSv≈15 km h^{-1}

ambient air, the O_2 content of which was assumed to be 20.9%, and a gas of known CO_2 concentration (5%) (K4 b^2 instruction manual). The turbine flow-meter of the K4 b^2 was calibrated with a 3-l syringe (Quinton Instruments, Seattle, Wash., USA).

The subjects first performed an incremental test to determine $\dot{V}O_{2,max}$, the velocity associated with $\dot{V}O_{2,max}$ (v$\dot{V}O_{2,max}$), the running velocity at the lactate threshold (vLT) and the CROv [28]. The first three stages lasted 6 min and were performed at 50, 60 and 70% of the v$\dot{V}O_{2,max}$ that had been estimated previously from the personal best 10 km time, which has been reported to be an average of 92% v$\dot{V}O_{2,max}$ [12]. The following stages lasted 3 min and were run at 80, 85, 90, 95 and 100% of the estimated v$\dot{V}O_{2,max}$. Five subjects reached 105% of the estimated v$\dot{V}O_{2,max}$. Each stage was separated by a 30-s rest during which a capillary blood sample was obtained from the finger tip and analysed for lactate concentration. A plateau of $\dot{V}O_2$ was identified if the $\dot{V}O_2$ of the most recent stage was less than 2.1 ml kg^{-1} min^{-1} greater than the previous one. $\dot{V}O_{2,max}$ was defined as the highest 30-s $\dot{V}O_2$ value reached during this incremental test with an RER ($\dot{V}CO_2/\dot{V}O_2$) greater than 1.05, blood lactate greater than 8 mM and a peak heart rate at least equal to 90% of the age-predicted maximum. When subjects did not reach a $\dot{V}O_2$ plateau, we considered the value to be $\dot{V}O_{2,peak}$ rather than $\dot{V}O_{2,max}$. v$\dot{V}O_{2,max}$ was defined as the lowest running speed maintained for more than 1 min that elicited $\dot{V}O_{2,max}$. For the sake of simplicity, we have used the acronym $\dot{V}O_{2,max}$ for all subjects. If, during the last stage, an athlete achieved $\dot{V}O_{2,max}$ which was not sustained for at least 1 min, the speed during the previous stage was regarded as his v$\dot{V}O_{2,max}$. vLT was defined as the speed at which a lactate concentration increase of 1 mM occurs between 3.5 and 5 mM [1] and was determined by two independent experimenters. Blood was collected by the experimenters placed on the track according to the stage's speed and duration of the protocol. For instance, running at 16 km h^{-1} for 3 min the runners covered a distance of 800 m (two laps).

The second test was performed to determine MLSSv and MLSSc. The runners performed two 20-min runs, one below (−5%) and the other above (+5%) vLT, as determined in previous incremental tests [10]. During a rest period of 40 min, which separated the two stages, the subjects were only allowed to drink water (ad libitum) [10]. Blood was collected by the experimenters every 5 min. The experimenters were positioned on the track according to the stage's speed and duration of the protocol.

The third test was the time-to-exhaustion run at MLSSv. After a 15-min warm-up at 80% MLSSv, subjects then ran until exhaustion at MLSSv. Blood lactate concentration was collected at the end of the warm-up and every 10 min during the exhaustive run at MLSSv, stopping the subjects for only 10 s. Since time-to-exhaustion was not a multiple of 10 (min), the blood lactate accumulation, expressed in millimoles/litre per minute, was calculated taking into account the Δlactate concentration between the end of exercise and the last collected sample. Note that the Δtime for the final exercise period was sometimes less than 10 min (for instance 8 min for the subject

Table 1 Training log at the velocity at maximal lactate steady state (*MLSSv*). For this subject, MLSSv was 4.3 m s^{-1}. An example (*in bold*) is shown for the 1st week, during which the subject underwent two training sessions at MLSSv: the first comprising three sets of 10-min runs at MLSSv, giving a distance of 2,532 m. Recovery comprised a 5-min run at 70% MLSSv. The second weekly session involved two repetitions of 15 min at MLSSv (for a total 3798 m). The total duration of the run at MLSSv was the 30 min/session in the 1st week. In subsequent weeks, the total time run at MLSSv was increased by 6 min/week

Week	First training session of the week	Distance per session (m)	Second training session of the week	Distance per session (m)	Total time run at MLSSv per session
1	**3×10 min**	**2532**	**2×15 min**	**3798**	**30 min**
2	3×12 min	3038	2×18 min	4558	36 min
3	3×14 min	3545	2×21 min	5317	42 min
4	3×16 min	4051	2×24 min	6077	48 min
5	3×18 min	4558	2×27 min	6836	54 min
6	3×20 min	5064	2×30 min	7596	60 min

who gave up at 58 min). We checked that the Δtime between the last time blood collection and time-to-exhaustion was not significantly different in the pre vs. post tests (11 min 46 s±2 min 56 s vs. 12 min 13 s±2 min 41 s, P=0.80). Time-to-exhaustion at MLSSv was determined between the start and the time when the subject was no longer able to follow the pacemaker (cyclist). As for the incremental test, each subject was encouraged to perform at his maximum effort. The distance run at MLSSv was the product of the time-to-exhaustion at MLSSv and MLSSv.

Training

Between the two sets of the three tests (pre and post tests), the runners carried out the following training procedure. The subjects normally ran 5 times a week, but only at a low pace below their half-marathon pace of 15.3±1 km h^{-1}. For the experimental training lasting 6 weeks, the two shortest weekly sessions (lasting40–60 min) were replaced by two sessions at MLSSv according to the schedule described in the Table 1. Each training session was supervised by a physical education student and the coach of the club.

Data analysis

Anthropometry

Height and weight were measured. Five skin-fold measurements were made (triceps, biceps, supra-iliac, sub-scapular, mid-thigh) and the percentage body fat calculated using the formula of Durnin and Womersley [18].

Determination of MLSSv and MLSSc

The principle of the method depends on the variation of blood lactate concentration ($[la^-]_b$) with time during exercise at two steady-state intensities for 20 min performed at low and high speed as reported below. The difference between $[la^-]_b$ at 5 min and at 20 min was calculated (20th−5th min). At the moderate exercise intensity, $[la^-]_b$ decreased with time but increased at the highest intensity. From these two distinctly different changes in $[la^-]_b$ with time, we calculated the exact maximal intensities at which $[la^-]_b$ would not change and the $[la^-]_b$ corresponding to the stabilization at MLSSv (MLSSc) [10] (Figs. 1 and 2).

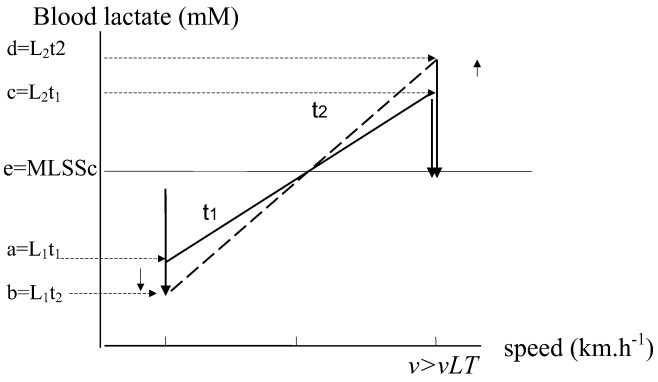

Fig. 2 Graphical representation of the determination of $[la^-]_b$ at maximal lactate steady state (MLSSc) from $[la^-]_b$ at two different times (t_1 and t_2) during two runs, one at 95% vLT, the other at 105% vLT. The letters *a–d* on the *ordinate* indicate $[la^-]_b$ at the respective times. MLSSc (*e* on the ordinate) is the point at which the line connecting the two values of $[la^-]_b$ at t_1 (*solid line*) intersects with that connecting the values of $[la^-]_b$ at t_2 (*dashed line*), at which exercise intensity is equal to MLSSv. From the geometry of the figure, $(d-e)/(e-b)=(c-e)/(e-a)$, i.e. e (MLSSc)=(da−bc)/[(d+a)−(c+b)]. From the numerical example in Fig. 1, $[la^-]_b$ at 5 and 20 min at 13.8 km h^{-1} was 4 and 3 mM respectively and 4.0 and 6.5 mM respectively at 15.2 km h^{-1}. Inserting these values in the algebraic function yields an MLSSc of 4.0 mM

Calculation of substrate oxidation balance during exercise

CHO and lipid oxidation rates were calculated from gas exchange measurements according to the non-protein respiratory quotient technique [28]:

$$\text{CHO oxidation rate} = 4.585 - 3.22 \cdot \dot{V}CO_2 - 3.22 \cdot \dot{V}O_2 \quad (1)$$

and

Table 2 Effect of 12 training sessions at MLSSv (over 6 weeks) on the physiological characteristics of expert long-distance runners (*vLT* velocity at lactate threshold, $\dot{V}O_{2,max}$ maximal O_2 uptake, $v\dot{V}O_{2,max}$ minimal velocity associated with $\dot{V}O_{2,max}$ in an incremental test, $[la^-]_b$ blood lactate concentration, *HR* heart rate, *MLSSc* $[la^-]_b$ at the maximal lactate steady state, *RER* respiratory exchange ratio)

Variables	Pre-training	Post-training	Change (% of the pre-training value)	P^b
Weight (kg)	69.3±5.7	68.6±5.6	−1.1±1.4	0.04
$\dot{V}O_{2,max}$ (ml kg^{-1} min^{-1})	55.1±4.2	57.6±3.9	+3.6±4.3	<0.01
Fat mass (% body mass)	17.2±2.6	16.2±2.6	−6.7%±9.5	0.06
$\dot{V}O_{2,max}$ (ml kg^{-1} min^{-1})[a]	66.6±5.7	68.8±4.9	+3.5±3.7	0.02
$\dot{V}O_{2,max}$ (ml min^{-1})	3824±504	3959±506	+4.4±3.6	0.03
$v\dot{V}O_{2,max}$ (km h^{-1})	16.8±1.3	17.5±1.1	+3.8±3.6	0.03
vLT (km h^{-1})	14.5±1.6	15.0±1.5	+3.3±3.8	0.03
MLSSv (km h^{-1})	13.8±1.5	15.2±1.6	+4.2±3.9	<0.01
MLSSv (%$v\dot{V}O_{2,max}$)	85.2±4.5	85.3±5.2	-	0.93
MLSSc (mM)	3.7±0.8	4.3±1.4		0.90
Peak $[la^-]_b$ at $v\dot{V}O_{2,max}$ (mM)	12.3±2.3	11.9±1.5	-	0.33
RER_{max} at $v\dot{V}O_{2,max}$	1.12±0.03	1.12±0.04	-	0.73
Peak HR at $v\dot{V}O_{2,max}$ (bpm)	181±9	181±10	-	0.5
Cross-over point velocity (km h^{-1})	8.9±1.4	9.9±1.8	-	0.1
Cross-over point velocity (%$v\dot{V}O_{2,max}$)	53.2±6.1	56.6±10.1	+6.8±17.9	0.29
Crossover point velocity (%MLSSv)	62.4±6.4	66.3±11.5	+6.8±20.7	0.36

[a] referred to lean body mass
[b] paired *t*-test, pre- vs. post-training

$$\text{Lipid oxidation rate} = -1.7012 \cdot \dot{V}CO_2 + 1.6946 \cdot \dot{V}O_2 \quad (2)$$

with $\dot{V}O_2$ and $\dot{V}CO_2$ in millilitres/minute and oxidation rates in milligrams/minute.

$\dot{V}O_2$ and $\dot{V}CO_2$ were determined during the 5th and 6th min of each state according to [28]. This technique yields CHO and lipid oxidation rates at different levels of exercise. These values were then converted into kilojoules. According to the concept of Brooks and Mercier [13] the cross-over point of substrate utilization is defined as the speed (power) at which energy (in kilojoules) from CHO-derived fuels predominates (>50% of the total energy spent) over energy from lipids (<49% of the total energy spent).

Statistical analysis

Linear regression analysis, Pearson's correlation test, Student's *t*-test for paired data and one-way ANOVA with repeated measures were used for data analysis. Results are presented as mean±SD. $P<0.05$ was regarded as significant.

Results

The training effect on $\dot{V}O_{2,max}$, v$\dot{V}O_{2,max}$, MLSSv and the cross-over point velocity (CROv)

Training at MLSSv increased $\dot{V}O_{2,max}$ per unit body mass (+4.4±3.6%), v$\dot{V}O_{2,max}$ (+3.8±3.6%) and MLSSv (+4.2±3.9%) significantly (Table 2). However, these increases were not correlated. MLSSv expressed relative to v$\dot{V}O_{2,max}$ did not increase significantly as both v$\dot{V}O_{2,max}$ and MLSSv increased similarly (MLSSv=85.2±4.5 vs. 85.3±5.2% v$\dot{V}O_{2,max}$ in pre- and post training conditions, P=0.93, Table 2). Heart rate at the same absolute velocity (the pre-training 60, 70, 80, 85, 90 and 95% of v$\dot{V}O_{2,max}$, i.e. 10.4±0.9, 12.1±1.1, 13.8±1.2, 14.7±1.3, 15.5±1.4 and 16.3±1.5 km h^{-1} respectively) decreased significantly after training (Fig. 3). Training did not increase CROv significantly (8.9±1.4 vs. 9.9±1.8 km h^{-1}, P=0.1, i.e. 62.4±6.4 vs. 66.3±11.5% of MLSSv, P=0.136, Table 2).

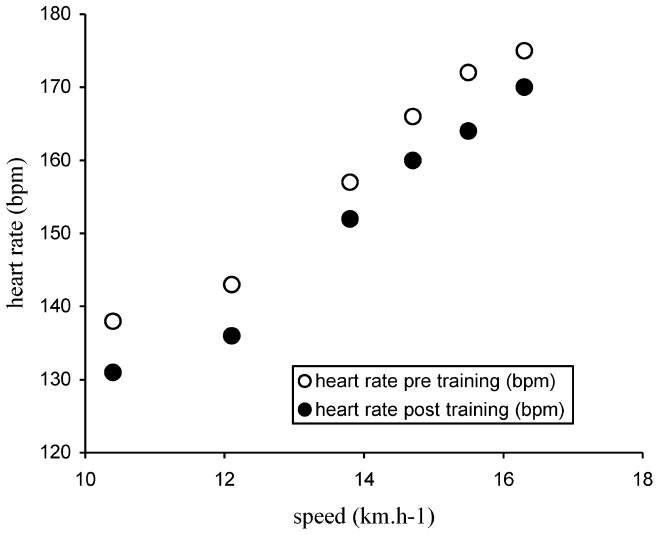

Fig. 3 Relationship between heart rate and the running speed during the incremental test to determine $\dot{V}O_{2,max}$ before and after 6 weeks training at MLSSv

Table 3 Effect of 12 training sessions at MLSSv (over 6 weeks) on physiological responses during a run to exhaustion at MLSSv. (*tlim MLSSv*, time-to-exhaustion at MLSSv, *dlim MLSSv*, distance covered in run to exhaustion at MLSSv, *CHO%* percentage of energy yielded by the carbohydrates)

Variables	Pre-training values at the pre-training MLSSv	Post-training values at the post-training MLSSv	P^a
MSSLv (km h^{-1})	13.8±1.5	15.2±1.6	<0.01
tlim MLSSv (min)	44±10	63±12	<0.01
dlim MLSSv (km)	10.4±2.2	15.7±2.5	<0.01
$[la^-]_b$ at 10th min of tlim MLSSv (mM)	3.6±1.2	4.0±0.4	0.5
$[la^-]_b$ 10 min before the end of tlim MLSSv (mM)	3.8±0.4	5.5±1.8	0.02
$[la^-]_b$ at the end of tlim MLSSv (mM)	3.9±0.4	6.4±1.5	<0.01
HR at 10th min of tlim MLSSv (bpm)	160±7	163±12	0.26
HR 10 min before the end of tlim MLSSv (bpm)	163±8	169±13	0.06
HR at the end of tlim MLSSv (bpm)	166±7	171±9	0.03
HR at MLSSv (calculated) (bpm)	164±9	170±8	<0.01
RER at 10th min of tlim MLSSv	0.94±0.04	0.93±0.02	0.17
RER 10 min before the end of tlim MLSSv	0.93±0.04	0.93±0.02	0.53
RER at the end of tlim MLSSv	0.93±0.04	0.94±0.04	0.58
$\dot{V}O_2$ at 10th min of tlim MLSSv (ml kg^{-1} min^{-1})	46.6±5.6	48.9±4.8	0.08
$\dot{V}O_2$ 10 min before the end of tlim MLSSv (ml kg^{-1} min^{-1})	47.0±5.1	47.7±3.6	0.40
$\dot{V}O_2$ at the end of tlim MLSSv (ml kg^{-1} min^{-1})	47.1±5.2	48.2±3.4	0.33
%CHO at 10th min of tlim MLSSv	83.5±11.5	79.6±6.7	0.17
%CHO at 10 min before the end of tlim MLSSv	81.5±12.4	83.3±11.0	0.56
%CHO at the end of tlim MLSSv	80.1±12.2	83.3±16.0	0.30

[a]Pre vs. post-training, paired *t*-test

MLSSc (Table 2) was not significantly modified by training (3.7±0.84 vs. 4.3±1.4 mM, P=0.09).

The training effect on time-to-exhaustion at MLSSv and on the substrate balance and physiological responses during the exhaustive run at MLSSv

Time-to-exhaustion at MLSSv increased 10 times more than MLSSv (in kilometres/hour) (+50.6±41.8% i.e. from 44±11 min before to 63±12 min after training, P<0.01, Table 3). The increase in time-to-exhaustion at MLSSv was not related to the increase in MLSSv (r=0.39, P=0.32) or to that of $\dot{V}O_{2,max}$ (r=0.02, P=0.96) or v$\dot{V}O_{2,max}$ (r=0.25, P=0.52).

In the pre-training run to exhaustion at MLSSv, the subjects maintained a lactate steady state at a concentration not significantly different from MLSSc (3.6±1.2, 3.8 ±0.4, 3.9±0.4 mM at the 10th min, 10 min before the end and at the end of the run at MLSSv, P=0.80, compared with MLSSc 3.7±0.84 mM, P=0.92). In contrast, in the post-training run to exhaustion at MLSSv, blood lactate concentration increased throughout the test (4.0±0.9, 5.5 ±1.8, 6.4±1.5 mM at the 10th min, 3–10 min before the end and at the end of the respectively, P<0.01, Table 3). However, the rate of blood lactate accumulation in the last 3–10 min of the exhaustive run at MLSSv still met the criteria defining MLSS, i.e. a rate of blood lactate accumulation by no more than 0.05 mM min^{-1} [6] in the pre-training MLSSV run (+0.01±0.04 mM min^{-1}). In the post-training test the rate of blood lactate accumulation was higher than in the pre-training test (0.10±0.08 mM min^{-1}, P=0.07) and five subjects were above the MLSS criteria (by 0.07–0.23 mM min^{-1}). However, this increase in the rate of blood lactate accumulation in the post- vs. pre-training test was correlated with the increase in time-to-exhaustion at MLSSv after training (r=0.37, P=0.34). Therefore the 50% longer run at MLSSv was not associated with the ability to sustain a higher blood lactate accumulation. However, in the post-training time-to-exhaustion run at MLSSv, the blood lactate concentration at the end of the run was significantly higher than MLSSc (6.4±1.5 vs. 4.3±1.4 mM, P<0.01). Both in the pre- and post-training conditions, the rate of blood lactate accumulation was not related to the time-to-exhaustion at MLSSv (r=−0.44, P=0.25 and r=−0.28, P=0.49 in pre- and post-training tests respectively).

$\dot{V}O_2$ kinetics followed a mono-exponential function with a time constant not significantly different before and after training (29±11 vs. 33±16 s, P=0.3). Thus, $\dot{V}O_2$ did not increase during the time-to-exhaustion run at MLSS (P=0.3 and P=0.4 respectively in the ANOVA test for repeated measurements during the run to exhaustion at MLSSv) and stayed submaximal, representing a fraction of 84.5±4.8 and 83.7±5.0% of $\dot{V}O_{2,max}$ before and after training respectively. In contrast to $\dot{V}O_2$, heart rate increased significantly throughout the time-to-exhaustion run at MLSSv both before and after training (P<0.01, Table 3).

Training at MLSSv did not modify substrate usage during the run to exhaustion at MLSSv (Table 3). Indeed, both before and after training, CHO yielded 80% of the energy at the 10th min. This was the same both at 10 and 1 min before the end of the time-to-exhaustion run at MLSSv while RER remained around 0.93 (Table 3). The five subjects who were, after training, above the MLSS criteria, also obtained 80% of their energy from CHO (79.1±8.6, 79.7±6.6, 81.5±10.4 at 10 min, 10 min before the end and in the last minute of the run to exhaustion at MLSSv) and did not modify this proportion.

Relationship between time-to-exhaustion at MLSSv and substrate used during the exhaustive run at MLSSv

Only after training was there a significant inverse relationship between the percentage of energy yielded by CHO 10 min before exhaustion at MLSSv and time-to-exhaustion (r=−0.72, P=0.03, Fig. 4). This relationship was not observed before training. Time-to-exhaustion at MLSSv was well predicted (69% of the variance) by the blood lactate concentration 10 min before the end of the exhaustive run at MLSSv (r=−0.83, P<0.01, Fig. 5). Time-to-exhaustion at MLSSv also correlated with the blood lactate concentration at exhaustion (r=0.72, P=0.03). This means that the higher the blood lactate concentration during the last 10 min of the exhaustive run at MLSSv, the longer the endurance run at MLSSv. Blood lactate concentration at the 10th min of the exhaustive run at MLSSv correlated with the percentage of energy yielded

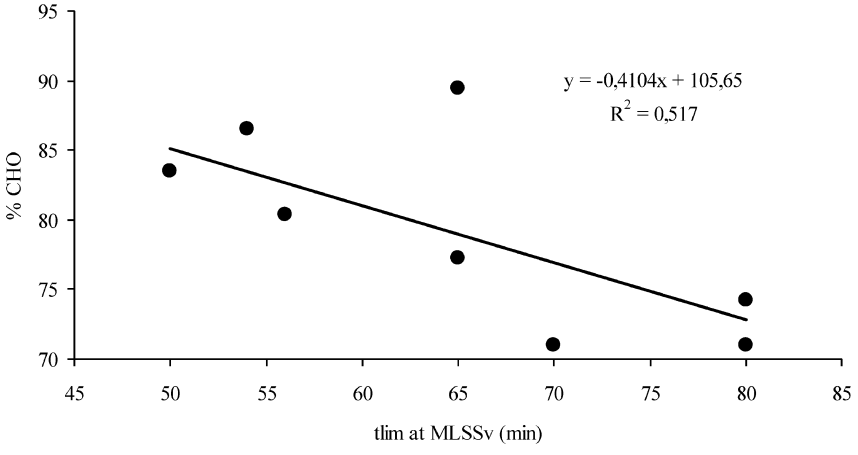

Fig. 4 Relationship between the percentage of the energy derived from carbohydrates (% CHO) during the exhaustive run at MLSSv and the corresponding time-to-exhaustion (*tlim*) at MLSSv after 6 weeks training at MLSSv

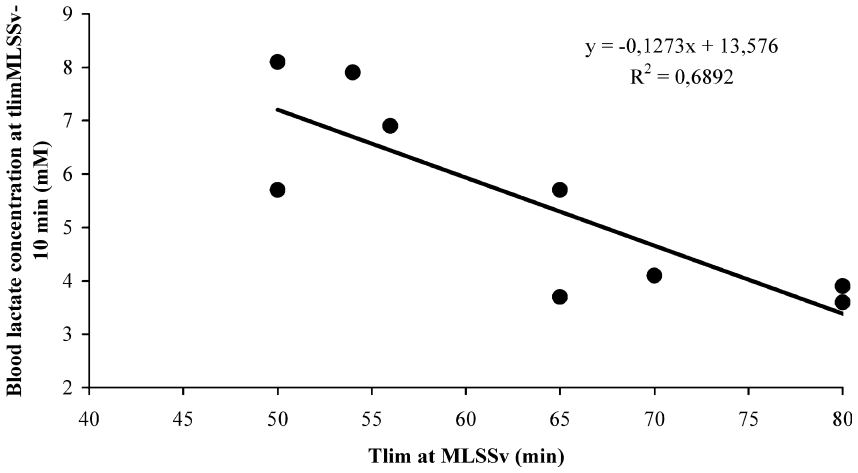

Fig. 5 Relationship between blood lactate concentration at the 10th min of a run to exhaustion at MLSSv and the time-to-exhaustion at MLSSv after 6 weeks training at MLSSv

by CHO (r=0.75, P=0.02). These relationships were not observed before training. Before and after training, time-to-exhaustion at MLSS was not related to CROv expressed as a fraction of MLSSv (r=0.15, P=0.72 and r=0.51, P=0.16 in pre- and post-training, respectively).

Discussion

The main results of the exercise protocol used in this study were small increases in MLSSv and $\dot{V}O_{2,max}$, a large increase of endurance (running time) with no effect on the relative fractions of substrate utilization at MLSS speed and no change of the cross-over point that discriminates between fat- and CHO-dominated metabolism. Before continuing with the discussion it is necessary to discuss some methodological points concerning the determination of MLSSv.

Determination of MLSSv has, in the past, required several work grades lasting 10–30 min [26]. To avoid such a long procedure, we determined MLSSv according to Billat et al. [10]. This protocol allows an immediate estimation of the exercise intensity corresponding to the MLSS using only two sub-maximal intensities each lasting 20 min [10]. The blood lactate steady state was obtained in the runs to exhaustion at MLSSv both before and after training. Subjects ran at the estimated MLSSv, which was higher after training. In addition to this 4% increase in velocity, the runners were able to sustain a 50% longer duration than before training. Both before and after training, $\dot{V}O_2$ was in steady state and the measurement of RER and thus of the CHO oxidized was not affected by any slow component of $\dot{V}O_2$ [33]. Indeed, the time course of $\dot{V}O_2$ was mono-exponential even for the five subjects who had a rate of blood lactate accumulation higher than 0.05 mM min^{-1}. The subjects did not increase $\dot{V}O_2$ during the all-out run at MLSSv in contrast to exhaustive runs at a velocity intermediate between MLSSv and v$\dot{V}O_{2,max}$ (vΔ50) [11, 15].

As a final methodological consideration, we did not measure CROv under conditions of fasting. CROv is thus rather a cross-over point under normal conditions for a runner. The subjects were not fasted so as to be in a condition similar to that during normal training and competition. This was done to avoid any alterations in the exercise endurance time due to modification of substrate utilization [41]. Indeed, we were especially interested in simulating conditions occurring normally during training and competition. Each subject was encouraged to perform at maximum effort in the tests. The most important fact was that subjects had the same nutritional regime, including the timing of nutritional intake, both before and after training, as well as before and after the exercise bout [23].

In discussing the main findings of this study we shall consider firstly the effect of training on the time and the substrate balance at MLSSv and secondly, the effect of training on MLSSc in relation to the improvement in time-to-exhaustion at MLSSv.

Training increased the time-to-exhaustion at MLSS but did not modify the substrate balance during the incremental test and during the exhaustive run at MLSSv

The time-to-exhaustion at MLSSv increased tenfold more than MLSSv but the balance of substrate during this exhaustive run was not changed. Since MLSSv and v$\dot{V}O_{2,max}$ increased similarly with training, the exhaustive run at MLSSv was performed at the same relative intensity to v$\dot{V}O_{2,max}$ (85%). We can therefore consider that the subjects improved their endurance i.e. their ability to sustain the same fraction of v$\dot{V}O_{2,max}$ for a longer time. Given that this endurance run was performed at the same relative value of v$\dot{V}O_{2,max}$ and, in addition, at the maximal lactate steady-state condition, it is not surprising that the balance of substrate was unchanged. In addition, MLSSv expressed as a percentage of CROv was also not modified significantly by training (160±16 vs. 151±17%, P=0.4). The cross-over point was around 55% $\dot{V}O_{2,max}$ both before and after training, in agreement with previous findings showing the relative contribution of carbohydrate to total energy expenditure to be 50% during a 25-min exercise at 62% of $\dot{V}O_{2,max}$ [35]. In the present study, both before and after training, RER stayed around 0.94 throughout the exhaustive run at MLSSv. This is lower than our

hypothesis would suggest and lower than that usually expected (RER=1) [6]. In addition, the subjects were able to sustain their level of exercise using the same source of energy (CHO for 80%) at a higher blood lactate concentration during the last 10 min of exercise. These findings are in accordance with others demonstrating that training decreases glucose flux for a given power output but not for the same relative exercise intensity (65% of $\dot{V}O_{2,max}$) [20].

The ability of cyclists to sustain higher percentages of the peak power output in a 40-km time-trial (TT_{40}) cycling performance performed after an interval training program is not due to lower rates of CHO oxidation [40]. Higher relative work rates in the TT_{40} rides following training increased the estimated rates of CHO oxidation by 18%. In addition, supplementary CHO does not improve 1-h cycle time-trial distance and, furthermore, the substantial muscle CHO reserves observed at the termination of exercise indicated that whole-muscle glycogen depletion does not determine fatigue at this exercise intensity and duration [22].

The question of why subjects cease exercise after 1 h at MLSSv remains open and is beyond the scope of this study. In this study 69% of the variance of the time-to-exhaustion at MLSSv was predicted by the blood lactate concentration 10 min before the end of exercise. This is in accordance with one classical explanation for fatigue, which is the possibly higher than expected glycolytic rate for the substantial non-oxidative ATP supply and the accumulation of inhibitory by-products during sustaining contractions [36]. It is also possible that at 85% $\dot{V}O_{2,max}$ muscle is not in a metabolic steady-state, as indicated by a fall in the concentration of phosphocreatine [PCr] and pH [14]. The drop of PCr necessary to enhance $\dot{V}O_2$ could be lower after training and could explain the endurance increase at MLSSv.

However, the most probable explanation for improvement of endurance at MLSSv is the increase of the lactate clearance after training, as demonstrated in numerous studies [8, 9, 16, 17, 27, 31, 32, 38, 39]. Endurance training also enhances lactate clearance during rest [39], and this is accompanied by a greater gluconeogenesis from lactate [17]. Lactate uptake and conversion to glucose in perfused livers is also enhanced by endurance training [39]. After only 10 consecutive days of cycling for 2 h/day at 59% $\dot{V}O_{2,max}$, moderately active, but untrained, men increased the metabolic clearance rate of lactate during exercise from 50% [27]. A more recent study has demonstrated that 9 weeks training with five sessions per week at 75% $\dot{V}O_{2,peak}$ increases both whole body and leg lactate clearance during a cycling exercise at 65% $\dot{V}O_{2,peak}$ [9]. During the same time $\dot{V}O_{2,peak}$ increased by 15% and lactate threshold by 22% in terms of absolute power output and by 7% when expressed as a percentage of $\dot{V}O_{2,peak}$. In our study, MLSSv did not increase relative to $\dot{V}O_{2,max}$ but the subjects were already endurance-trained and we oriented their training toward a shorter and higher intensity than their previous training. This training increased MLSSv little by itself but rather the time-to-exhaustion at MLSSv. No previous studies have focused on the relationship between lactate clearance and endurance at MLSSv. This could be a way to explore and to better understand the relationship between training, endurance at MLSSv and performance during long-distance running.

The effect of training on MLSSc

Training did not affect the blood lactate concentration at MLSSv (MLSSc). Due to the fact that the determination of the maximal lactate steady state depends on the time course of blood lactate concentration at two intensities of exercise (above and below the lactate threshold), we suggest that this time course was not modified by training at MLSSv. While the kinetics of blood lactate generally have been studied in incremental intensity exercise models, no studies have reported a difference in blood lactate concentration kinetics during constant work-rate exercise [19].

The present study shows that training at MLSSv did not decrease the large inter-individual difference in MLSSc. Indeed, before and after training, the average MLSSc was around 4 mM but varied greatly between subjects (2.0–5.0 mM and 2.0–6.0 mM before and after training, respectively) in accordance with other findings in cyclists and triathletes [10] and in elite athletes [25]. Even if distinct methods of MLSS determination as well as the power output (load) per unit mass of working muscles result in different values for MLSSc [2, 3, 7], our MLSSc data are in accordance with those of previous studies [5, 10, 21, 30, 37]. Beneke et al. [4] have reported a maximal stable lactate equal to 4.2±0.7 mM with extreme values of 2.8–5.5 mM, without this value varying with age in adolescents (10–20 years). To our knowledge there are no data on MLSS in older persons. Our values are comparable with those obtained in younger subjects and training did not change the blood lactate concentration at the maximal lactate steady state. In addition, we are not aware of any data on the effect of training on the MLSSc (for constant work-rate exercise).

In conclusion, the present study showed that 6 weeks training with two weekly sessions at MLSSv increased MLSSv and $\dot{V}O_{2,max}$ and greatly improved the time-to-exhaustion at MLSSv (from 40 to 60 min). The percentage of CHO oxidation at MLSSv was not changed by training, but running time at MLSSv did. Indeed, before and after training CHO was responsible for the major and same sources of energy (80%) of the endurance run at MLSSv. Perhaps even highly trained humans cannot sustain a greater rate of CHO oxidation because the prerequisite glycolytic flux inevitably results in metabolic acidosis? This means that training increases lactate clearance so that higher glycolytic and oxidative fluxes can be sustained since velocity and $\dot{V}O_{2,max}$ increased. The blood lactate concentration at the MLSSv was not modified after training. There was therefore a considerable increase in endurance at MLSSv in the absence of a relative increase in CROv expressed as a fraction of MLSSv and substrate balance. Our data from master long-distance runners are in general agreement with those obtained in other groups according to age, gender, level of performance and habits

for intensity of training, especially at velocities faster than MLSSv widely used by elite male and female marathon runners [2].

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