Running Interval Training and Estimated Plasma-Volume Variation

Abderraouf Ben Abderrahman, Jacques Prioux, Karim Chamari, Omar Ben Ounis, Zouhair Tabka, and Hassane Zouhal

The effect of endurance interval training (IT) on hematocrit (Ht), hemoglobin (Hb), and estimated plasma-volume variation (PVV) in response to maximal exercise was studied in 15 male subjects (21.1 ± 1.1 y; control group n = 6, and training group, n = 9). The training group participated in interval training 3 times a week for 7 wk. A maximal graded test (GXT) was performed to determine maximal aerobic power (MAP) and maximal aerobic speed (MAS) both before and after the training program. To determine Ht, Hb concentration, and lactate concentrations, blood was collected at rest, at the end of GXT, and after 10 and 30 min of recovery. MAP and MAS increased significantly (P < .05) after training only in training group. Hematocrit determined at rest was significantly lower in the training group than in the control group after the training period (P < .05).

IT induced a significant increase of estimated PVV at rest for training group (P < .05), whereas there were no changes for control group. Hence, significant relationships were observed after training between PVV determined at the end of the maximal test and MAS (r = .60, P < .05) and MAP (r = .76, P < .05) only for training group. In conclusion, 7 wk of IT led to a significant increase in plasma volume that possibly contributed to the observed increase of aerobic fitness (MAP and MAS).

Keywords: endurance, maximal exercise, performance, athletes, oxygen uptake

Aerobic metabolic process governed by oxygen delivery to the mitochondria of the working muscle cell represents the main determinant factor of performance during continuous whole-body exercise.1 It is commonly accepted that cardiac output represents, in humans, one of the main limits to high-intensity aerobic performance. Cardiac output is determined by heart rate, myocardial contractility, and end-diastolic volume, which is influenced by total volume of blood.2 In this context, it is well known that blood volume (BV) and hemoglobin concentration ([Hb]) play important roles in oxygen transport and aerobic capacity, for example, maximal oxygen uptake (VO2max).3-5 Manipulation of both BV and [Hb] can markedly affect systemic oxygen transport and VO2max. However, the role of BV in oxygen transport and aerobic performance is not well understood.5 It has recently been postulated that an acute expansion of BV using plasma volume (PV), independent of changes in [Hb], may represent a potential ergogenic property.5 Several studies demonstrated that induced PV expansion was accompanied by an increase in VO2max and exercise performance,6,7 while other studies failed to do so.2 Similarly, it was concluded5 that volume loading does not result in an improvement in VO2max or endurance performance in highly endurance-trained athletes; it may improve VO2max in untrained individuals but does not bring these individuals to the aerobic capacity of endurance-trained athletes.

It is well known that endurance training, especially continuous exercise training (cycling or running), is accompanied by increases in PV, hematocrit (Ht), and [Hb] both at rest and in response to exercise.8-10 However, this condition of PV expansion, known as pseudoanemia, rather than being “pathological,” is thought to enhance endurance performance by increasing stroke volume, improving thermoregulatory efficiency, and decreasing blood viscosity.1,11 Nevertheless, Freund et al12 observed no significant differences in PV responses to a maximal continuous incremental protocol on a treadmill between trained and untrained subjects. PV reduction was also observed after detraining.6

On the other hand, it is established that, in response to training, these PV variations are influenced by the differences in intensities, frequencies, durations, and modes of exercise.9 In this context, several studies observed a significant increase of PV in response to long-term or
relatively short-term continuous exercise training. In fact, this result was observed after a variety of training programs: after training on a cycle ergometer, running and walking training programs, or after general exercise. Several other studies have shown that a short training period involving intermittent exercises (especially cycling) may result in significant changes in PV. Indeed, Green et al. observed in moderately trained subjects an increase of 11.6% and 4.5% of PV and BV, respectively, in response to only 3 consecutive days of intermittent supramaximal (120% VO2max) interval cycling exercise. In the same way, Warburton et al. examined the effects of 12 weeks of continuous and interval training performed on a cycle ergometer on BV and cardiorespiratory function in untrained males (mean VO2max = 39.3 ± 7.0 mL/kg/min); they observed similar improvements in VO2max and left-ventricle function, and they concluded that training-induced hypervolemia accounts for approximately 47% of the changes in VO2max after continuous and interval training. In athletic events and running events, intermittent running exercise (interval training [IT]) is commonly used to enhance VO2max and aerobic performance in both untrained and trained subjects. However, little is known about the effects of running IT on PV changes, Ht, and Hb and their eventual link to the improvement of endurance aerobic fitness or performance in well-endurance-trained subjects. Consequently, the aim of this work was to study the effect of 7 weeks of IT on Ht, Hb, and PV variation (PVV) at rest and in response to maximal exercise in well-trained male subjects. We hypothesized that a short IT period (7 wk) would induce a significant increase in aerobic power (VO2max and maximal aerobic speed) and calculated PV.

**Methods**

**Subjects**

Fifteen active adult male subjects, students in physical and sport education (20–23 y old), volunteered to participate in this study. All subjects were well trained, but none were specialized in middle- or long-distance running, none had ever practiced interval exercises as a training program, and none had undergone any IT for 3 months before this study. They practice several sports (soccer, judo, swimming, athletic events, etc) in their institute of physical and sports education for several hours a week (8–12 h/wk).

Their morphological characteristics measured before and after training are displayed in Table 1. The subjects were assigned in randomized order to a control group (n = 6) or a training group (n = 9). Before participation, the participants underwent a medical examination, were fully informed about the experimental procedures, and signed informed consent. The study protocol was conducted in accordance with the ethical standards and guidelines of the Ethical Committee of the University of Rennes 2, France, which approved the experimental protocol and the procedures involved.

**Experimental Procedure**

Each subject came to the laboratory for a medical examination and anthropometric measurements performed by a person trained in anthropometric assessment before and after the training period. Body mass was measured to the nearest 0.1 kg with a digital scale (Ohaus, Florham Park, NJ). Height was measured with a standing stadiometer and recorded with a precision of 1 mm. Skinfold thicknesses were determined in triplicate at 4 sites (triceps, biceps, suprailliac, and subscapular) using a standard, recently calibrated Harpenden caliper (Holtain, Crosswell, UK). These skinfolds were measured with the subject in a sitting position with the whole body well relaxed and right arm along the body. Subjects’ percentage body fat was determined using the method proposed by Durnin and Womersley. All subjects performed a maximal graded field test until exhaustion to measure their VO2max and their maximal aerobic speed before and after the 7-week training program. The maximal test was carried out on a 200-m outdoor tartan track (of which 60 m were indoors) calibrated with cones. Blue cones were set at 50-m intervals along the track (inside the first line), while red cones were set 2 m behind the blue cones. The running pace was set by an examiner equipped with a whistle and a chronometer, emitting a short sound when the subject had to pass by a cone to be able to maintain a constant speed for each test stage. At each sound, the subject had to be within 2 m of the blue cones. When subjects were behind a red cone 3 consecutive times or stopped the exercise, judging themselves exhausted, the test ended. The initial speed was set at 8 km/h and was increased by 1 km/h every 2 minutes. The velocity at the last completed stage was considered maximal aerobic speed. It is important to note that this maximal test was performed without warm-up and cooldown. The maximal test was carried out under similar environmental conditions (temperature ranged from 14°C to 18°C, and humidity from 50% to 70%, with wind speed <2 m/s). During the maximal graded test, respiratory-gas exchange was measured by breath by using a calibrated portable telemetric system (Cosmed K4b2, Rome, Italy). The test took place in the morning 3 to 4 hours after having consumed a standardized breakfast (10 kcal/kg, 55% of which came from carbohydrates, 33% from lipids, and 12% from proteins, as determined by an experienced nutritionist). Between the end of the standardized breakfast and the beginning of the test, the subjects were allowed to drink water ad libitum without exceeding 250 mL/h.

Our study took place on January and March in temperatures between 15°C and 20°C and humidity between 50% and 70%.

**Blood Analysis.** For lactate, Ht, and Hb analysis and PVV calculation, 10 mL of venous blood was collected at rest from the antecubital vein, immediately at the end of the maximal graded test and after 10 and 30 min of passive recovery, both before and after the 7-week
Table 1  Morphological Characteristics of the Subjects, Mean ± SD

<table>
<thead>
<tr>
<th></th>
<th>Height (cm)</th>
<th>Body Mass (kg)</th>
<th>Fat Mass (%)</th>
<th>Fat-Free Mass (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age (y)</td>
<td>Before training</td>
<td>After training</td>
<td>Before training</td>
</tr>
<tr>
<td>TG, n = 9</td>
<td>21.1 ± 1</td>
<td>179.2 ± 4.1</td>
<td>179.0 ± 3.7</td>
<td>76.8 ± 10.9*</td>
</tr>
<tr>
<td>CG, n = 6</td>
<td>21.1 ± 1</td>
<td>176.8 ± 4.5</td>
<td>176.7 ± 4.4</td>
<td>66.5 ± 6.1</td>
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</tbody>
</table>

Abbreviations: TG, trained group; CG, control group.
*Significant differences between TG and CG, \( P < .05 \).
training program. Ht was determined in triplicate and Hb in duplicate.

Blood lactate was determined enzymatically using a lactate analyzer (Microzym, Cetrix, France). Ht and Hb were determined directly in quadruplicate, at the same time and in the same blood volume, automatically by using standard laboratory procedures (Automat MS9-5). Determinations of both Ht and Hb were performed to estimate PV changes according to the method developed by Dill and Costill\textsuperscript{21} and used by other authors in response to physical exercise and after training adaptations\textsuperscript{9}:

$$\% \Delta PV = 100 \left[ \frac{\text{Hb}_{B} \times (1 - \text{Ht}_{A} \times 10^{-2})}{\text{Hb}_{A} \times (1 - \text{Ht}_{B} \times 10^{-2})} \right] - 100$$

where ($\Delta PV$) is expressed as $\% PVV$, B is the value measured at rest, A is the value measured at the end of the exercise or after 10 and 30 min of recovery, Ht is hematocrit in %, and Hb is hemoglobin in g/100 mL.

**Training Program.** The training group participated to an IT program 3 times per week (Monday, Wednesday, and Friday) for 7 weeks (21 intermittent training sessions in total; Figure 1). The training sessions were exclusively composed of intermittent exercise with active recovery and were performed outside on a 400-m track. All sessions included 3 different periods: The sessions were preceded by a standardized warm-up, which consisted of 15 minutes of continuous jogging, followed by 5 minutes of stretching exercises and 5 short bursts of accelerations on the track. Then, the subjects performed their training program. For these exercises, the subjects’ pace was given by an examiner emitting sounds at regular intervals up to the end of the exercise. During the 30-second active recovery, the training group had to cover a distance determined according to their own maximal aerobic speed. During the recovery period, a longer sound was made at midperiod (15 s) to inform the subjects of the remaining time for the end of recovery. At the end of the training session subjects cooled down for about 15 minutes, running at low intensity and performing static stretching. Two members of our laboratory supervised all IT program sessions. At the midpoint of the training program (on the Wednesday of the fourth week), the training group performed a maximal graded test (without respiratory-gas-exchange measurements) to assess maximal aerobic speed to update each subject’s training speed. All the training sessions were controlled and monitored using a heart-rate monitor (Polar S 810 i). During this experimental period the control group did not participate in any physical training program, but they continued their physical practices at the Institute of Physical and Sports Education.

**Statistical Analysis**

Data were expressed as mean ± SD. Normal distribution of data was tested by Kolmogorov-Smirnov test. Morphological characteristics and performances were analyzed using a repeated-measures analysis of variance (ANOVA on ranks) and Student’s t test for paired observation. Metabolic data were analyzed using ANOVAs with repeated measures on both factors, and the Newman-Keuls post hoc test was used in the event of significance. Relationships between parameters were assessed using Pearson product–moment correlation. A value of $P < .05$ was accepted as the minimal level of statistical significance. Statistical analyses were carried out with the SigmaStat 1.0 program (Jandel Scientific, San Rafael, CA).

![Figure 1](image-url) — Training program for the trained group. MAV, maximal aerobic velocity; R, passive recovery between series; TL, training load; ATU, arbitrary training units. Example: $2 \times (8 \times 30$ s IE) $100\%/50\%$ MAV. $R = 5$ min. It means that the subject had to run 2 series of 8 times 30 s IE composed of 30 s running at 100% of MAV and 30 s active recovery at 50% of MAV. The subject recovers passively 5 min between each two series. Each session is repeated 3 times a week. Example of training load calculation for training sessions during the first week: $((100 + 50)/2) \times 4 \times 2 = 600$ ATU.
Results

The morphological characteristics of the trained group and control group measured before and after the 7 weeks of IT are presented in Table 1. The subjects in the training group were heavier than those in the control group both before and after training. However, the training period induced no effect on body composition for either group.

The 7 weeks of IT induced a significant increase of maximal aerobic speed and VO2max only for the training group (Table 2).

Ht values are displayed in Table 3. Only for training group, the training period was accompanied by a significant decrease of Ht at rest, at the end of the maximal exercise, and after 10 minutes of recovery. Ht determined at rest was significantly lower in the training group than in the control group after the training period.

There were no significant differences between the training group and the control group before or after training. The 7 weeks of training did not induce any changes in Hb values in the training group (Table 3).

Before and after the 7 weeks of training the estimated PV decreased significantly at the end of the maximal exercise for both groups. There was a significant posttraining increase of PV at rest for the training group, whereas there were no changes for the control group (Figure 2).

Significant relationships were observed after the 7 weeks of training between PVV determined at the end of the maximal test and VO 2max (r = .76, P < .05) and maximal aerobic speed (r = .60, P < .05) only in the training group.

Discussion

This study clearly shows that 7 weeks of IT induced a significant increase of resting PV along with increases in VO2max and maximal aerobic speed. There was also

<table>
<thead>
<tr>
<th>Trained Group, n = 9</th>
<th>Control Group, n = 6</th>
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<tbody>
<tr>
<td><strong>Before training</strong></td>
<td><strong>After training</strong></td>
</tr>
<tr>
<td>Maximal aerobic speed (km/h)</td>
<td>16.1 ± 1.7</td>
</tr>
<tr>
<td>VO2max (L/min)</td>
<td>4.5 ± 0.7</td>
</tr>
<tr>
<td>VO2max (mL · min⁻¹ · kg⁻¹)</td>
<td>59 ± 9</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>198 ± 7</td>
</tr>
<tr>
<td>Respiratory-exchange ratio</td>
<td>1.1 ± 0.03</td>
</tr>
<tr>
<td>[La]post (mmol/L)</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>[La]post (mmol/L)</td>
<td>6.5 ± 1.8</td>
</tr>
<tr>
<td>[La] 10’ (mmol/L)</td>
<td>5.6 ± 1.4</td>
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</tbody>
</table>

Abbreviations: VO2max, maximal oxygen consumption; [La], blood lactate.

*Significant differences between TG and CG after training, P < .05.

<table>
<thead>
<tr>
<th>Hemoglobin (g/100 mL)</th>
<th>Hematocrit (%)</th>
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<tr>
<td><strong>Before training</strong></td>
<td><strong>After training</strong></td>
</tr>
<tr>
<td>Trained Group, n = 9</td>
<td>Rest 14.6 ± 0.6</td>
</tr>
<tr>
<td>End exercise 14.8 ± 0.8</td>
<td>14.4 ± 0.8</td>
</tr>
<tr>
<td>10’ 14.9 ± 0.7</td>
<td>15.1 ± 0.7</td>
</tr>
<tr>
<td>30’ 14.6 ± 0.7</td>
<td>15.1 ± 0.5</td>
</tr>
<tr>
<td>Control Group, n = 6</td>
<td>Rest 14.8 ± 0.6</td>
</tr>
<tr>
<td>End exercise 15.0 ± 0.6</td>
<td>15.0 ± 0.7</td>
</tr>
<tr>
<td>10’ 15.0 ± 0.8</td>
<td>15.3 ± 0.3</td>
</tr>
<tr>
<td>30’ 15.0 ± 0.5</td>
<td>15.0 ± 0.5</td>
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</tbody>
</table>

*Significant differences from before training for trained group, P < .05.
†Significant differences between trained group and control group at rest after training, P < .05.
a significant correlation between the PVV at the end of maximal graded exercise and VO$_{2\text{max}}$ and maximal aerobic speed.

The increase of maximal aerobic speed and VO$_{2\text{max}}$ observed only in the training group is in agreement with other studies’ results. Indeed, in sedentary recreationally active individuals significant increases of VO$_{2\text{max}}$, maximal aerobic speed, and time to exhaustion were observed after 6 to 7 weeks of high-intensity interval training. The same results were also observed in highly trained runners and cyclists. Thus, the IT used in the current study was of a sufficient load (intensity, volume, and frequency of sessions and training-program duration) and induced aerobic-fitness improvements according to other studies in the literature.

Ht values measured at rest before the training period are comparable to those observed in the literature. Only for the training group, the training period was accompanied by a significant decrease of Ht at rest, at the end of the maximal exercise, and after 10 minutes of recovery. Several studies showed that the exercise repetitions, which develop endurance, induced a decrease of Ht. Others observed that the Ht values of trained subjects are always lower than those of untrained ones. This decrease of Ht in response to endurance training is due to a disproportional increase of PV compared with the increase in red blood cells and is well known as sportsmen’s anemia. For Kargotich et al, this “pseudoanemia” must be considered a physiological adaptation rather than a pathological adaptation. The Hb values measured at rest before the training period are comparable to those observed in the literature for untrained men. There were no significant differences between the training group and the control group before or after the training period. In response to maximal exercise, before and after the training period, the PVV decreased significantly in both groups after training. This result is in accordance with those reported in the literature after continuous training.

The training program induced a significant increase of estimated PV at rest for the training group, whereas there were no changes for the control group. These results agree with those observed in the literature. Indeed, in response to cycling, for a long- or short-term period of continuous training, several authors observed a significant increase of PV. Luetkemeier and Thomas observed in moderately trained cyclists an increase of 9.4% of BV in response to short-term cycling training at low intensities (67–70% VO$_{2\text{max}}$). Williams et al observed a 22% increase of PV by day 5 after 7 consecutive days of strenuous hill walking (17,500 kJ/d). In the same way, Allen et al recorded a 5.9% increase in PV in response to 3 weeks of general mild aerobic exercise (eg, swimming, running, etc). To the best of our knowledge few studies have focused on the effect of IT on PVV, and only in cycling, and the results remained conflicting. Indeed, some studies observed in moderately trained subjects an increase of PV and BV (11.6% and 4.5%, respectively) in response to only 5 consecutive days of intermittent supramaximal (120% VO$_{2\text{max}}$) interval cycling exercise. In the same way, Warburton et al using Evan’s blue method to determine PVV, reported that 12 weeks of continuous and interval aerobic training performed on a cycle ergometer result in similar elevations in vascular volumes in untrained male subjects. But, more recently, studying 8 habitually active male subjects, Hodges et al observed no significant changes in estimated PV after 3 days of interval cycling training. In the current study, which to the best of our knowledge is the first
on the effect of IT running on PVV in highly trained subjects, we noted an increase of about 5% in PV after 7 weeks of IT. The magnitude of this increase differs from those observed in other studies.6,10,17,31,32 These discrepancies in the results can mainly be explained by the experiment protocols involved in the different studies (eg, training intensities, durations, frequency and the subjects’ level of training).

**PVV and Aerobic Performance**

It has been demonstrated that the enhancement of aerobic exercise performance is associated with an increase in BV, which consists of an increase in PV and red cells. These changes can be induced by either long-term exercise training or, artificially, by blood doping.33,34 Changes in PV independent of Hb have a more complicated effect on both VO2max and endurance performance due to the concomitant hemodilution effect.1 In the current study and as in other several studies, a significant increase in PV after exercise training was observed.10,17,27,31,32 In the training group, we also observed a significant increase of VO2max and maximal aerobic speed and a significant correlation after training between PVV and VO2max (r = .76, P < .05) and maximal aerobic speed (r = .60, P < .05). These results were also observed in other studies. Indeed, Carroll et al32 reported a significant increase of PV (+11.2%), which was concomitant with an increase of VO2max (+ 11.2%). Luetkemeier and Thomas7 observed after 3 days of submaximal cycling (92.9 min at 68% VO2max) a reduction in the time to reach a target work goal and an increase in average cycling power output. Several other studies conducted at the laboratory of Green et al and involving relatively short-term training observed an increase in VO2max of 17.2%27 and an increase in peak power output of 23 W.17 However, these changes are not consistent with another study conducted at the same laboratory,13 in which no change in VO2max was observed. More recently, Gibala and colleagues35 conducted several experiments and demonstrated that 3 to 6 very high-intensity training sessions over 2 weeks led to a significant increase in endurance performance. However, in the latter studies, skeletal-muscle metabolism was investigated and the PV changes were not studied. Nevertheless, one might note that there are multiple determinants of VO2max, and when examining exercise performance the only link to PVV is certainly tenuous.1

**Practical Applications**

The findings of this study showed that only 7 weeks of IT running may increase aerobic fitness of well-trained male adult subjects. Trainers and coaches can use the simple training program presented in this study to enhance aerobic fitness for trained and nonelite endurance subjects. This training program is easy and respects the major training principles of progressivity and specificity.

**Conclusion**

In conclusion, the current results clearly demonstrate that only 7 weeks of running IT led to a significant increase in aerobic fitness (VO2max and maximal aerobic speed) in highly trained subjects, and this increase may be linked to the increase of PV observed. Indeed, these increases in aerobic-fitness variables concomitant with those of PV were only observed in the trained group. Consequently, coaches can use the simple training program presented in this study to enhance aerobic fitness for trained and nonelite endurance subjects. This training program is easy and respects the major training principles of progressivity and specificity.

**Acknowledgments**

This article is dedicated to our young colleague Délphine Thevenet (PhD, 31 y), who died in November 2010. Délphine participated in this study from the design to the draft of the manuscript, and she conducted all the experiments on the track.

The authors would like to thank all the students from the ISSEP of Tunisia for their participation, as well as the staff of the CNMSS.

**References**


