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Serum iron and transferrin during an exhaustive session of interval training

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Summary. Conflicting data have been reported on "sports anaemia" and anaemia during physical training. Most of these results are of studies at rest before or after training. The aim of this investigation was to further study the profiles of serum iron (Se Fe) and transferrin (Se Tr), in 14 physically trained men (28 ± 6 years) during an exhaustive interval training session. The 45 min Square-Wave Endurance Exercise Test (SWEET) was performed on a cycle ergometer. To the SWEET base, established as a % of individual $\dot{V}_{O_{2max}}$, a peak of 1 min at $\dot{V}_{O_{2max}}$ was added every 5 minutes. Arterial blood samples were taken at rest, during the SWEET at the 14th, 15th, 29th, 30th, 44th and 45th minutes, just before and after the peaks, and at the 15th min of recovery. Lactate, acidity [H^+], $PaCO_2$, PaO_2, Haematocrit (Hct), Haemoglobin (Hb), Se Fe and Se Tr were measured. After the SWEET, weight loss was 0.89 ± 0.15 kg. Lactate and serum iron rose progressively at the base levels and at the peaks, while $PaCO_2$ and bicarbonate fell progressively. Hct, [Hb], serum transferrin and [H^+] increased significantly at the 14th min of SWEET and thereafter no change was observed. At the 45th min with respect to the value at rest, Se Fe increased as much as +32%, Se Tr +13% and [Hb] +8%. Haemoconcentration could explain the changes in Se Tr but not the total significant increase in Se, Fe, which moreover is not explained by acidosis [H^+]. If serum transferrin was saturated, the transitory iron changes during SWEET would make way for a readily available iron reserve for synthesis of myoglobin, and moreover it is probable that a certain quantity of iron lost in the sweat and in the urine could explain the iron deficiency observed with training programmes.

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Introduction

Conflicting data have been reported regarding the iron status of subjects involved in training programmes. Kilbom (1971) reported that serum-iron concentrations (Se Fe) in women were significantly lower after physical training, while Pate et al. (1979), and Frederickson et al. (1983) found that training did not alter Se Fe. More recently Dufaux et al. (1981) have shown that serum ferritin, Se Fe or haemoglobin concentration [Hb], were significantly lower in middle and long-distance runners than in the other three groups: control students, rowers or professional racing cyclists (Yoshimura 1970, Hunding et al. 1981, Ehn et al. 1980, Hegenauer et al. 1983, Wishmitzer et al. 1983). Liesen et al. (1977) revealed an acute increase in transferrin in response to exercise after one day. However after an arduous triathlon competition Rogers et al. (1986) observed that serum iron was significantly reduced after the race in which the mean time expended was 11 h.

No published studies have yet addressed the question of the importance of Se Fe and serum transferrin (Se Tr) variations during a vigorous laboratory exercise test simulating a training session. The purpose of this study was therefore to further investigate the profile of serum iron and transferrin in physically trained men during an exhaustive bicycle exercise test lasting 45 min (Gimenez et al. 1982a).

Materials and methods

Fourteen normal male subjects (mean age 28 ± 6 years) volunteered for this study after having been informed of the protocol involved. They completed a medical questionnaire and underwent a cardiopulmonary and electrocardiographic examination, all of which were normal for all subjects. They had practiced sport, for five or more hours per week for more than three months. Firstly, maximal oxygen uptake ($\dot{V}_{O_{2max}}$) was measured directly with a progressive test: an initial load of 30 W was increased by 30 W every three min (Gimenez et al. 1981). The highest level maintained for 2 or 3 min, referred to as "maximum tolerated power" (MTP) is an essential measurement for the experimental test to be described. The Square Wave Exercise Endurance Test (SWEET), of 45 min duration, consisted of a submaximal base level, at a % of MTP depending on the degree of the subject's training (aerobic level). A 60 s peak at MTP was superimposed on the base level activity every 5 min (anaerobic level). Maximum Intensity of Endurance during the SWEET (MIE_{45}) is defined by both maximal heart rate at the end of the test (220-age), and the impossibility of maintaining 5% above the percent MTP of the MIE_{45}. Exhaustion would be reached at the end of the MIE_{45}, which is expressed as total mechanical work (TMW) in $kJ \cdot kg^{-1}$ (Gimenez et al. 1982a). None of the subjects participated in any regular physical training or competition for at least three days before the study.

The subjects arrived at the laboratory at 8 a.m. after having had a light breakfast with no fat content two hours before. After half an hour of rest, a soft catheter was placed in the radial artery. The first blood sample was taken after 15 to 20 min of rest. The subject was then seated on a bicycle ergometer (Jaeger) and performed the 45 min Square-Wave Endurance Exercise Test (SWEET) at the Maximal Intensity of Endurance (Gimenez et al. 1982a). The blood samples were taken every fifteen minutes of exercise, before and after the peak, i.e. 14th and 15th minutes, 29th and 30th minutes and 44th and 45th minutes. The last blood sample was taken during the recovery phase, 15 min after the end of exercise.

Ventilation ($\dot{V}_E$), respiratory exchanges:oxygen uptake ($\dot{V}_{O_2}$), CO_2 output ($\dot{V}_{CO_2}$) and respiratory rate, were measured continuously using a Jaeger Ergopneumotest with a Dataspir EDV 70 data processing system (E. Jaeger, Würzburg, FRG). The electrocardiogram was continuously monitored on a screen and recorded (Hellige-France, Multiscriptor EK33).

The following analyses were made in the blood samples; blood gases, using an ABL Radiometer (pH, PaO_2, $PaCO_2$) and lactic acid, as previously described (Gimenez et al. 1982a). The concentration of bicarbonate was calculated from the pH and $PaCO_2$. Whole blood hemoglobin concentration [Hb] was measured with a Radiometer OSM_2, hematocrit (Hct) by the microcapillary method; serum iron (Se Fe) by a modified method of Ichida et al. (1968) adapted to Prisma, and serum transferrin (Se Tr) using a nephelometer laser Hoechst Behring (Marburg, FRG) and Behring's antibodies. All assays were carried out in duplicate. Quality controls were included in all sets of measurements, and the coefficients of variation were 4.49% for Se Fe and 3.1% for transferrin.

The paired t test difference and an analysis of variance were used for statistical purposes.

Results

Table 1 shows the mean values of physical characteristics, $\dot{V}_{O_{2max}}$ and the Maximal Intensity of Endurance during 45 min (MIE_{45}) expressed as $kJ \cdot kg^{-1}$. $\dot{V}_{O_{2max}}$ and MIE_{45} values correspond to those subjects who trained regularly (Gimenez et al. 1982b). Sweat output, as clinically observed, and weight loss as measured, were 0.89 ± 0.15 kg.

Table 1. Physical characteristics, $\dot{V}_{O_{2max}}$ and maximal intensity of endurance during 45 min (MIE_{45}) of the 14 subjects studied

	Age (year)	Weight (kg)	Height (cm)	$\dot{V}_{O_{2max}}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	MIE_{45} TMW ($kj \cdot kg^{-1}$)
$\bar{X}$	28	71	174	58	7.8
SD	6	5	5	9	0.6

TMW = Total Maximal Work performed during the MIE_{45}

Figure 1 represents the evolution of $[H^+]$, [lactate] and [bicarbonate]:[lactate] rose significantly at the 14th min. There was a significant increase in the peaks of the 15th, 30th and 45th minutes in relation to the preceding base (14th, 29th and 44th min respectively). Moreover [lactate] rose progressively at the base levels and at the peaks, the highest values corresponding to those of the 44th and

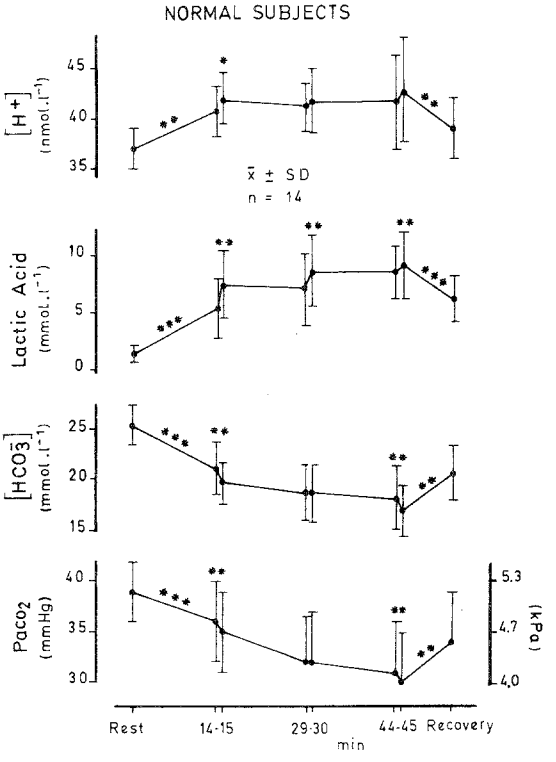

Fig. 1. Arterial H^+ concentration $[H^+]$, lactic acid, bicarbonate $[HCO_3^-]$, and carbon dioxide arterial pressure ($PaCO_2$) at rest, during the 45 min square-wave endurance exercise test, and at the 15th min of recovery. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

45th min (8 ± 2.5 and 9.4 ± 2.4 mEq·l^{-1} respectively). A significant diminution was observed at the 15th min of recovery (4.87 mEq·l^{-1}). Bicarbonate and $PaCO_2$ fell at the 14th min and both present an evolution inverse to that of [lactate] (Fig. 1). There was a diminution at the 15th and 45th min in relation to the preceding bases (14th and 44th min). At the end of exercise (last peak), the values were 17 ± 2.5 mEq·l^{-1} for the bicarbonate and 30 ± 5 mm Hg for $PaCO_2$. At the 15th min of recovery the values rose to 20.8 ± 2.7 mEq·l^{-1} and 34 ± 5 mm Hg respectively. The value of $[H^+]$ increased significantly at the 14th and 15th min, but no differences were observed between the 14th and the 29th and 44th min, or the 15th and 30th and 45th min. In about 80% of the subjects (11/14) $[H^+]$ at the end of exercise was within the normal range of variations observed at rest (37—43 nEq·l^{-1}). PaO_2 (92 ± 6 mm Hg at rest) showed a slight fall at the 14th min (86 ± 5 mm Hg, $p < 0.01$) but did not change thereafter until the end of exercise. Figure 2 represents the evolution of transferrin, serum iron and [Hb]. Transferrin increased significantly at the 14th min (+13%) and thereafter no changes were observed with the exception of the difference between base and peak at the 29th—30th min of exercise. The variance analysis does not show any variations in the values of bases or peaks and no differences existed between the 14th and 44th min or the 15th and 45th min. In relation to the end of exercise, transferrin fell significantly in the 15th min of recovery but still remained significantly higher ($p < 0.001$) than the values at rest. Serum iron from rest rose at the 14th min (+13%). There was a significant difference between the 14th and 15th min and between the 29th and 30th min of exercise, the highest values being those of the peaks (15th and 30th min respectively). The variance analysis test showed a significant increase as much for the bases (14th, 29th and 44th min F=2.5%) as for the peaks (15th, 30th and 45th min F=1%). At the 45th min with respect to the value at rest, serum iron increased by as much as 32%. No change was observed between the end of exercise (45th min) and the 15th min of the recovery phase, values of which were significantly higher than those observed at rest ($p < 0.001$). Haemoglobin concentration and haematocrit increased in the 14th min of exercise (+8%) and thereafter no change was observed, with the exception of the recovery phase, in which [Hb] fell significantly (Fig. 2). The values of haematocrit were, at rest: 43.20 ± 0.9; during exercise: 46.7 ± 1.2, 46.9 ± 1.1, 46.4 ± 1.0, 46.7 ± 1.3, 46.9 ± 0.8, 47 ± 1.2 respectively and, at the 15th minute of recovery: 45.6 ± 1.3. Only between the rest and 14th min of exercise were values different ($p < 0.001$) and likewise between the 45th min of exercise and recovery values.

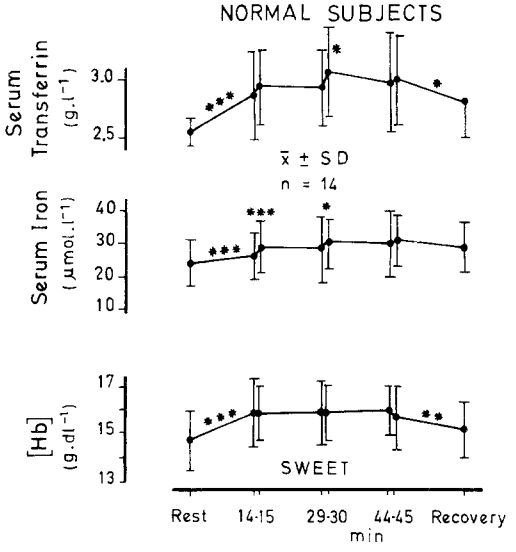

Fig. 2. Evolution of the mean values of serum transferrin, serum iron and haemoglobin concentration during the exhaustive session of interval training (45 min Square-Wave Endurance Exercise Test). * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

Discussion

Because "sports anaemia", a reduction in [Hb], Se Fe and transferrin (Yoshimura 1980; Pate 1983) is most commonly associated with strenuous training (Yoshimura 1970; Puhl and Runyan 1980; Hunding et al. 1981), the total amount of work performed at the Maximal Intensity of Endurance of SWEET (Table 1) represents a good endurance training session (Gimenez et al. 1982a) and allows analysis of haematological responses in well trained subjects.

The main features of the results presented in this study are the different patterns of change in serum iron and serum transferrin during the exhaustive session of training with the MIE_{45}. Both Se Fe and Se Tr rose at the 14th and 15th min of the SWEET. Thereafter Se Tr does not change during exercise, while Se Fe continues to increase progressively until the end of the test when the total increase represents twice that of serum transferrin. Taking into account that an increase of 8% in haemoglobin concentration in whole-blood represents approximately a double increase in the plasma concentration of nonpermeating sub-

stances (Van Beaumont et al. 1973), the change in Se Tr could be explained by a haemoconcentration effect during exercise. Moreover, both Se Tr and [Hb] decreased significantly during recovery, while iron did not change. This suggests that in this study, after the 15th min of exercise, the iron increase overtakes that of transferrin. This evolution of Fe Se could not be explained by the metabolic acidosis as measured by the $[H^+]$, and haemoconcentration is not the only mechanism of this profile (Fig. 1).

Changes in iron status, which could be evaluated by assessing indices of red-cell iron and iron availability, were evaluated only by serum iron, and thus the interpretation is more complex. However, this release of iron during exercise can follow two paths.

If we consider transferrin during the exhaustive session of MIE_{45} (Fig. 2) at maximum saturation (Haralambie 1975), then transferrin is ineffective in promoting the absorption of iron (Banister et al. 1985; Haralambie 1975). Depressed iron absorption in male athletes during strenuous training has been reported (Ehn et al. 1980) and significant iron loss in sweat has also been documented (Vellar 1968). Although not measured in this study, there is probably a certain low of iron in the sweat during the MIE_{45}, but excretion of iron shows definite limits (Bowering et al. 1976). Also, without signs of bleeding, haemolysis or increased excretion of haemoglobin via the urine, Ehn et al. (1980) showed increased elimination of radio iron at a rate corresponding to 2 mg iron/day. This fact seems to be related to the persistance of the high values of Se Fe observed during recovery in this study (Fig. 2), and suggests an increased iron elimination during training, explaining the empty iron stores described in the literature (Yoshimura 1970; Pate 1983; Rogers et al. 1986).

It is known that training causes an increase in myoglobin concentration [Mb] within skeletal muscles in man (Astrand et al. 1960; Astrand and Rodahl 1977) and animals (Pattengale and Holloszy 1967; Hickson 1981; Booth 1978). Booth (1978) found an increase in Mb in the gastrocnemius of the guinea pigs subjected to training on a treadmill after only two days. In previous studies (Gimenez and Florentz 1979a, 1979b), we observed in rats exercising in situ with electrical stimulation for 10 min, which corresponds from the biochemical results (lactate and glycogen) to those observed in the rat on a treadmill at a speed of 48 $m \cdot min^{-1}$ (Baldwin et al. 1977), that myoglobin concentration increased significantly in soleus. The fact that there is an increase in Mb after 10 min stimulation evokes the possibility of either the existence of [Mb] ready to be produced, or more probably a complete Mb synthesis. One of the components necessary for Mb synthesis is iron: the level of [Mb] varies with a diet lacking in, or rich in iron (Kagen 1973). As shown in the present study, the iron increases progressively during intensive exercise and overtakes transferrin saturations during exercise of short duration (Haralambie 1975). Exercise can cause a loss of sarcoplasmic proteins (myoglobin) into the blood of animals or normal healthy subjects (Demos et al. 1974; Fowler et al. 1962) and a physiological myoglobinuria in the hours following intensive or moderate exercise in healthy subjects has also been described (Abraham 1977). Finally, physical training produces a decrease in blood iron in both men (Yoshimura 1970; Ehn et al. 1980; Dickson et al. 1982; Banister and Hamilton 1985; Rogers et al. 1986) and women (Kilbom 1971; Pate et al. 1979; Frederikson et al. 1983; Hegenauer et al. 1983). If the mechanisms of this apparent Mb synthesis are not clear, such an acute increase would help to explain the Mb increase observed after training (Astrand and Rodalh 1977; Pattengale and Holloszy 1967; Booth 1979; Hickson 1981). If so, the increased iron requirement for synthesis of Mb may temporarily take precedence over erythropoiesis so as to facilitate oxygen delivery to the exercising muscle. Iron may then become available again for red cell synthesis when Mb has stabilized. Elevated free erythrocyte porphyrin (FEP) concentrations are an indication of abnormalities in haeme synthesis. The significant increases that occurred in a runner's FEP reported by Frederickson et al. (1983) provides more evidence that there is a reduction in iron reserves or at least a demand for erythropoiesis during training. Because it integrates both iron supply and iron demand, FEP concentration, which was not measured during this study, is, as pointed out by Frederickson et al. (1983), perhaps the best index of iron adequacy to be studied during exercise.

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