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LONGITUDINAL STUDY OF RELATIONSHIPS BETWEEN RED CELL AGGREGATION AT REST AND LACTATE RESPONSE TO EXERCISE AFTER TRAINING IN YOUNG GYMNASTS

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ABSTRACT

Increased red cell aggregation appears to experimentally impair muscular microcirculation and thus O_2 supply to muscles. In sportsmen, we reported in three different cross-sectional studies a correlation between RBC aggregation and lactate release during exercise, which could be explained by this mechanism. This study aimed at confirming this finding in a follow up study of young gymnasts submitted to a 6 months training session. 11 gymnasts (age 12-14.5 yr; 7 girls and 4 boys; weight 33-60.5 kg; height 1.44-1.7m) underwent a 15 min submaximal incremental exercise-test on cycloergometer before and after the training session, as part of a check-up for detecting adverse effects of training on growth and puberty. The difference between RBC aggregation (measured with the Myrenne erythroaggregometer) before and after training was correlated to the difference in blood lactate area under the curve during exercise before and after training ('M' index which measures aggregation during stasis after disaggregation at 600 s^{-1}: $r=0.727$ $p<0.02$; 'M1' index which measures RBC aggregation at low shear rate after disaggregation: $r=0.832$ $p<0.01$). Changes in plasma viscosity during the same period are also positively correlated to changes in lactate area: $r=0.717$ $p<0.02$). Since changes in aggregation and changes plasma viscosity are not correlated, they appear to be independent determinants of lactate response during exercise. Thus, decreases in RBC aggregation and/or plasma viscosity after training in young gymnasts are associated with an improvement in aerobic metabolism during exercise. Although a causal relationship remains to be demonstrated, this study, in agreement with previous ones showing a correlation between RBC aggregation and lactate response, suggests a possible involvement of RBC aggregation in O_2 transfer to exercising muscles.

Key words: Blood viscosity, hematocrit, exercise, gymnasts, hemorheology, erythrocyte deformability, erythrocyte aggregation, blood lactate.

INTRODUCTION

Exercise training induces metabolic and circulatory adaptations that improve an individual's ability to sustain high rates of aerobic metabolism (1). One of the most widely investigated biological parameters affected by training is blood lactate. Its concentration after training is lower at a given submaximal exercise power output than before training (2-6). Classically, lactate accumulation into blood was supposed to reflect a shift towards 'anaerobic' metabolism, i.e. a relative deficiency in O_2 supply to muscle at high power intensities of exercise. Clearly, individuals can perform larger amounts of work than that which is reflected by their O_2 consumption i.e. there can be during exercise an O_2 deficit (7). However, the concept of anaerobiosis has been widely criticized, and blood lactate accumulation is rather interpreted as the consequence of differences in metabolite flux rates through the glycolytic pathways in various exercise conditions (8).

Actually, muscles experimentally submitted to hypoxia release higher amounts of lactate (9), and even more if they are exercising (10-13). Thus, if a lower O_2 supply to muscle is no longer the only explanation of blood lactate accumulation during exercise, it remains true that reducing O_2 transfer to muscle cells will result in increased production of lactate. In addition, hypoxia experimentally seems to decrease also lactate clearance, resulting in an even higher hyperlactatemia (9).

One factor which is potentially important for O_2 distribution to tissues is blood viscosity (14-15). More specifically, excessive erythrocyte aggregation has been reported to result in inhomogeneous O_2 supply (16) and to impair microcirculatory blood flow in muscle (17).

Consistent with these concepts, we reported in three different cross-sectional studies in sportsmen a correlation between RBC aggregation and lactate accumulation into blood during exercise (18, 19, 20).

This study aimed at confirming this finding of cross-sectional studies in a follow up study of young gymnasts submitted to a 6 months training session.

SUBJECTS AND METHODS.

Subjects.

11 gymnasts (age 12-14.5 yr; 7 girls and 4 boys; weight 33-60.5 kg; height 1.44-1.7m) underwent a 15 min submaximal incremental exercise-test on cycloergometer before and after the training session, as part of a check-up for detecting adverse effects of training on growth and puberty. At the time the study was performed, they were submitted to 12 hr training each week.

Laboratory measurements

Pubertal stage was precised by the measurement of dehydroepiandrosterone (DHA) sulphate (in both sexes), testosterone (in boys) and estradiol (in girls) by standardized routine radioimmunoassay techniques.

Hemorheological measurements

Blood samples for hemorheological measurements (7 ml) were drawn with potassium

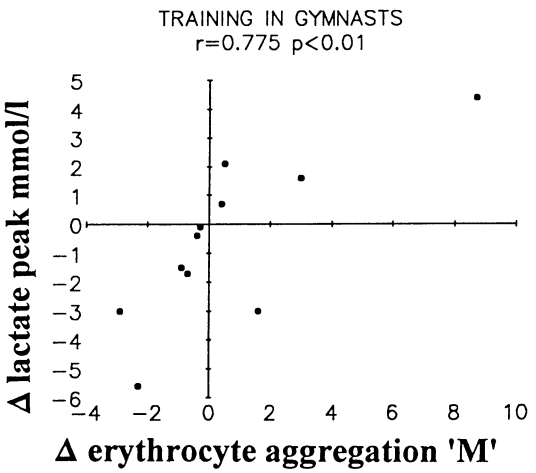

FIG. 1.
Correlation between changes in preexercise RBC aggregation 'M' index and changes in lactate peak during exercise (r=0.775 p<0.01).

EDTA as the anticoagulant in a vacuum tube (Vacutainer). Viscometric measurements were done at very high shear rate (1000 s^{-1}) with a falling ball viscometer (MT 90 Medicatest, F-86280 Saint Benoit) (21-22). Accuracy of the measurements was regularly controlled with the Carrimed Rheometer 'CS' (purchased from Rhéo, 91120 Palaiseau, France)(23). The coefficient of variation of this method ranges between 0.6 and 0.8% (24). We measured with this device apparent viscosity of whole blood at native hematocrit, plasma viscosity, and blood viscosity at corrected hematocrit (45%) according to the equation of Quemada (25):

$$\eta b = \eta pl \,.\, (1 - 1/2\ k.h)^{-2}$$

where ηs is blood viscosity, ηpl plasma viscosity, h the hematocrit and k a shear dependent intrinsic viscosity of the red cells according to Quemada. Two indices of erythrocyte rigidity were calculated: Dintenfass' 'Tk' (26-27) and Quemada's 'k' (25). RBC aggregation was assessed with the Myrenne aggregometer (28) which gives two indices of RBC aggregation: 'M' (aggregation during stasis after shearing at 600 s^{-1}) and 'M1' (facilitated aggregation at low shear rate after shearing at 600 s^{-1}).

Biochemical analyses

The sampled blood was centrifuged and the plasma assayed for diverse parameters by well standardized and routine techniques, on an automatic clinical analizer (DuPont de Nemours). Lactate was assayed with the kit from DuPont specially adapted to this analyzer. This assay was based on NADH production by rabbit lactate dehydrogenase. Coefficients of variation range beween 0,7 and 5.6 %.

Statistics

Correlations were tested by linear regression analysis. Results are presented as mean ± the SE of the mean. Modifications of parameters before and after training were

assessed using the two tailed nonparametric test of Wilcoxon for paired data. Significance was defined as $p<0.05$.

TABLE I.

	before	after
$\dot{W}170$ (w/kg)	1.74(0.16)	1.71(0.09)
lactate peak (mmol/l)	5.69(0.48)	5.48(0.42)
blood viscosity (mPa.s)	2.18(0.08)	2.11(0.05)
corrected viscosity η_{45} (mPa.s)	2.36(0.08)	2.36(0.04)
plasma viscosity (mPa.s)	1.22(0.02)	1.22(0.02)
"Tk" (RBC rigidity)	0.50(0.02)	0.51(0.01)
hematocrit (%)	40.62(1.13)	38.07(0.93)
aggregation 'M'	4.51(0.6)	5.76(1.09)
aggregation 'M1'	6.23(0.73)	7.08(1.49)

Modifications (mean ± SEM) of rheologic parameters before and after training. No significant improvement on the whole group.

Other calculations

Area under the curve of blood lactate over 25 min (15 min exercise and 10 min recovery) was calculated with the trapezoidal rule. Physical working capacity $\dot{W}_{170}$ was calculated, as the work in watts that the subjects can perform at a heart rate of 170 $b.min^{-1}$ (29-30), interpolated after linear regression with the least squares method.

RESULTS

The maximum lactate value during exercise ranged between 2.7 and 8.4 $mmol.l^{-1}$ before training and between 2.8 and 8 $mmol.l^{-1}$ after training. $\dot{W}_{170}$ ranged between 0.9 and 2.84 watt/kg before training and 1.24 and 2.35 watt/kg after training. Evolution of parameters during training is shown on table 1. On the whole, there was no uniformous evolution of hemorheologic parameters, as well as no uniformous improverment in fitness parameters. $\dot{W}_{170}$ increased in 4 subjects and decreased in 5. Lactate accumulation into blood (as assessed by both lactate peak and lactate area) decreased in 7 subjects and increased in 4. The most interesting findings were the correlations between changes in hemorheologic parameters and changes in lactate accumulation after the training period. Changes in lactate peak value were correlated to changes in preexercise values of the following parameters: M ($r=0.775$ $p<0.01$ see fig.1), M1 ($r=0.630$ $p<0.05$) and plasma viscosity ($r=0.672$ $p<0.05$). Changes in lactate area under the curve over 25 min were correlated to changes in preexercise values of the following parameters: M1 ($r=0.727$ $p<0.02$), M ($r=0.832$ $p<0.01$ see fig.2), plasma viscosity ($r=0.717$ $p<0.05$ see fig.3), and whole blood viscosity at native hematocrit ($r=0.635$ $p<0.05$ see fig.4). Changes in plasma viscosity were correlated with neither changes in M ($r=0.296$) nor changes in M1 ($r=0.331$) nor changes in whole blood viscosity ($r=0.333$).

DISCUSSION

The main finding of this study is that training-induced changes in erythrocyte aggregability as assessed by the Myrenne apparatus parallel modifications in blood

lactate accumulation. When erythrocyte aggregability decreases, blood lactate accumulation is also reduced. Changes in preexercise plasma viscosity are also positively correlated with changes in lactate accumulation, and are not correlated with changes in M.

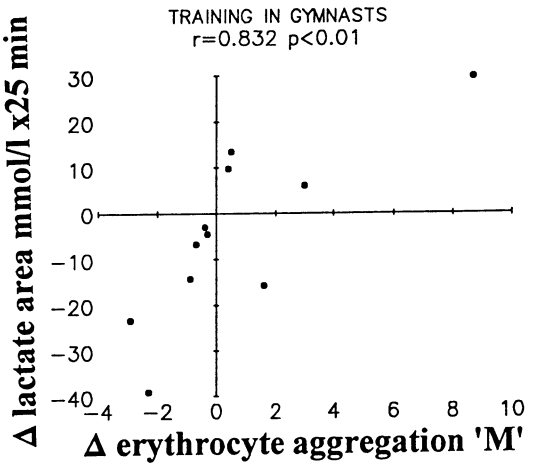

FIG. 2.
Correlation between changes in preexercise RBC aggregation 'M' index and changes in lactate area under the curve over the 25 min of exercise and recovery (r=0.832 p<0.01).

There is also a less significant correlation between these changes in lactate accumulation and changes in preexercise whole blood viscosity at native hematocrit. Therefore, erythrocyte aggregability, plasma viscosity, and to a lesser extent whole blood viscosity seem to be independent determinants of postexercise hyperlactatemia. This longitudinal study confirms the results of our previous cross-sectional ones and further supports the concept of an influence of blood rheology on muscle 'aerobic' metabolism.

This study is a part of a follow-up protocol of high level young gymnasts submitted to a hard training program. This protocol aims at determining to which extent this hard training is harmful for growth and puberty, and which specific testing can be proposed for such adolescents submitted to high level training. Some alterations in trace element status (31) as well as in the growth hormone/somatomedin axis (32-33) have already been found. Blood rheology was also attractive to study since it is now largely demonstrated that improvements in fitness are correlated to a decrease in blood viscosity (34-38). This decrease is largely explained by the "autohemodilution" which occurs when plasma volume increases after training (39) and makes exercise a "hemorheological treatment" (40).

In this protocol, one cannot describe a standard evolution of children during training. Some improve their W_{170} while some others decrease it. Similarly, lactate response improves in some children and worsens in some others. Apparently, this training improves fitness in some subjects, but is too hard and results in "overtraining" in others. This response to training was interesting for our purpose of looking at the parallel evolution of some fitness parameters and some rheologic ones. In addition, it suggests that plasma viscosity and erythrocyte aggregation might be interesting markers of the effects of training in children. Whether this measurement can be proposed for the follow-up of adolescents (as well as adult athletes) in sports medicine

requires to be further studied, but is supported by the current findings.

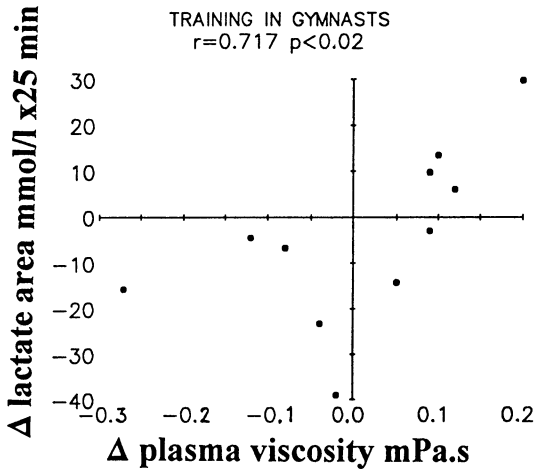

FIG. 3.
Correlation between changes in preexercise plasma viscosity and changes in lactate area under the curve over the 25 min of exercise and recovery (r=0.717 p<0.02).

The precise meaning of changes in blood lactate concentration in response to exercise is extremely complex, and it is clear that lactate flux rates cannot be determined solely from changes in blood lactate concentrations (2, 5, 8). Further studies with a more sophisticated protocol will be needed for determining if lactate production by muscles, or lactate removal from blood, or both, are influenced by changes in erythrocyte aggregation.

At present, we can only speculate that the relationship we have observed in four separate studies between erythrocyte aggregation and lactate accumulation into blood is explained by a lower supply of O_2 to exercising muscles. Although there is no direct demonstration of this, we think that several data from the literature support this explanation. First, experimental limitation of O_2 supply to muscles results in increase lactate production (9-13). Moreover, the experiments of Vicaut (17) indicate that increasing aggregation of the red cells decreases microvascular perfusion in skeletal muscles. In addition, the importance of circulatory mechanisms in the "warm-up" process of muscles which protects against excessive lactate accumulation has been recently emphasized (41).

In conclusion, this longitudinal study provides further evidence of a close relationship between erythrocyte aggregation and lactate production by muscles during exercise. While additional studies are necessary for giving a precise explanation of this relationship, we suggest that an influence of erythrocyte aggregation on microcirculatory adaptation of the exercising muscle may be the underlying mechanism. Thus, beside the balance between metabolic pathways (8) and the speed of enzymatic reactions involved in glucose oxidation (2), we speculate that there would be truly some degree of 'anaerobiosis' involved in the physiological process of exercise-induced lactate accumulation.

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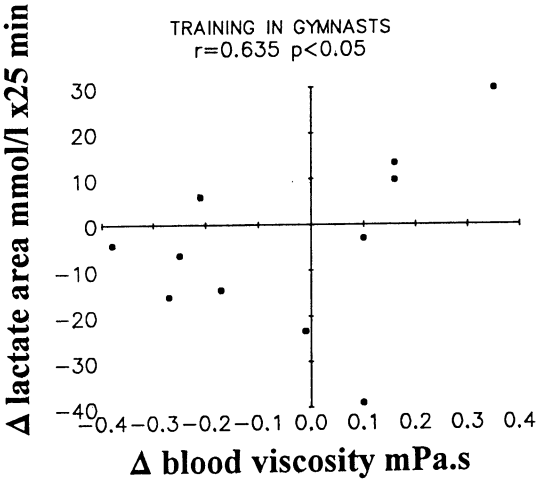

FIG. 4.
Correlation between changes in preexercise blood viscosity and changes in lactate area under the curve over the 25 min of exercise and recovery (r=635 p<0.05).

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