BEHAVIOUR OF THE ERYTHROCYTE DEFORMABILITY AND NITRIC OXIDE METABOLITES IN UNPROFESSIONAL ATHLETES / COMPORTAMENTO DA DEFORMABILIDADE ERITROCITÁRIA E DE METABOLISMOS DO MONÓXIDO DE AZOTO EM ATLETAS AMADORES

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ABSTRACT

We examined, in 81 athletes subdivided into three groups according to the practised sport (endurance, mixed, power), erythrocyte deformability and nitric oxide metabolites (NOx). In the whole group and in athletes that practised endurance and mixed sports we observed, in comparison with sedentary controls, an increase in erythrocyte deformability. In the same groups we found an increase in plasma NOx level although we did not note any significant correlation between these parameters.

Physical training induces significant effects on the haemorheological pattern¹ of which the major components are haematocrit, plasma viscosity, red cell aggregation and deformability. Each of these rheological parameters acts in a specific area of the circulatory system in relation to the velocity gradient and so pointing out the strong link between non-newtonian blood viscosity and haemodynamic profile.

Red cell deformability, that together with plasma viscosity plays a pivotal role in the microcirculation, physiologically depends on the surface-volume ratio, internal viscosity and membrane dynamic properties but also by pH, osmolarity, mechanical factors and nitric oxide (NO). The influence of NO on red cell deformability is related to dose^{2,3} and partly dependent on guanylate cyclase activity⁴; its influence is significantly reduced by the employment of NO synthesis inhibitors^{4,5}.

Up to now the literature data show that in several sports at rest an increase of red cell deformability has

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Corresponding author: Prof. Gregorio Caimi Via Leonardo da Vinci, 52 90145 Palermo, Italy Fax: +39 91 6554535 *e-mail:* caimigre@unipa.it been observed in comparison with sedentary controls⁶⁻¹² as well as in athletes a greater percentage of younger cellular elements has been found^{9,10,13}. On the other hand the exercise training causes a significant increase in NO production¹⁴⁻¹⁷.

Considering these aspects we examined erythrocyte deformability and NO metabolites $(NO_2^- + NO_3^- = NOx)$ in 81 athletes (55 men and 26 women; mean age 31.5 ± 8.7 years) subdivided into 3 subgroups. The first group included 28 subjects (23 men and 5 women; mean age 35.9 ± 10.0 years) who practised endurance sports (14 cyclists, 14 endurance swimmers). The second group included 30 subjects (20 men and 10 women; mean age 28.5 ± 7.8 years) who practised mixed sports (11 basket players, 10 judoists, 9 water polo players). The third group included 23 subjects (19 men and 4 women; mean age 30.6 ± 6.1 years) who practised power sports (4 sprint runners, 5 weightlifters, 14 sprint swimmers).

The control group included 27 healthy sedentary subjects (20 men and 7 women; mean age 33.2 ± 5.6 years).

The erythrocyte deformability was examined using the diffractometer Rheodyn SSD of Myrenne^{18,19}. This instrument measures the diffraction pattern of a laser beam passing through erythrocytes suspended in a viscous medium and deformed by a force with defined shear stresses. A measure of erythrocyte deformation is the Elongation Index (EI) = (L-W)/ (L+W)×100, where L = length and W = width of the erythrocytes. We considered the EI at the shear stress of 60 Pascal (Pa).

The NO production was evaluated by a micromethod which measures

the concentration of both NO metabolites (nitrite plus nitrate). At first nitrate was converted into nitrite by a nitrate reductase, then nitrite was assessed by spectrophotometry after addition of the Griess reagent²⁰.

The values were expressed as means \pm standard deviation. The difference between sedentary controls and athletes was evaluated according to the Student's t test for unpaired data.

Erythrocyte deformability, expressed as EI, was significantly increased in athletes (Controls: $43.85 \pm$ 4.48; Athletes: 47.21 ± 4.46 ; p < 0.01). Subdividing the whole group of athletes into three subgroups according to the practised sport, we noted (Fig. 1) in the endurance athletes an increase in erythrocyte deformability (EI: 46.98 ± 4.03 ; p < 0.05 vs controls); the same behaviour was even more evident in the mixed athletes (EI: 49.52 ± 3.42 ; p < 0.001 vs controls) while in the power athletes no difference was evident in comparison with control subjects (EI: 44.45 ± 4.66).

The evaluation of NOx showed an increase in the whole group of athletes (controls: 26.67 ± 18.63 micromol/l; Athletes: 41.16 ± 24.87 micromol/l; p < 0.01); this increase (Fig. 2) was statistically significant only in endurance and mixed athletes (endurance athletes: 43.01 ± 23.08 micromol/l; p < 0.01 vs controls; mixed athletes: 45.85 ± 30.63 micromol/l; p < 0.01 vs controls) and not in power athletes (33.00 \pm 16.45).

From the obtained data it was evident that erythrocyte deformability, at rest, distinguished sedentary controls only from athletes that prac-

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Fig. 1 - Erythrocyte deformability, expressed as elongation index, in sedentary controls and in the three subgroups of athletes



Fig. 2 - Nitric oxide metabolites (NOx) in sedentary controls and in the three subgroups of athletes

tised aerobic and mixed sports. Also a NOx increase was present in these groups of athletes, although we did not find, using linear regression, any correlation between NOx values and elongation index. Our observations confirm several findings concerning the behaviour of erythrocyte deformability found in athletes⁶⁻¹², also considering that, in athletes who practise power sports, the erythrocyte turnover is not particularly accelerated, differently from endurance athletes²¹. Our data also underline the role of training on the NOx level. The datum in fact confirms the strong link between exercise and endothelium and in particular how regular exercise seems to upregulate eNOS expression, even if up to now it is not sure if this upregularion is due to the shear stress or metabolic factors¹⁷.

In conclusion, these data show that in athletes that practise aerobic and mixed sports the increase of NOx plasma level is accompanied by an increase of erythrocyte deformability; the latter, as it is known, plays a pivotal role in the microcirculation system and influences the tissue oxygen transport. These results may contribute to explain the significant role of the aerobic exercise in the cardiovascular prevention.

BIBLIOGRAPHY

- Marton Z, Toth K. Exercise and hemorheology. In: Baskurt OK, Hardeman MR, Rampling MW, Meiselman HJ (Eds.). Handbook of hemorheology and hemodynamics. Amsterdam: IOS Press, 2007:422.
- Kleinbongard P, Schulz R, Rassaf T, *et al.* Red blood cells express a functional endothelial nitric oxide synthase. Blood 2006; 107:2943.
- Carvalho FA, Maria AV, Braz Nogueira JM, *et al.* The relation between the erythrocyte nitric oxide and hemorheological parameters. Clin Hemorheol Microcirc 2006; 35:341.
- Bor-Kucukatay M, Wenby RB, Meiselman HJ, Baskurt OK. Effects of nitric oxide on red blood cell deformability. Am J Physiol 2003; 284: H1577.
- Bor-Kucukatay M, Meiselman HJ, Baskurt OK. Modulation of density-fractionated RBC deformability by nitric oxide. Clin Hemorheol Microcirc 2005; 33:363.
- Ernst E, Weihmayr T, Schmid M, et al. Cardiovascular risk factors and hemorheology. Physical fitness, stress and obesity. Atherosclerosis 1986; 59:263.

- Wood SC, Doyle MP, Appenzeller O. Effects of endurance training and long distance running on blood viscosity. Med Sci Sports Exerc 1991; 23:1265.
- Kamada T, Tokuda S, Aozaki S, Otsuji S. Higher levels of erythrocyte membrane fluidity in sprinters and long-distance runners. J Appl Physiol 1993; 74:354.
- Smith JA, Martin DT, Telford RD, Ballas SK. Greater erythrocyte deformability in world-class endurance athletes. Am J Physiol 1999; 276: H2188.
- Nakano T, Wada Y, Matsumura S. Membrane lipid components associated with increased filterability of erythrocytes from long-distance runners. Clin Hemorheol Microcirc 2001; 24:85.
- Cazzola R, Russo-Volpe S, Cervato G, Cestaro B. Biochemical assessments of oxidative stress, erythrocyte membrane fluidity and antioxidant status in professional soccer players and sedentary controls. Eur J Clin Invest 2003; 33:924.
- Melnikov AA, Vikulov AD, Bagrakova SV. Relationships between von Willebrand factor and hemorheology in sportsmen. Clin Hemorheol Microcirc 2003; 29:19.
- Muravyov AV, Draygin SV, Eremin NN, Muravyov AA. The microrheological behavior of young and old red blood cells in athletes. Clin Hemorheol Microcirc 2002; 26:183.
- 14. Maeda S, Miyauchi T, Kakiyama T, et al. Effects of exercise training of 8 weeks and detraining on plasma levels of endothelium-derived factors, endothelin-1 and nitric oxide, in healthy young humans. Life Sci 2001; 69:1005.
- Maeda S, Tanabe T, Otsuki T, *et al.* Moderate regular exercise increases basal production of nitric oxide in elderly women. Hypertens Res 2004; 27:947.
- Franzoni F, Galetta F, Morizzo C, *et al.* Effects of age and physical fitness on microcirculatory function. Clin Sci 2004; 106:329.
- Haram PM, Kemi OJ, Wisloff U. Adaptation of endothelium to exercise training: insights from experimental studies. Front Biosci 2008;13:336.
- Ruef P, Pöschl JMB, Linderkamp O, Schmid-Schönbein H. The shear stress diffractometer Rheodyn SSD for determination of erythrocyte deformability. II. Sensitivity to detect abnormal erythrocyte deformability. Clin Hemorheol 1996; 16:749.
- Schmid-Schönbein H, Ruef P, Linderkamp O. The shear stress diffractometer Rheodyn SSD for determination of erythrocyte deformability. I. Principles of operation and reproducibility. Clin Hemorheol 1996; 16:745.
- Nims RW, Darbyshire JF, Saavedra JE, et al. Colorimetric methods for the determination of nitric oxide concentration in neutral aqueous solutions. Methods 1995; 7:48.
- Beneke R, Bihn D, Hutler M, Leithauser RM. Haemolysis caused by alterations of alpha- and beta-spectrin after 10 to 35 min of severe exercise. Eur J Appl Physiol 2005; 95:307.