

# Reduction of red blood cell disaggregability during submaximal exercise: Relationship with fibrinogen levels

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**Abstract.** Effects of exercise on erythrocyte aggregation were investigated in 19 elite athletes. High shear rate viscometry ( $1000\text{ s}^{-1}$ ) evidenced an increase in blood viscosity explained by an increase in hematocrit ( $+8\%$   $p < 0.01$ ) and plasma viscosity ( $+7\%$   $p < 0.01$ ). Erythrocyte rigidity index and erythrocyte aggregability measured using the Myrenne erythroaggregometer did not change. However, using the laser backscattering technique (SEFAM erythroaggregometer), we observed significant changes in aggregability and disaggregability after 25 min of exercise. The initial aggregation time (TA) decreased by 33% ( $p < 0.01$ ), while the final aggregation time decreased by 13.6% ( $p < 0.01$ ). TA was correlated with aerobic working capacity ( $r = 0.73$ ;  $p = 0.005$ ), which was negatively correlated with blood viscosity at rest ( $r = -0.57$ ;  $p = 0.043$ ). A significant relationship was observed between TA and the initial fibrinogen levels ( $r = 0.71$ ;  $p < 0.01$ ). The plasma volume contraction during exercise was found to be statistically explained by the water loss proportional to the total work load. Thus, laser backscattering demonstrates an increase in aggregability and a decrease in disaggregability of red cells during exercise, proportional to baseline fibrinogen values.

**Keywords:** Exercise, erythrocyte deformability, erythrocyte aggregability, fibrinogen, rheology

## 1. Introduction

Exercise, whatever its intensity, induces acute modifications in the rheologic properties of blood, resulting in increased blood viscosity [1–3]. Beside changes in hematocrit and plasma viscosity, which are likely to mostly reflect fluid shifts [4], there are also alterations in erythrocyte physiological properties. The best established one is a decrease in erythrocyte deformability [5,6]. Concerning erythrocyte aggregation, conflicting results were reported since the most widely used technique (i.e., the light transmission analysis using a Myrenne aggregometer [7]), generally fails to detect any change in this parameter during exercise [8,9]. Recently, Hardeman et al. [9], using ektacytometry, reported a 18% increase of erythrocyte aggregation after physical stress. Another sensitive and well standardized technique which gives a quantitative assessment of the red blood cell aggregation process is the measurement of laser light backscattering under controlled flow conditions (i.e., the SEFAM erythroaggregometer [10–14]). This technique gives also an evaluation of the erythrocyte disaggregability process. Thus, the aim of this

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study was to investigate the effects of a submaximal standardized exercise test on red cell aggregation and disaggregation in elite athletes.

## 2. Methods

### 2.1. Study subjects

Nineteen elite sportsmen (national level, 14 footballers and 5 karatekas) were enrolled into the study. Their age ranged from 19 to 33 yr, and their weight from 64 to 95.5 kg. The subjects were checked to be on good health and were free of medication.

During the run-in period, 3 subjects were discontinued due to missing data. Thus, 16 athletes completed the study. Red blood cell (RBC) aggregability from 2 subjects were not taken into account in the study due to anti-inflammatory drug intake that modified RBC aggregation.

### 2.2. Bioelectrical impedance measurements

Body composition was assessed using a multifrequency bioelectrical impedancemeter Dietosystem Human IM Scan that used low intensity (100–800  $\mu$ A) at the following frequencies: 1, 5, 10, 50 and 100 kHz. The four electrode method minimizes contact impedance and skin–electrode interactions. Measurements were made in fasting subjects after 15 min resting in a supine position. The measurement of the drop in voltage allowed the determination of total body reactance and impedance. From these values, using the software Master 1.0 provided by the manufacturer, intracellular and extracellular body water, fat mass, fat-free mass, and body cell mass were calculated [15,16].

### 2.3. Exercise-test

No diet restriction was imposed on days preceding the test. Subjects were asked to fast for 12 hr before commencement of the test at 8:30 A.M. A cannula was placed into the cephalic vein at the level of the cubital fossa for blood sampling. The exercise-test was performed 2 hrs after a standardized breakfast [17,18] which was composed of bread (80 g), butter (10 g), jam (20 g), skimmed concentrated milk (80 ml) (Gloria SA, Paris, France), sugar (10 g) and powder coffee (2.5 g). This breakfast comprised 2070 kilojoules with 9.1% proteins, 27.5% lipids, and 63.4% carbohydrates. The standardized exercise-test was performed on a cycloergometer (Bodyguard, Jonas Oglænd A.S, N4301 Sandnes, Norway) as previously described [19]. It consisted of a 25 min cycling session, with the last 15 minutes at 85% of the theoretical maximal heart rate given by the tables of the American Heart Association, and was followed by a 10 minutes recovery. Pedal speed was kept constant at 60 rpm by the subject. Physical working capacity,  $W_{170}$ , was calculated. It corresponds to the work, in watts, that the subject was able to perform at a heart rate of 170  $\text{b}\cdot\text{min}^{-1}$  [20].  $\dot{V}_{\text{O}_2 \text{ max}}$  was measured from the submaximal steps according to Astrand's normograms [21]. Handgrip and adductor isometric strength were measured using home-made dynamometers [22,23]. Water loss during cycling was evaluated by accurate weighing (Sartorius model F 150-S-F2, France). Changes in plasma volume (%  $\Delta$ PV) during exercise were evaluated from hematocrit changes using a formula developed at the NASA-Ames Research Center [24,25] and that has been demonstrated to be valid in moderate as well as maximal exercise:

$$\% \Delta \text{PV} = 100 / (100 - \text{Ho}) \times 100 [(\text{Ho} - \text{H}) / \text{Ho}],$$

where  $H_0$  is the hematocrit at rest and  $H$  is the hematocrit during exercise. Hematocrit (packed cell volume) was evaluated by a microhematocrit technique on a Hellige autocrit centrifuge.

#### 2.4. Laboratory measurements

Blood samples for hemorheological measurements (7 ml) were drawn with potassium EDTA as the anticoagulant in a vacuum tube (Vacutainer). Measurements were performed within 2 hours after venepuncture to avoid influence of red blood cell “senescence” [14–26]. Viscometric measurements were done at very high shear rate ( $1000 \text{ s}^{-1}$ ) using a falling ball viscometer (MT 90 Medicatest, Saint Benoit, France) [27,28]. Accuracy of the measurements was regularly controlled using the Carrimed Rheometer “CS” (Rhéo, Palaiseau, France) [29]. The precision of this method was 0.6–0.8%. Using this device, the following parameters: apparent viscosity of whole blood at native hematocrit, plasma viscosity, and blood viscosity at corrected hematocrit (45%) were measured according to the equation of Quemada [30]:

$$\eta_{\text{blood}} = \eta_{\text{pl}}(1 - 0.5k \times \text{Ht})^{-2},$$

where  $\eta_{\text{blood}}$  is the whole blood viscosity,  $\eta_{\text{pl}}$  is the plasma viscosity,  $k$  is the index of erythrocyte rigidity of Quemada and  $\text{Ht}$  is the native hematocrit.

The Dintenfass “Tk” index of erythrocyte rigidity was calculated [31,32] according the following formula:

$$\text{Tk} = \frac{\eta_r^{0.4} - 1}{\eta_r^{0.4} \times \text{Ht}},$$

where  $\eta_r$  is the ratio  $\eta_{\text{blood}}/\eta_{\text{pl}}$ .

RBC aggregation was measured using two well-standardized methods. The first one was the Myrenne aggregometer [7] which gives two indices of RBC aggregation: “M” (aggregation during stasis after shearing at  $600 \text{ s}^{-1}$ ) and “M1” (facilitated aggregation at low shear rate after shearing at  $600 \text{ s}^{-1}$ ). The second one was the SEFAM aggregometer, based upon the experiments of Mills [10–14] on cell disaggregation behavior in shear flow, which gives two aggregation indexes “S<sub>10</sub>” and “S<sub>60</sub>” (measurements of the extent of erythrocyte aggregation, relative surface area above the curve calculated over the first respectively 10 and 60 seconds), the aggregation times of red cells “TA” and “TF” (respectively primary and final) and disaggregation shear rates “ $\gamma_s$ ” and “ $\gamma_D$ ” (respectively total and partial disaggregation thresholds).

Fibrinogen was measured with the method of Von Clauss.

#### 2.5. Statistics

Values are presented as mean  $\pm$  standard deviation (SD). The relationships between fibrinogen level and RBC aggregability, between working capacity and RBC aggregability, between working capacity and body water losses, and between hematocrit and body water losses were explored. The data were modelled using a linear, an exponential and an logarithmic models. A one-way analysis of variance (ANOVA) for repeated measures was performed to compare the variations with time of the hemorheological parameters during exercise. Significance level was defined as  $p < 0.05$ .

### 3. Results

Ergometric parameters are presented in Table 1.

Biological responses to exercise are shown in Table 2. Hematocrit did not increase significantly after a standardized breakfast. Mean values were  $41.7 \pm 1.7$  before breakfast and  $42.3 \pm 1.5$  after breakfast, just before test-exercise. However, hematocrit increased significantly during exercise. As shown in this table, high shear rate viscometry evidenced the well known increase in blood viscosity (from  $3.07 \pm 0.19$  baseline to  $3.59 \pm 0.22$  after 25 min, i.e. +17%  $p < 0.01$ ). This was explained by an increase in both hematocrit (from  $42.30 \pm 1.5$  baseline to  $45.70 \pm 1.5$  after 25 min, i.e. +8%  $p < 0.01$ ) and plasma viscosity (from  $1.34 \pm 0.07$  baseline to  $1.44 \pm 0.07$  after 25 min, i.e. +7%  $p < 0.01$ ).

Table 1

Clinical and anthropometric and ergometric data of study subjects ( $n = 19$ )

Age (years)	$25 \pm 1$
Weight (kg)	$77.2 \pm 1.9$
Height (cm)	$180 \pm 1.3$
Body mass index ( $\text{kg}/\text{m}^2$ )	$24 \pm 0.3$
Waist/hip ratio	$0.8 \pm 0.01$
Fat mass (%)	$12.4 \pm 0.3$
Total body water (%)	$60.4 \pm 0.7$
Total water/fat-free mass (%)	$70.3 \pm 0.6$
Body cell mass (%)	$49.9 \pm 0.6$
$W_{170}$ (watt/kg)	$2.6 \pm 0.11$
$V_{O_2 \text{ max}}$ ( $\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ )	$45.3 \pm 2.39$
Handgrip strength (N)	$444 \pm 20.3$
Adductor isometric strength (N)	$520 \pm 8.9$

Table 2

Modifications (mean  $\pm$  SD) of rheologic parameters during submaximal exercise in study subjects

	Time			
	Baseline	10 min	25 min	35 min
Blood viscosity $1000 \text{ s}^{-1}$ (mPa.s)	$3.07 \pm 0.19$	$3.42 \pm 0.19^{***}$	$3.59 \pm 0.22^{***}$	$3.26 \pm 0.22^*$
Corrected viscosity $\eta_{45}$ (mPa.s)	$3.16 \pm 0.22$	$3.28 \pm 0.15^{***}$	$3.48 \pm 0.11^{***}$	$3.52 \pm 0.11^{***}$
Plasma viscosity (mPa.s)	$1.34 \pm 0.07$	$1.41 \pm 0.07^{***}$	$1.44 \pm 0.07^{***}$	$1.40 \pm 0.07^*$
"Tk" (RBC rigidity)	$0.65 \pm 0.04$	$0.66 \pm 0.04$	$0.66 \pm 0.04$	$0.67 \pm 0.04$
Hematocrit (%)	$42.30 \pm 1.50$	$44.10 \pm 1.50^{***}$	$45.70 \pm 1.50^{***}$	$42.90 \pm 1.50^{***}$
RBC aggregation "M"	$3.90 \pm 1.12$	$4.80 \pm 1.12$	$4.30 \pm 1.12$	$4.60 \pm 1.50$
RBC aggregation "M1"	$7.50 \pm 1.87$	$8.90 \pm 1.87$	$8.30 \pm 2.62$	$8.80 \pm 1.87$
TA (s)	$3.25 \pm 1.32$	$2.57 \pm 0.67^{***}$	$1.99 \pm 0.67^{***}$	$2.67 \pm 0.91^*$
TF (s)	$41.1 \pm 8.42$	$37.8 \pm 8.40^{***}$	$33.5 \pm 8.37^{***}$	$38.1 \pm 5.49^*$
$S_{10}$	$23.6 \pm 4.88$	$24.5 \pm 4.32^{***}$	$27.1 \pm 4.81^{***}$	$23.5 \pm 4.56^*$
$S_{60}$	$40.7 \pm 4.32$	$41.4 \pm 3.96^{***}$	$43.7 \pm 4.62^{***}$	$41.5 \pm 3.84^{***}$
$\gamma_D$ ( $\text{s}^{-1}$ )	$45.7 \pm 3.67$	$47.1 \pm 4.53^{***}$	$48.5 \pm 3.83^{***}$	$46.7 \pm 4.32^{***}$
$\gamma_S$ ( $\text{s}^{-1}$ )	$95.4 \pm 24.36$	$83.5 \pm 20.46$	$96.4 \pm 18.13$	$84.1 \pm 21.97$
$\Delta\text{PV}$ (% from baseline)		$-7.90 \pm 3.60^{***}$	$-13.40 \pm 3.20^{***}$	$-3.00 \pm 2.40^{***}$

$\Delta\text{PV}$ : changes in plasma volume compared to rest as assessed by Greenleaf's equations.

\*  $p < 0.05$ ; \*\*\*  $p < 0.01$  vs. baseline.

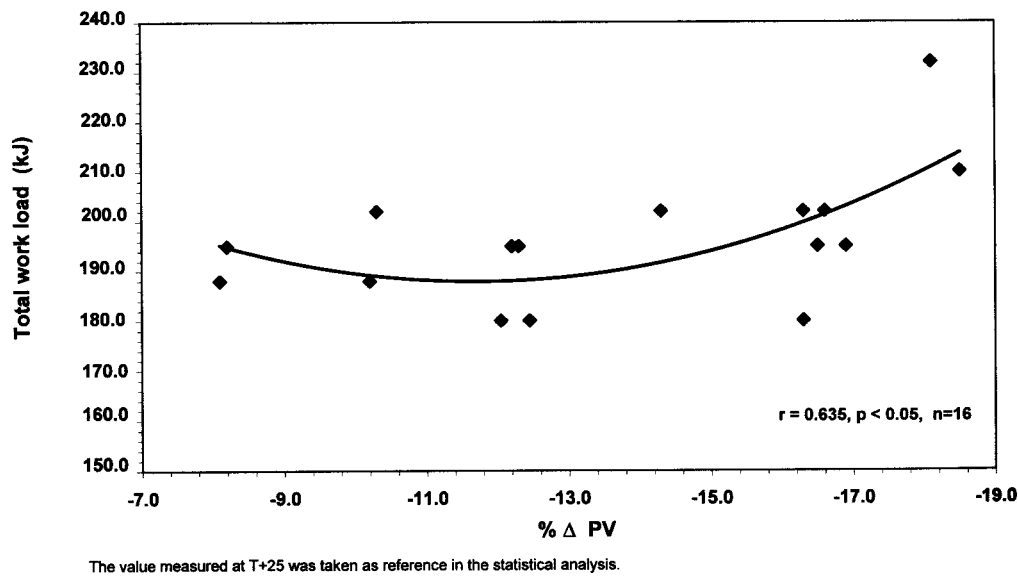


Fig. 1. Correlation between plasma volume contraction (%  $\Delta$ PV) and total work load (WF) during the exercise test. There is a curvilinear relationship between the two parameters ( $r = 0.63$ ;  $p < 0.05$ ).

Correlations between fibrinogen levels and aggregation parameters (TA and  $S_{10}$ ) were found.

### 3.1. Hematocrit and water loss after exercise

At rest, a weak negative correlation was observed between hematocrit and total body water in l/kg measured by bioelectrical impedance ( $r = -0.5$ ;  $p < 0.05$ ). Likewise, hematocrit was correlated with the ratio total body water/fat free mass ( $r = -0.471$ ;  $p < 0.05$ ).

The total water loss was correlated to plasma volume contraction (%  $\Delta$ PV) ( $r = 0.594$ ;  $p < 0.034$ ) and to the total work load (WF) calculated from the area of power plotted against time ( $r = 0.68$ ;  $p < 0.01$ ). This total work load was also correlated to plasma volume contraction ( $r = 0.63$ ;  $p < 0.05$ ): as shown in Fig. 1, there were a curvilinear relationship between WF and %  $\Delta$ PV.

Accordingly, a partial correlation analysis [33] shows that the correlation between the total work load and %  $\Delta$ PV is statistically “explained” by the correlation between %  $\Delta$ PV and total water loss. In other terms, the proportionality between %  $\Delta$ PV and work load is due to the workload-induced water loss.

### 3.2. Measures with laser backscattering technique SEFAM

During this experiment, neither the erythrocyte rigidity index “Tk” nor the erythrocyte aggregability index were significantly modified. However, we observed significant changes in both aggregability and disaggregability during exercise (Fig. 2). The aggregation time (TA) decreased by 33% ( $p < 0.01$ ) after a 25 min exercise and the final aggregation time (TF) decreased by 13.6% ( $p < 0.01$ ). If these data were expressed as aggregation index, there was a significant increase in the aggregation index both at 10 s (+13%  $p < 0.01$ ) and at 60 s (+6%  $p < 0.01$ ). The partial disaggregation threshold  $\gamma_D$  increased from 45.7 to 48.7  $s^{-1}$  ( $p < 0.01$ ).

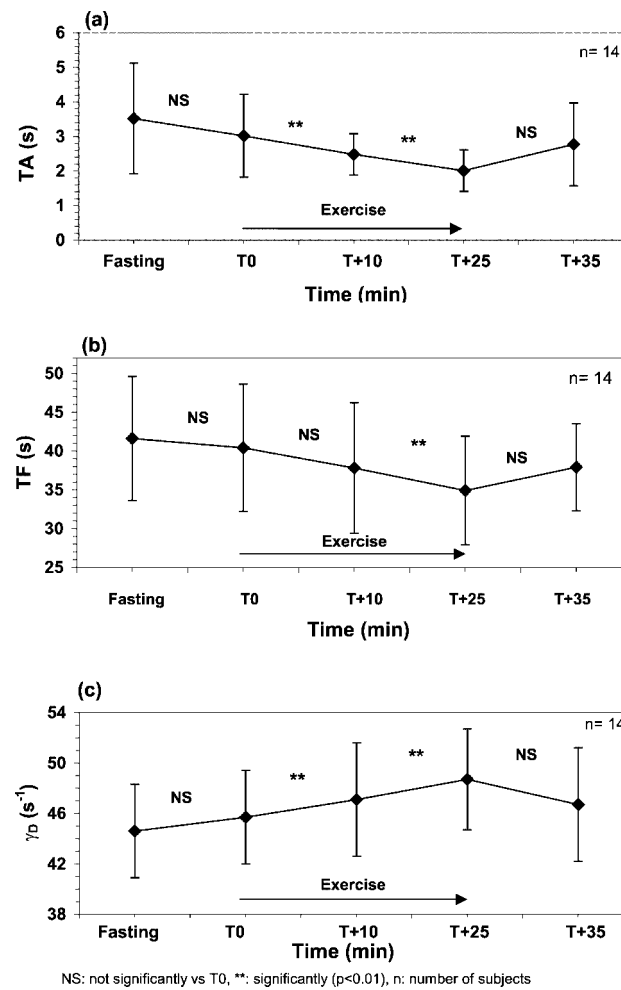


Fig. 2. Variations with time of red cell aggregation time (TA) (a), final red cell aggregation time (TF) (b), and partial disaggregation threshold  $\gamma_D$  (c).

### 3.3. Fibrinogen and aggregation

Correlations between fibrinogen levels at rest and hemorheological parameters were investigated. While no correlation was observed at rest after 25 min of exercise, a strong correlation was found between aggregation (TA) and initial fibrinogen levels ( $r = -0.71$ ;  $p < 0.01$ ) (Fig. 3). We also found a correlation between  $S_{10}$  and fibrinogen levels ( $r = 0.60$ ;  $p < 0.05$ ).

### 3.4. Working capacity and aggregation

There were highly significant correlations between fitness and blood fluidity. As shown in Fig. 4, the initial aggregation time TA at rest was correlated with aerobic working capacity  $W_{170}$  ( $r = 0.73$ ;  $p = 0.005$ ).  $W_{170}$  was also negatively correlated with blood viscosity at rest ( $r = -0.57$ ;  $p = 0.043$ ) and with viscosity at corrected hematocrit 45% ( $r = -0.65$ ;  $p = 0.016$ ).

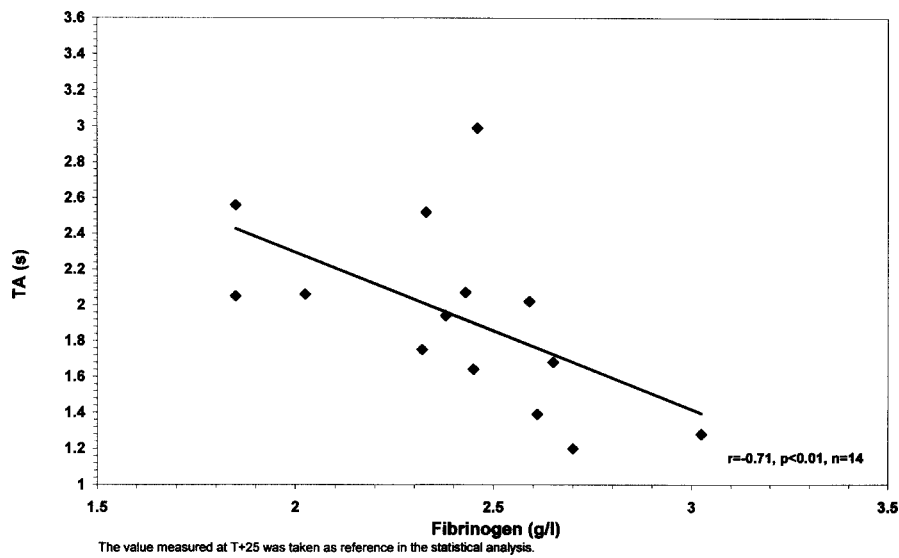


Fig. 3. Correlation between plasma fibrinogen at rest and aggregation index at the peak of exercise after 25 min of exercise ( $r = -0.71$ ;  $p < 0.01$ ).

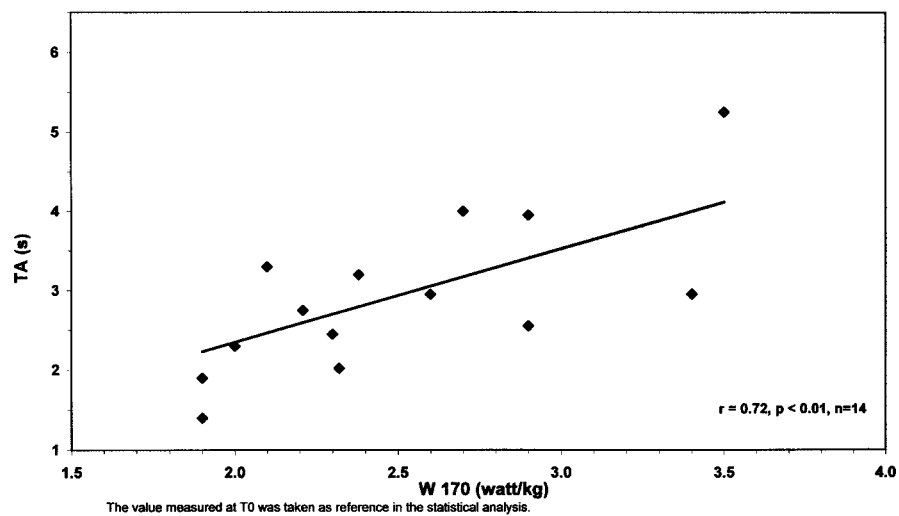


Fig. 4. Correlation between the initial aggregation time TA and the aerobic working capacity  $W_{170}$  ( $r = 0.73$ ;  $p = 0.005$ ) performed on 14 elite athletes. This correlation means that aggregability is negatively related to fitness.

#### 4. Discussion

This study shows that (a) the plasma volume contraction observed during exercise is explained by exercise-induced water loss proportional to the work load; (b) exercise induces a highly significant increase in red cell aggregability and a decrease in red cell disaggregability, both of them being proportional to baseline fibrinogen levels.

We also find that exercise-related erythrocyte hyperaggregation is correlated to fibrinogen levels measured at rest before the exercise test. Subjects with high fibrinogen rate seem to have an aggregation index

$S_{10}$  more important and a higher aggregation time TA. Thus, initial fibrinogen levels seem to influence at least in part RBC aggregation during exercise. Even if correlation between fibrinogen level and aggregation parameters is well known, one can think that other parameters, such as inflammatory proteins, could be taken into account. Is the increase of fibrinolytic activity during intense exercise a response to increase of fibrinogen by hemoconcentration? This hypothesis is attractive, since two studies shown that fibrinogen level remain constant before and after exercise even if the subject drink during it [4–34].

Techniques used to measure red cell aggregability remain a matter of controversy [35]. Clearly, the Myrenne light transmission analysis, that is useful to detect an increase in red cell aggregation due to (i) peripheral obliterative arterial disease [36], (ii) pregnancy [37] or (iii) obesity [38], is not very sensitive to detect alterations in RBC aggregability during exercise [8]. Using this technique in previous studies, we did not found any change in red cell aggregation during a standardized cycling session in either professional sportsmen [5] or healthy sedentary children [2] but we detected a change in rugby players submitted to a longer, steady-state exercise protocol [39]. Using the SEFAM erythroaggregometer, the data of this study demonstrate that exercise acutely increases erythrocyte aggregability and decreases erythrocyte disaggregability. These results were consistent with those found by Hardeman et al. [9]. These authors used the Laser-assisted Optical Rotational Cell Analyzer (LORCA) to measure RBC aggregation which has some degree of similarity with the SEFAM erythroaggregometer. The reason for the discrepancy between light transmission and laser backscattering values is not clear. The process of aggregation studied, which is fully physiological, is poorly detected using the light transmission analysis which in contrast clearly detects hyperaggregation in pathologic states. These two methods, which indirectly assess aggregation, may be sensitive to different aspects of the aggregation process. Similar remark has been made when comparing different methods for the measurement of erythrocyte deformability, e.g., viscometry and filtration [28,32].

Nonetheless, laser backscattering demonstrates clearly an increase in aggregability and a decrease in disaggregability of RBC during exercise. The physiological relevance of this finding remains a matter of speculation. Erythrocyte aggregation experimentally impairs RBC distribution in muscular microcirculatory networks [40]. Therefore, an increase in aggregability during exercise could be hypothesized to induce alterations in oxygen transfer to tissues at the microcirculatory level. The highly significant correlation found between the initial aggregation time TA at rest and aerobic working capacity  $W_{170}$  suggests that fitness is associated with a low aggregability. Thus, an alteration of this low aggregability state may represent a limiting factor for body's adaptation to exercise. Increased erythrocyte aggregation during exercise may also be hypothesized to play a physiological role in the regulation of microcirculatory hemodynamics. According to Johnson et al. [41], erythrocyte aggregation increases postcapillary pressure, counteracting the effects of blood pressure on the arteriolar side of microcirculation and thus maintaining capillary pressure homeostasis [42]. Such a mechanism has been suggested to be physiologically important in horses, which are prone to develop very high pressures in their pulmonary circulatory bed and are thus at risk for alveolar hemorrhage during races. In these animals, erythrocyte aggregation which is very high may represent a protection against excess capillary pressure [43].

## 5. Conclusion

This study shows some interesting aspects of exercise hemorheology.

First, the high shear rate viscometry evidences the well known increase in blood viscosity explained by an increase in both hematocrit (+8%) and plasma viscosity (+7%) [5,44–47]. If we assumed, according to classical circulatory physiology, a linear relationship between viscosity factors and muscle



hemodynamics, it is very surprising to notice that doped athletes, with high Hct and presumably increased blood viscosity, are actually able to achieve remarkable performances. By contrast, the concept of a non-linear influence of viscosity factors described by the percolation theory [48,49] makes all this very easy to understand. Briefly, the application of the percolation theory to hemorheology means that (a) whole blood viscosity by its own is not a relevant factor in microcirculation; (b) in high shear/high flow situations, Hct and erythrocyte rheology do not markedly influence circulatory parameters; (c) by contrast, these parameters are critical in low flow/low shear conditions. In others terms, in the exercising muscle, blood rheology has little circulatory influence, and the most relevant hemorheological factor that is able to increase local resistance is plasma viscosity. By contrast, at rest, high Hct is likely to promote a self-potentiating viscidation process that may markedly impede local flow. It is interesting to point out that this theoretical description is in agreement with the clinical picture of Epo-doped athletes that are both extremely fit at exercise and prone to venous insufficiency at rest [50].

Then, it shows that data on fluid status calculated from venous blood measurements are consistent with that calculated from whole body measurements; indeed, hematocrit is negatively correlated with the bioimpedance measurement of body water, while plasma volume contraction calculated from hematocrit changes is correlated with water loss measured by accurate weighing.

On the whole, this study shows that acute submaximal exercise increases both erythrocyte aggregability and disaggregability. The extent of this increase is proportional to pre-exercise fibrinogen levels. This hyperaggregation and hypodisaggregation is likely to be of physiological importance but needs to be more extensively studied.

## References

- [1] E. Ernst, Changes in blood rheology produced by exercise, *J. Am. Med. Ass.* **253** (1985), 2962–2963.
- [2] J.F. Brun, C. Lagoueyte, M. Rauturier, C. Fédou and A. Orsetti, Hématocrite, viscosité plasmatique et activité musculaire chez l'enfant, *Science & Sports* **4** (1989), 53–63.
- [3] J.F. Brun, J.P. Micallef and A. Orsetti, Hemorheologic effects of light prolonged exercise, *Clin. Hemorheol.* **14** (1994), 807–818.
- [4] H. Vandewalle, C. Lacombe, J.C. Lelièvre and C. Poirot, Blood viscosity after a 1-hr submaximal exercise with and without drinking, *Int. J. Sports Med.* **9** (1988), 104–107.
- [5] J.F. Brun, C. Fons, I. Supparo, C. Mallard and A. Orsetti, Could exercise-induced increase in blood viscosity at high shear rate be entirely explained by hematocrit and plasma viscosity changes?, *Clin. Hemorheol.* **13** (1993), 187–199.
- [6] E. Ernst, L. Daburger and T. Saradeth, The kinetics of blood rheology during and after prolonged standardized exercise, *Clin. Hemorheol.* **11** (1991), 429–439.
- [7] H. Schmid-Schönbein, E. Volger and H.J. Klose, Microrheology and light transmission of blood III: the velocity of red cell aggregate formation, *Pflügers Arch.* **254** (1975), 299–317.
- [8] J.F. Brun, S. Khaled, E. Raynaud, D. Bouix, J.P. Micallef and A. Orsetti, Triphasic effects of exercise on blood rheology: which relevance to physiology and pathophysiology?, *Clin. Hemorheol. Microcirc.* **19** (1998), 89–104.
- [9] M. Hardeman, H.P.F. Peters and P.T. Goedhart, Low hematocrit and plasma fibrinogen in trained athletes increase hemorheological tolerance for physical stress, *Biorheology* **32** (1995), 401 (abstract).
- [10] P. Mills, D. Quemada and J. Dufaux, Etude de la cinétique d'agrégation érythrocytaire dans un écoulement Couette, *Rev. Phys. Appl.* **15** (1980), 1357–1366.
- [11] P. Snabre, M. Bitbol and P. Mills, Cell disaggregation behavior in shear flow, *Biophys. J.* **51** (1987), 795–807.
- [12] J.F. Stoltz, Validation d'une méthode d'approche de l'agrégation érythrocytaire par rétrodiffusion laser in: *Hémorhéologie et agrégation érythrocytaire*, Vol. 2: applications cliniques, J.F. Stoltz, ed., Editions Médicales Internationales, Paris, 1988, pp. 65–74.
- [13] M. Donner, M. Siadat and J.F. Stoltz, Erythrocyte aggregation: approach by light scattering determination, *Biorheology* **25** (1988), 367–375.
- [14] A. Chabanel and M. Samama, Evaluation of a method to assess red blood cell aggregation, *Biorheology* **26** (1989), 785–797.
- [14a] A.L. Copley, On erythrocyte aggregation and disaggregation, *Clin. Hemorheol.* **7** (1987), 3–14.

- [15] J.F. Monnier, E. Raynaud, J.F. Brun and A. Orsetti, Evaluation de la répétabilité moyenne d'une technique d'impédancemétrie appliquée à la détermination de la composition corporelle, *Sciences & Sports* **12** (1997), 208–209.
- [16] E. Varlet-Marie, J.-F. Brun, C. Blachon and A. Orsetti, Relations entre la composition corporelle mesurée par impédancemétrie et la performance motrice sur ergocycle, *Sciences & Sports* **12** (1997), 204–206.
- [17] J.F. Brun, I. Supparo and A. Orsetti, Effects of a standardized breakfast compared to fasting on the hemorheologic responses to submaximal exercise, *Clin. Hemorheol.* **15** (1995), 213–220.
- [18] J.F. Brun, C. Fédou, O. Bouix, E. Raynaud and A. Orsetti, Evaluation of a standardized hyperglucidic breakfast test in postprandial reactive hypoglycaemia, *Diabetologia* **38** (1995), 494–501.
- [19] E. Raynaud, J.F. Monnier, J.F. Brun, M. Solère and A. Orsetti, Biochimie et hormonologie de l'exercice submaximal; standardisation d'un test d'effort chez le sportif, *Sciences & Sports* **12** (1997), 72–74.
- [20] H. Wahlund, Determination of physical working capacity, *Acta Med. Scand.* **215** (1948), 1–78.
- [21] P.O. Astrand and K. Rodahl, *Textbook of Work Physiology. Physiological Bases of Exercise*, McGraw-Hill book Company, New York, 1977.
- [22] J.F. Brun, C. Blachon, J.P. Micallef, C. Fédou, A. Charpiat, O. Bouix and A. Orsetti, Protéines porteuses des somatomédines et force isométrique de préhension dans un groupe de gymnastes adolescents soumis à un entraînement intensif, *Sciences & Sports* **11** (1996), 157–165.
- [23] J.F. Brun, C. Dieu-Cambrézy, A. Charpiat, C. Fons, C. Fédou, J.P. Micallef, M. Fussellier, L. Bardet and A. Orsetti, Serum zinc in highly trained adolescent gymnasts, *Biological Trace Element Research* **47** (1995), 273–278.
- [24] J.E. Greenleaf, V.A. Convertino and G.R. Mangseth, Plasma volume during stress in man: osmolality and red cell volume, *J. Appl. Physiol.* **47** (1979), 1031–1038.
- [25] W. Van Beaumont, J.E. Greenleaf and L. Juhos, Disproportional changes in hematocrit, plasma volume and proteins during exercise and bed rest, *J. Appl. Physiol.* **33** (1972), 55–61.
- [26] B. Pignon, S. Muller, D. Jolly, M. Siadat, E. Petitfrère, B. Vessel, M. Donner, G. Potron and J.F. Stoltz, Validation d'une méthode d'approche de l'aggrégation érythrocytaire par rétrodiffusion laser, in: *Hémorhéologie et aggrégation érythrocytaire*, Vol. 2, J.F. Stoltz, ed., Editions Médicales Internationales, Paris, 1988, pp. 65–74.
- [27] J. Doffin, R. Perrault and G. Garnaud, Blood viscosity measurements in both extensional and shear flow by a falling ball viscometer, *Biorheology* **21**(Suppl. 1) (1984), 89–93.
- [28] C. Fons, J.F. Brun, I. Supparo, C. Mallard, C. Bardet and A. Orsetti, Evaluation of blood viscosity at high shear rate with a falling ball viscometer, *Clin. Hemorheol.* **13** (1993), 651–659.
- [29] J. Bouton and M. Ansermin, Rhéomètre Carrimed CS, Appareil à contrainte imposée pour mesure de fluides viscoélastiques et de fluides à seuil in: *Techniques en Biorhéologie*, J.F. Stoltz, M. Donner and E. Puchelle, eds, *Séminaire INSERM*, Vol. 143, 1986, pp. 121–124.
- [30] D. Quemada, Rheology of concentrated disperse systems. II. A model of non-newtonian shear viscosity in steady flows, *Rheol. Acta* **17** (1978), 632–642.
- [31] L. Dintenfass, *Blood Viscosity, Hyperviscosity & Hyperviscosaemia*, MTP Press, Melbourne, 1985, 482 pp.
- [32] L. Dintenfass, Red cell rigidity, "Tk" and filtration, *Clin. Hemorheol.* **5** (1985), 241–244.
- [33] D. Schwartz, Méthodes statistiques à l'usage des médecins et des biologistes, *Flammarion Paris* **23** (1981), 239–341.
- [34] W. Drygas, Changes in blood platelet function and fibrinolytic activity in response to moderate, exhaustive and prolonged exercise in normal men, Abstracts of the 4th European Congress of Sport Medicine, Prague, 1985.
- [35] G. Mchedlishvili, Attempt to score various techniques applied for examination of the erythrocyte aggregability in blood, *Clin. Hemorheol.* **16** (1996), 193–195.
- [36] C. Dupuy-Fons, J.F. Brun, F. Pellerin, J.C. Laborde, L. Bardet, A. Orsetti and C. Janbon, Relationships between blood rheology and transcutaneous oxygen pressure in peripheral occlusive arterial disease, *Clin. Hemorheol.* **15** (1995), 191–199.
- [37] J.F. Brun, P. Boulot, M.N. Hédon, J.L. Viala and A. Orsetti, Modifications physiologiques de la rhéologie sanguine au cours de la grossesse et de l'accouchement : résultats préliminaires, *Artères et Veines* **9** (1989), 552–557.
- [38] J.F. Brun, C. Fons, I. Supparo, C. Mallard and A. Orsetti, Relationships between metabolic and hemorheologic modifications associated with overweight, *Clin. Hemorheol.* **13** (1993), 201–213.
- [39] J.F. Brun, C. Dupuy-Fons, J.F. Monnier, J.P. Micallef, D. Bouix, C. Peyreigne and A. Orsetti, Exercise hemorheology and dehydration in rugby players, *Biorheology* **32** (1995), 400–401.
- [40] E. Vicaud, X. Hou, L. Decuypere, A. Taccoen and A. Duvelloy, Red blood cell aggregation and microcirculation in rat cremaster muscle, *Int. J. Microcirc.* **14** (1994), 14–21.
- [41] P.C. Johnson, J.J. Bishop, P. Nance, A.S. Popel and M. Intaglietta, Red cell aggregation in vivo, *Biorheology* **36** (1999), 61.
- [42] P.C. Johnson, Differentiating hemorheological adaptations in nature, *Biorheology* **36** (1999), 69.
- [43] J.H. Boucher, Could circulatory curiosities in exercising horses be a consequence of blood pathorheology?, *Biorheology* **36** (1999), 68.

- [44] R.L. Letcher, T.G. Pickering, S. Chien and J.H. Laragh, Effects of exercise on plasma viscosity in athletes and sedentary normal subjects, *Clin. Cardiol.* **4**(4) (1981), 172–179.
- [45] G. Galea and R.J. Davidson, Hemorheology of marathon running, *Int. J. Sports Med.* **6** (1985), 136–138.
- [46] S.C. Wood, M.P. Doyle and O. Appenzeller, Effects of endurance training and long distance running on blood viscosity, *Med. Sci. Sports Exerc.* **23** (1991), 1265–1269.
- [47] J. Nosadova, The changes in hématocrit, hemoglobin, plasma volume and proteins durionf and after differents types of exercice, *Eur. J. Appl. Physiol.* **36** (1977), 223–230.
- [48] H. Schmid-Schönbein, Wege des Infarzierens. Neue Erkenntnisse durch die In-vivo-Rheoskopie, *Internist* **42** (2001), 1196–1218.
- [49] H. Schmid-Schönbein, M. Foerster, H. Heidtmann, J. Hektor, J. Hoymann, R. Grebe and T.H. Kirschkamp, Percolation theory as the rational basis of clinical hemorheology: the role of “abnormal” flow behaviour of blood elements on the homogeneity of perfusion, gas exchange and metabolism, *Biorheology* **39**(5) (2002), 627.
- [50] C. Le Devehat, M. Vimeux and A. Bertrand, Venous insufficiency and hemorheology, Influence of venous stasis on erythrocyte aggregation and disaggregation, *J. Mal. Vasc.* **14**(4) (1989), 307–311.