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# Insulin-like growth factor-binding protein 1 and blood rheology in athletes

A. Aïssa Benhaddad, J.F. Monnier, C. Fédou, J.P. Micallef and J.F. Brun *

*Service Central de Physiologie Clinique, Centre d'Exploration et de Réadaptation des Anomalies du Métabolisme Musculaire (CERAMM), CHU Lapeyronie 34295 Montpellier-cédex 5, France*

**Abstract.** The GH–IGF axis has been recently suggested to modulate blood rheology in trained athletes, via GH effects on body water status and a possible action of IGF-I on erythrocyte deformability and aggregability. Another potential candidate for such a rheologic effect of the GH–IGF axis is insulin-like growth factor binding protein-1 (IGF-BP1) which is increased in trained people and correlated to fitness: IGF-BP1 is elevated in patients with polycythemia vera and stimulates erythroid burst formation *in vitro*. We investigated the statistical relationships between IGF-BP1 and blood rheology in athletes. 21 soccer players, age $24.5 \pm 1.13$ yr; body mass index $23.7 \pm 0.38$ kg/m$^2$; $VO_{2\,max}$ $44.8 \pm 7$ ml.min$^{-1}$.kg$^{-1}$). The major statistical determinant of IGFBP1 (measured at rest after overnight fast) was age ($r = 0.752$, $p = 0.00013$) which was not correlated with rheological parameters. IGF BP1 was negatively correlated with blood viscosity $\eta$ (high shear rate $r = -0.516$, $p = 0.024$) and positively correlated with the percentage of extracellular water in total body water (ECW/TBW) ($r = 0.488$, $p = 0.039$). The previously reported correlations between IGF-I and both $\eta$ ($r = 0.637$, $p = 0.003$) and red cell rigidity "Tk" ($r = 0.696$, $p = 0.0137$) were observed, but IGF-I and IGF-BP1 were not correlated to each other ($r = -0.176$ ns) and their correlations with $\eta$ and Tk appeared to be independent when studied by multivariate analysis. Consistent with these correlations, subjects in the upper tertile of IGF-BP1 (>23.4 ng/ml) compared to those in the lower (<7.5 ng/ml) had a higher percentage of ECW/TBW ($40.8 \pm 0.4$ vs $38 \pm 0.8\%$, $p = 0.033$), a lower $\eta$ ($2.7 \pm 0.05$ vs $2.97 \pm 0.06$ mPa.s, $p = 0.016$), and a lower Tk ($0.54 \pm 0.05$ vs $0.63 \pm 0.01$, $p = 0.027$). Thus, beside GH and IGF-I, IGF-BP1, which is reported to act on erythroid progenitors, exhibits statistical relationships with blood fluidity and erythrocyte flexibility that may suggest a physiological role in improving blood rheology.

Keywords: Blood viscosity, hemorheology, erythrocyte deformability, erythrocyte aggregability, exercise training, overtraining, insulin-like growth factor binding protein 1, insulin-like growth factor binding protein 3, insulin-like growth factor I, growth hormone, body fluids

## 1. Introduction

While the growth hormone (GH)–insulin-like growth factor 1 (IGF-I) axis exerts almost ubiquitous effects, little is known about its potential influence on the rheological properties of blood. However, several lines of evidence support the hypothesis that this influence might be quite important [1].

We previously showed that GH response to exercise is a major determinant of the increase in blood viscosity during exercise [2] and that IGF-I correlates with blood viscosity, due to lower red cell deformability and increased red cell aggregability in subjects with high values of IGF-I [3]. These results were consistent with our working hypothesis of an involvement of the GH–IGF axis in the regulation of the rheological properties of blood.

*Corresponding author: Dr J.F. Brun, MD, PhD, Service Central de Physiologie Clinique, Centre d'Exploration et de Réadaptation des Anomalies du Métabolisme Musculaire (CERAMM), CHU Lapeyronie 34295 Montpellier-cédex 5, France. Tel.: +33 4 67 33 82 84; Fax: +33 4 67 33 89 86; Telex: CHR MONTP 480 766 F; E-mail: drjfbrun.@aol.com.

Beside IGF-I, its binding protein IGFBP-1, which physiologically traps IGF-I in order to inhibit its action [4] behaves also as a hormone which exerts a direct effect on erythrocyte progenitors [5]. This property indicates that IGFBP-1 could also be expected to be involved in such a regulation.

Exercise training, a situation which improves blood rheology [6], also increases IGFBP-1 [7]. Therefore, this study was undertaken in order to analyze whether IGFBP-1 and hemorheology are related in trained athletes.

## 2. Methods

Subjects used in this study were 21 male elite athletes (national level in football, volleyball and karate) submitted daily to a physical training program. Their characteristics are shown on Table 1. They underwent a standardized submaximal exercise session on cycloergometer over 25 min. Pedal speed was kept constant at 60 rpm by the subjects. Physical working capacity $W_{170}$ was calculated as the work in watts that subjects were able to perform at a heart rate of 170 $b.min^{-1}$ [8]. Body composition was assessed with a multifrequency bioelectrical impedancemeter Dietosystem Human IM Scan that uses low intensity (100–800 $\mu$A) at the following frequencies: 1, 5, 10, 50, and 100 kHz. Analysis was performed with the software Master 1.0 that gives the choice among 25 published equations for body composition calculations. The check-up included clinical examination and body composition evaluation by bioelectrical impedancemetry. The psychological scale for overtraining proposed by the consensus group on overtraining of the French Society of Sports Medicine was used [9]. Isometric strength was measured with home-made devices which are designed to assess handgrip strength and tight adductors isometric strength. Baseline samples for the measurement of zinc and various hormones (see below) were drawn.

### 2.1. Hemorheological measurements

Blood samples for hemorheological measurements (7 ml) were drawn with potassium EDTA as the anticoagulant in a vacuum tube (Vacutainer). Viscometric measurements were done at high shear rate (1000 $s^{-1}$) with a falling ball viscometer (MT 90 Medicatest, F-86280 Saint Benoit) [10]. Accuracy of the measurements was regularly controlled with the Carrimed Rheometer "CS" (purchased from Rhéo, 91120 Palaiseau, France) [11]. The coefficient of variation of this method ranged between 0.6 and 0.8% [12]. With this device we measured apparent viscosity of whole blood at native hematocrit, plasma viscosity, and blood viscosity at corrected hematocrit (0.45) according to the equation of Quemada [13]. Dintenfass' "Tk" index of erythrocyte rigidity was calculated [14]. RBC aggregation was assessed with the Myrenne aggregometer [15] 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 $s^{-1}$). The hematocrit/viscosity ($h/\eta$) ratio, an index of oxygen supply to tissues, was calculated according to Chien [16] and Stoltz [17], with hematocrit (as percentage) divided by viscosity at high shear rate determined as described above.

The SEFAM aggregometer was used for a more precise assessment of RBC aggregation. This device measures the changes in backscattered light which are observed when sheared RBC suspensions are abruptly brought to a full stop. The decrease in the optical signal reflects the formation of RBC aggregates [18,19]. Some parameters are derived from the curve of light intensity as a function of time. The aggregation time is the reciprocal of the initial slope (calculated between 0.5 and 2 s after the shear has stopped). The aggregation index at 10 s is a measurement of the extent of erythrocyte aggregation and

Table 1
Clinical characteristics (anthropometry and ergometry) of the 21 subjects of the study

| | |
|---|---|
| age (years) | 24.5±1.13 |
| weight (kg) | 78.8±1.94 |
| height (cm) | 182±1.31 |
| body mass index (kg/m$^2$) | 23.7±0.38 |
| overtraining score | 6.4±1.4 |
| fat mass (kg) | 10.6±0.41 |
| percentage of fat (%) | 13.3±0.35 |
| total body water (kg) | 48±1.1 |
| extracellular water (kg) | 19±0.6 |
| intracellular water (kg) | 29.2±0.69 |
| extracellular/total water (%) | 39±0.5 |
| water/fat free mass (%) | 69.6±0.69 |
| $W_{170}$ (w/kg) | 2.66±0.13 |
| $VO_{2\,max}$ (ml.min$^{-1}$.kg$^{-1}$) | 44.8±7 |
| $VO_{2\,max}/VO_{2\,max\ theor}$ (%) | 116.7±23.1 |
| $W_{max}$ (w) | 354.7±83.3 |
| $W_{max}/W_{max\ theor}$ (%) | 117.9±24.9 |
| isometric handgrip strength (N) | 527.5±21.4 |
| isometric adductor strength (N) | 647.7±61.4 |

is the relative surface area above the curve calculated over the first 10 s. This device measures also disaggregation thresholds, by submitting blood to a succession of shear rates from 600 to 7 s$^{-1}$. The total disaggregation threshold is the shear rate below which the backscattered light intensity starts to decrease, indicating that the shear stress applied to aggregates is no longer sufficient for allowing complete dispersion of RBC aggregates. The partial disaggregation shear rate is defined as the shear rate corresponding to the intersection point of the two asymptotes drawn from the extremes (maximum and minimum shear rate).

### 2.2. *Hormone and growth-factor assays*

Serum Somatomedin C/IGF-I was assayed with the INCSTAR IGF-I RIA (from INCSTAR Corporation, Stillwater, MN 55082-0285, USA, purchased from Sorin Biomedica, France, SA). This is a double antibody desequilibrium assay which includes an ODS-silica extraction procedure from serum samples. After the extraction procedure the RIA is performed employing addition of sample and rabbit anti-IGF-I, followed by a 2 hr incubation at 2–8°C. Iodine-125 IGF-I is then added followed by a second incubation for 20 hr at 2–8°C. Pre-precipitated carrier, second antibody and polyethylene glycol are added in a single step. The assay is centrifuged after the 2 hr second antibody incubation at 2–8°C. Detection limit is 2 nmol/l. This assay does not cross-react (<1%) with IGF-II, hGH, FGF, TGR, PDGF. Within assay CVs range between 9.1–10.1%, between-assay CVs range between 10.3–15.2%.

Serum IGF-binding protein-1 was assayed with the DSL ACTIVE IGFBP-1 coated tube immunoradiometric assay kit (from Diagnostic system laboratories Inc., PO Box 57946, Webster, TX 77598, USA, purchased from Chiron Diagnostics BP109, 95613 Cergy Pontoise, France, SA). This is a two site immunoradiometric assay (IRMA) in which the analyte to be measured is "sandwiched" between two antibodies. The first antibody is immobilized to the inside wall of the tubes. The other antibody is radi-

olabelled for detection. The analyte present in the patient samples, standards and controls is bound by both of the antibodies to form a "sandwich" complex. Unbound materials are removed by decanting and washing tubes. Detection limit is 0.01 ng/ml. Within assay CVs range between 3.4–6%, between-assay CVs range between 1–3.5%. No cross reactivity with IGFBP-2, 3 and 4 has been dectected.

Serum IGF-binding protein-3 was assayed with the DSL IGFBP-3 radioimmunoassay kit (from Diagnostic system laboratories Inc., PO Box 57946, Webster, TX 77598, USA, purchased from Chiron Diagnostics BP109, 95613 Cergy Pontoise, France, SA). This is a classical radioimmunoassay where there is competition between a radioactive and a non-radioactive antigen for a fixed number of antibody binding sites. The separation of free and bound antigen is achieved by using a double antibody system. Detection limit is 0.01 ng/ml. Within assay CVs range between 3.4–6%, between-assay CVs range between 1–3.5%. No cross reactivity with IGFBP-2, 3 and 4 has been dectected.

Serum zinc was measured by flame atomic spectrophotometry The lowest limit of sensitivity of this method is 0.0125 mg/l. Its coefficient of variation is 7.2% ($n = 9$).

## 3. Results

Mean laboratory measurements in the 39 subjects of the study are shown on Table 2 and hemorheologic parameters on Table 3.

### 3.1. Correlations on the whole sample

IGFBP-1 was correlated to age ($r = 0.752$, $p = 0.00013$) but not with BMI or fatness. Thus age appeared to be its main determinant in this sample of subjects. IGFBP-1 was correlated with the percentage of extracellular water in fat free mass (Spearman rank correlation coefficient $r = 0.488$, $p = 0.039$) and negatively correlated with blood viscosity (Spearman rank correlation coefficient $r = -0.516$, $p = 0.024$). The correlation between IGFBP-1 and blood viscosity was not likely to be explained by Hct or Tk since they are not correlated to IGFBP-1 ($r = -0.128$ ns and $-0.268$ ns) neither they are explained by a correlation with age (correlation between viscosity and age $r = -0.37$ ns) while age was a major determinant of IGFBP-1 ($r = 0.752$, $p = 0.00013$).

The previously reported [3] correlations between IGF-I and both $\eta$ ($r = 0.637$, $p = 0.003$) and red cell rigidity "Tk" ($r = 0.696$, $p = 0.0137$) were observed, but IGF-I and IGF-BP1 were not correlated

Table 2
Laboratory measurements in the 39 subjects of the study

| | |
|---|---|
| creatine kinase (UI/l) | 374.9±74.6 |
| ammonia (μg/l) | 82.7±2.8 |
| fibrinogen (g/l) | 2.36±0.08 |
| serum zinc (mg/l) | 0.80±0.03 |
| IGF-1 (ng/ml) | 258±18.8 |
| IGFBP-1 (ng/l) | 15.4±4 |
| IGFBP-3 (ng/ml) | 4±0.27 |
| IGF-1/IGFBP-3 (ng/ml) | 9.3±1 |
| GH at rest (ng/ml) | 1.3±0.5 |
| GH at peak exercise (ng/ml) | 26.9±3.8 |
| mean GH increase (ng/ml) | 25.6±3.6 |

Table 3
Hemorheologic parameters

| | |
|---|---|
| ferritin (ng/ml) | 77.6±8.5 |
| hematocrit (%) | 42±0.38 |
| $h/\eta$ ($mPa^{-1}.s^{-1}$) | 14.3±0.23 |
| blood viscosity $\eta_b$ (mPa.s) | 2.97±0.07 |
| $\eta_b$ at corrected hct 45% | 3.14±0.06 |
| plasma viscosity $\eta_p$ (mPa.s) | 1.38±0.03 |
| erythrocyte rigidity "Tk" | 0.62±0.02 |
| erythrocyte aggregation "M" | 4.4±0.68 |
| erythrocyte aggregation "M1" | 8.9±0.69 |
| aggregation kinetics "Tf" | 38.6±3.75 |
| aggregation kinetics "TA" | 3.04±0.31 |
| aggregation kinetics "S10" | 20.97±1.21 |
| aggregation kinetics "S60" | 40±1.2 |
| disaggregation $\gamma S$ ($s^{-1}$) | 83±5.9 |
| disaggregation $\gamma D$ ($s^{-1}$) | 44±1.6 |

to each other ($r = -0.176$ ns) and their correlations with $\eta$ and Tk appeared to be independent when studied by multivariate analysis. The ratio IGF-I/IGFBP-1 is not correlated to blood rheology: thus, it is not likely that our findings could be explained by a modulatory role of IGFBP-1 on the previously reported effect of IGF-I.

The RBC partial disaggregation threshold $\gamma D$ was negatively correlated to the percentage of water in fat free mass ($r = -0.6434$, $p = 0.022$).

### 3.2. *Tertiles of IGFBP-1*

Mean values (Table 2) of IGFBP-1 in this sample (15.4 ± 4 ng/l) are similar to those found with the same kit in our general population of athletes. It can thus be calculated that limits for the lower and the upper tertiles for IGF BP1 are respectively 7.47 and 23.4. When we divide this sample according to these cut-off values for tertiles (Table 4), we evidence differences consistent with these correlations. Subjects in the upper tertile of IGF-BP1 (>23.4 ng/ml) compared to those in the lower (<7.5 ng/ml) had a higher percentage of ECW/TBW (40.8 ± 0.4 vs 38 ± 0.8%, $p = 0.033$), a lower $\eta$ 2.7 ± 0.05 vs 2.97 ± 0.06 mPa.s, $p = 0.016$), and a lower Tk (0.54 ± 0.05 vs 0.63 ± 0.01, $p = 0.027$).

Subjects in the upper tertile of IGFBP-1 were 7 yr older than the others ($p < 0.001$), while height, weight and body mass were similar. They had a lower GH response to exercise and a lower serum zinc (0.7 ± 0.05 vs 0.83 ± 0.03, $p = 0.0287$).

## 4. Discussion

This study aimed at investigating relationships between IGFBP-1 and blood rheology. Our results show that IGFBP-1 is negatively correlated with blood viscosity and positively correlated with the percentage of extracellular water in total body water so that subjects in the upper tertile of IGFBP-1 have a higher percentage of extracellular water in total body water and a lower viscosity. Differences in blood viscosity are explained by a lower red cell rigidity in the upper tertile of IGFBP-1.

Table 4
Comparison of ergometric and anthropometric parameters according to the tertile of IGFBP1

| | Lower <7.47 ($n = 10$) | Middle 7.47–23.4 ($n = 6$) | Upper >23.4 ($n = 5$) |
|---|---|---|---|
| age | 21.3±0.9 | 25.3±1.9 | 31.2±0.8*** |
| weight | 76.6±3.2 | 77.6±2.7 | 85.9±1.7 |
| height | 181.3±2 | 181.2±2.8 | 185.7±1.1 |
| BMI | 22.2±0.6 | 23.6±0.6 | 25.2±0.5 |
| fat mass (kg) | 9.7±0.7 | 11.1±0.5 | 11.7±0.4 |
| percentage of fat (%) | 12.6±0.6 | 14.3±0.3 | 13.6±0.3 |
| total body water (kg) | 47±1.6 | 46.2±1.8 | 52±1.6 |
| extracellular water (kg) | 18.2±0.9 | 19±1.2 | 21.1±0.6 |
| intracellular water (kg) | 28.7±1.1 | 29±1 | 30.7±1 |
| extracellular/total water (%) | 38±0.8 | 39.4±0.8 | 40.8±0.4* |
| water/fat free mass (%) | 70±1 | 68.1±1.14 | 70.12±1.7 |
| $W_{170}$ (w/kg) | 2.43±0.16 | 2.91±0.21 | 2.82±0.3 |
| $VO_{2\,max}$ ($ml.min^{-1}.kg^{-1}$) | 37.1±3.4 | 41.3±4 | 50±11.1 |
| $W_{max}$ (w) | 250.2±5.3 | 354.7±83.3 | 459.1±140.8 |
| $W_{max}/W_{max\ theor}$ (%) | 85.8±6.6 | 118±25 | 150.1±40 |
| isometric handgrip strength (N) | 509±25.8 | 575.7±33.3 | 540.4±75.8 |

*$p < 0.05$, ***$p < 0.01$ vs the lowest tertile.

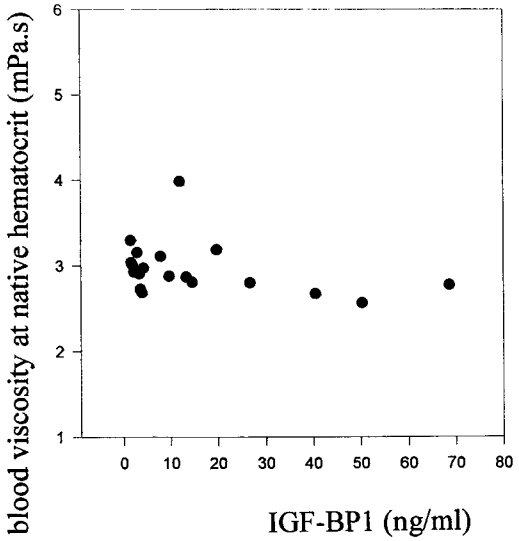


Fig. 1. Negative correlation between IGF-BP1 and blood viscosity at native hematocrit (Spearman rank correlation coefficient $r = -0.516$, $p = 0.024$).

Therefore, our results are consistent with our initial assumption that IGFBP-1 might be, as GH and IGF-I, a regulator of blood rheology. Moreover, given the existence of direct effects of IGFBP-1 on erythroid cells [5] via IGFBP-1 binding to receptors, these findings may reflect a direct effect of IGFBP-1 on erythrocyte rheology, as demonstrated for other hormones like insulin [20–22]. This point needs to be clarified by incubation studies *in vitro*.

Table 5
Comparison of hormonal, metabolic and hemorheologic parameters according to the tertile of IGFBP-1

| | Lower | Middle | Upper |
|---|---|---|---|
| | <7.47 | 7.47–23.4 | >23.4 |
| | ($n = 10$) | ($n = 6$) | ($n = 5$) |
| GH0 | 1.7±0.9 | 0.4±0.2 | 1.6±1.5 |
| GH peak | 34.3±5.3 | 27.6±6.7 | 11.1±4.9* |
| delta GH | 32.6±4.9 | 27.2±6.6 | 9.5±3.9** |
| overtraining score | 4.7±1.5 | 6.7±2.7 | 9.6±4 |
| ferritin (ng/ml) | 73.6±7.3 | 94.8±25.8 | 65±12.3 |
| NH4 | 85.2±3.9 | 73.2±3.5 | 89±5.7 |
| CPK | 516±123.6 | 254.8±56.5 | 172±48.6 |
| fibrinogen | 2.39±0.09 | 2.22±0.22 | 2.39±0.22 |
| zinc (mg/l) | 0.83±0.03 | 0.81±0.06 | 0.7±0.05* |
| IGF-I (ng/ml) | 267.2±22.5 | 271.6±50.9 | 223.2±29.4 |
| IGFBP-1 | 2.75±0.3 | 12.9±1.7 | 43.9±7.3 |
| IGF-1/BP3 | 9.1±1.4 | 12.3±2.4 | 6.5±0.6 |
| hematocrit (%) | 41.8±0.36 | 43.3±0.8 | 40.5±0.6 |
| $h/\eta$ ($mPa^{-1}.s^{-1}$) | 14.2±0.24 | 13.9±0.5 | 15±0.4 |
| blood viscosity $\eta_b$ (mPa.s) | 2.97±0.06 | 3.14±0.2 | 2.7±0.05* |
| $\eta_b$ at corrected hct 45% | 3.17±0.06 | 3.2±0.2 | 2.94±0.09 |
| plasma viscosity $\eta_p$ (mPa.s) | 1.37±0.04 | 1.33±0.02 | 1.45±0.07 |
| erythrocyte rigidity "Tk" | 0.63±0.01 | 0.66±0.03 | 0.54±0.05* |
| erythrocyte aggregation "M" | 3±0.7 | 6±1 | 3.8±2 |
| aggregation kinetics "Tf" | 39.4±4.7 | 36.3±12.4 | 39.9 |
| aggregation kinetics "TA" | 2.95±0.5 | 2.83±0.4 | 3.63±1 |
| aggregation kinetics "S10" | 21.4±2.2 | 21.7±1.4 | 18.9±2.55 |
| aggregation kinetics "S60" | 40.2±1.9 | 40.3±1.7 | 38.9±3.6 |
| disaggregation $\gamma S$ ($s^{-1}$) | 88.6±4.6 | 80±14.3 | 73.8±20.4 |
| disaggregation $\gamma D$ ($s^{-1}$) | 44.1±1.6 | 47±3 | 39±5 |

*$p < 0.05$ vs the lowest quintile.

Actually, most of the differences in blood rheology across tertiles of IGFBP-1 appears to be explained by differences in body fluids, as shown on Fig. 2. The reason for a higher extracellular water volume in subjects with high levels of circulating IGFPB-1 is not clear. We are not aware of reports of an involvement of this circulating protein on water metabolism.

A little difference in age between the highest tertile of IGFBP-1 and the two others can be noticed on Table 4. It seems very unlikely to explain the correlation between IGFBP-1 and blood rheology, since such a difference of age (24 vs 31 yr) has never been reported, as far as we know, to be associated with so pronounced hemorheological differences. However, age is an important determinant of IGFBP levels [23] even in athletes of this range of ages [24], so that another study designed to separately analyze the effects of training and age on IGFBP-1 and blood rheology will be useful to perform.

There is also a slightly lower serum zinc in the highest tertile of IGFBP-1. This difference is not likely to explain our results since the effects of zinc on blood rheology are rather a fluidification of red cells [25,26], so that hypozincemic athletes actually are characterized by a higher viscosity and red cell rigidity [27]. Thus a lower zinc is very unlikely to explain a lower red cell rigidity and blood viscosity, and should rather be expected to result in the opposite picture. Therefore, if this slight difference in zinc

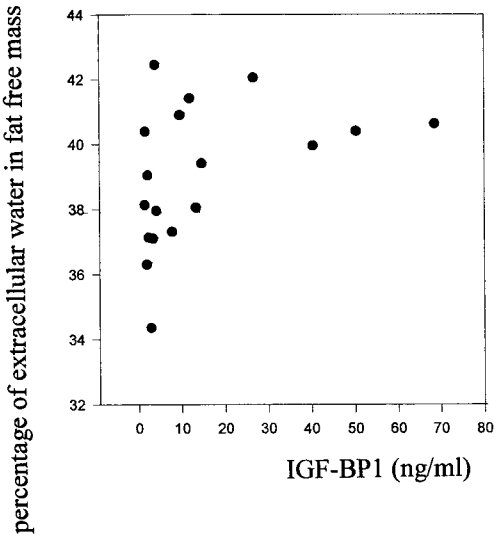


Fig. 2. Correlation between IGF-BP1 and the percentage of extracellular water in fat free mass (Spearman rank correlation coefficient $r = 0.488$, $p = 0.039$).

has any influence on red cell rigidity in this sample, it should actually be expected to rather counteract the effects of IGFBP-1 than to explain them.

In addition, the previously reported correlations between IGF-I and both $\eta$ and red cell rigidity are found again. Since IGF-I and IGFBP-1 are not correlated to each other, and that their correlations with $\eta$ and Tk are independent, they may thus be speculated to have separate effects on blood rheology.

In conclusion, we show statistical relationships between IGFBP-1 and blood fluidity (and erythrocyte flexibility) that support of working hypothesis of a physiological role of this hormone of the GH–IGF axis in the regulation of blood viscosity. This finding requires to be confirmed by further studies.

## References

[1] J.F. Brun, A. Aïssa Benhaddad, C. Fédou, E. Pierrisnard, J.F. Monnier, J.P. Micallef and J. Mercier, Is the growth hormone–Insulin-Like Growth Factor I (GH–IGF-I) axis a physiological regulator of blood rheology?, *J. Mal. Vasc.* **25** (2000), 159.

[2] C. Peyreigne, D. Bouix, J.P. Micallef, J. Mercier, J. Bringer, C. Préfaut and J.F. Brun, Exercise-induced growth hormone secretion and hemorheology during exercise in elite athletes, *Clin. Hemorheol. Microcirc.* **19** (1998), 169–176.

[3] J.F. Monnier, J.F. Brun, A. Aïssa Benhaddad, J.P. Micallef and J. Mercier, Relationships between blood viscosity and insulin-like growth factor I status in sportsmen, *Clin. Hemorheol. Microcirc.* **21** (2000) (accepted).

[4] R.C. Cuneo and L.D. Wallace, Growth hormone, insulin-like growth factors and sports, *Endocrinol. Metab.* **1** (1994), 3–13.

[5] A.M. Mirza, S. Ezzat and A.A. Axelrad, Insulin-like growth factor binding protein-1 is elevated in patients with polycythemia vera and stimulates erythroid burst formation in vitro, *Blood* **89** (1997), 1862–1869.

[6] 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.

[7] J. Manetta, J.F. Brun, L. Maimoun, C. Fédou, J. Mercier and C. Préfaut, Effect of 4 month intensive training on insulin-like growth factor (IGF) 1 and IGF-binding protein 1, and 3 in elite cyclists: relationships with glucose assimilation, Submitted for publication in *Hormone and Metabolic Research.*

[8] H. Wahlund, Determination of physical working capacity, *Acta Med. Scand.* **215** (1948), 1–78.

[9] P. Legros and the groupe "surentraînement" (A. Orsetti, M. Bedu, J.F. Brun, F. Brue, Y. Desmarais, E. Jousselin, P. Legros, J. Medelli, C. Paruit and B. Serrurier) Le surentraînement: diagnostic des manifestations psychocomportementales précoces, *Science & Sports* **8** (1993), 71–74.

[10] J. Doffin, R. Perrault and G. Garnaud, Blood viscosity measurements in both extensional and shear flow by a falling ball viscometer, *Biorheology* (1984) (Suppl. 1), 89–93.
[11] J. Bouton and M. Ansermin, Rhéomètre Carrimed CS. Appareil à contrainte imposée pour mesure de fluides viscoéastiques et de fluides à seuil. Techniques en Biorhéologie, J.F. Stoltz, M. Donner, E. Puchelle, eds, *Séminaire INSERM*, Vol. 143, 1986, pp. 121–124.
[12] 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.
[13] D. Quemada, Rheology of concentrated disperse systems. II. A model of non-newtonian shear viscosity in steady flows, *Rheol. Acta* **17** (1978), 632–642.
[14] L. Dintenfass, Blood viscosity, *Hyperviscosity & Hyperviscosaemia*, MTP Press, Melbourne, 1985, 482pp.
[15] 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.
[16] S. Chien and L.A. Sung, Physicochemical basis and clinical implications of red cell aggregation, *Clin. Hemorheol.* **7** (1987), 71–91.
[17] J.F. Stoltz, M. Donner and S. Muller, Introduction de la notion de profil hémorhéologique.: "Hémorhéologie et Facteurs de Risque" 7e Réunion Conjointe de la Société d'Hémorhéologie de l'Ouest et de la Société de Biorhéologie de Langue Française. Rennes (France) May 18th, 1990, J.M. Bidet, D. Boudart, M. Delamaire and F. Durand, eds, pp. 12–25.
[18] M. Donner, M. Siadat and J.F. Stoltz, Erythrocyte aggregation: approach by light scattering determination, *Biorheology* **25** (1988), 367–375.
[19] A. Chabanel and M. Samama, Evaluation of a method to assess red blood cell aggregation, *Biorheology* **26** (1989), 785–797.
[20] S. Scalesse, J.F. Brun and A. Orsetti, Short term in vitro influence of different glucose and insulin levels on red blood cell filterability, *Clin. Hemorheol.* **3** (1983), 269.
[21] I. Juhan, M. Buonocore, R. Jouve, P. Vague, J.P. Moulin and B. Vialettes, Abnormalities of erythrocyte deformability and platelet aggregation in insulin-dependent diabetes corrected by insulin in vivo and in vitro, *Lancet* **1982** (March 6), 535–537.
[22] M. Bryszewska and W. Leyko, Effects of insulin on human erythrocyte membrane fluidity in diabetes mellitus, *Diabetologia* **24** (1983), 311–313.
[23] A. Juul, P. Dalgaard, W.F. Blum, P. Bang, K. Hall, K.F. Michaelsen, J. Müller and N.E. Skakkebaek, Serum levels of insulin-like growth factor binding protein 3 (IGFBP3) in healthy infants, children and adolescents: the relation to IGFI, IGFII, IGFBPI, IGFBP2, age, sex, body mass index and pubertal maturation, *J. Clin. Endocr. Metab.* **80** (1995), 2534–2542.
[24] C. Fédou, J.F. Brun, S. Lumbroso, Ch. Sultan and J. Mercier, Déterminants des paramètres de l'axe GH-IGF-I-IGFBPs chez des sportifs entraînés, *Annales d'Endocrinologie* **61**(4) (2000), 386.
[25] C. Dupuy-Fons, J.F. Brun, C. Mallart, J. Carvajal, M. Fussellier, L. Bardet and A. Orsetti, In vitro influence of zinc and magnesium on the deformability of red blood cells artificially hardened by heating, *Biol. Trace Elem. Res.* **47** (1995), 247–255.
[26] S. Khaled, J.F. Brun, G. Cassanas, L. Bardet and A. Orsetti, Effects of zinc supplementation on blood rheology during exercise, *Clin. Hemorheol. Microcirc.* **20** (1999), 1–10.
[27] S. Khaled, J.F. Brun, J.P. Micallef, J.F. Monnier and A. Orsetti, Serum zinc and blood rheology in sportsmen (football players), *Clin. Hemorheol. Microcirc.* **17** (1997), 47–58.