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Relationships between blood viscosity and insulin-like growth factor I status in athletes

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Abstract. Exercise training is known (1) to enhance the function of the GH–IGF-I system, which has profound effects on body fluid status; (2) to increase blood fluidity. Thus, we investigated during an exercise-test in 39 male elite sportsmen (age 23.7 ± 0.72 years; body mass index 23.7 ± 0.28 kg/m^2) the possible relationships between GH and IGF-I status and the rheological properties of blood. Two correlations indicate a relationship between body hydration and fitness: isometric handgrip strength is correlated with the percentage of extracellular water in total body water ($r = 0.432$, $p = 0.02$) and the aerobic working capacity W_{170} is negatively correlated with hematocrit ($r = -0.341$, $p = 0.039$). Water loss during exercise appears to be inversely related to fitness as evaluated by W_{170} ($r = -0.529$, $p = 0.05$), and is positively correlated with the score of signs of overtraining ($r = 0.725$, $p = 0.003$) and with the red boood cell aggregation index ($r = 0.584$, $p = 0.036$). Finally, while the GH peak value is correlated with the extracellular water volume ($r = 0.393$, $p = 0.02$), IGF-I is correlated with blood viscosity ($r = 0.546$, $p = 0.0003$), suggesting that when IGF-I values are within the upper quintile (>340 ng/ml) IGF-I may unfavourably affect blood rheology. Among factors of blood viscosity, IGF-I exhibits a borderline correlation ($p = 0.05$) with "Tk" and the ratio IGF1/IGFBP3 which reflects free circulating IGF-I is correlated with red cell aggregability measured with the Myrenne "M" ($r = 0.485$, $p = 0.014$) and S_{60} ($r = 0.396$, $p = 0.494$). These findings confirm the importance of hydration and dehydration as determinants of both blood rheology and exercise performance. Moreover, they suggest that values of IGF-I within the upper quintile are associated with an impairment of blood fluidity, possibly due to a direct effect of IGF-I on red cell deformability and aggregability.

Keywords: Blood viscosity, hemorheology, erythrocyte deformability, erythrocyte aggregability, human, male, 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, sweating

1. Introduction

The effects of exercise training on body composition and fuel metabolism appear to be mediated in part by circulating levels of insulin-like growth factor-I (IGF-I) [1,2] which depend upon both growth hormone (GH) and nutritional equilibrium [3]. Insulin-like growth factor-I (IGF-I) is associated with muscle size and fitness, as demonstrated by correlations between peak V_{O_2} and circulating IGF-I [1,2,4, 5]. Among its various effects, IGF-I acts on erythrocyte physiology, via type-I IGF receptors [6] that exert important effects on erythroid progenitor cells [7] and can be studied on mature erythrocyte, providing

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a reflect of IGF-I binding in the whole body [8]. Whether these receptors and their activation by IGF-I exert an influence on blood rheology remains unknown.

Therefore, keeping in mind that exercise training, beside its effects on the GH-IGF-I system [1,2,4,5], induces profound hemorheological alterations resulting in an increased blood fluidity [9], we investigated in trained sportsmen the possible relationships between IGF-I status and the rheological properties of blood.

2. Methods

Subjects used in this study were 39 male elite sportsmen (national level in football, volleyball and karate) submitted daily to a physical training program. Subjects were informed of the protocol and gave their consent according to the local ethical regulations. Their characteristics are shown in 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 [10]. 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} [11]. Body composition was assessed with a multifrequency bioelectrical impedancemeter Dietosystem Human IM Scan that uses low intensity (100–800 μA) 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 [12]. 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) [13,14]. Accuracy of the measurements was regularly controlled with the Carrimed Rheometer "CS" (purchased from Rhéo, 91120 Palaiseau, France) [15]. The coefficient of variation of this method ranged between 0.6 and

Table 1
Clinical characteristics (anthropometry and ergometry) of the 39 subjects of the study (mean ± SEM)

age (years)	23.7 ± 0.72
weight (kg)	77.7 ± 1.22
height (cm)	180.7 ± 0.94
body mass index (kg/m^2)	23.7 ± 0.28
percentage of fat (%)	13.1 ± 0.38
W_{170} (w/kg)	2.82 ± 0.096
V_{O_2} max (ml.min^{-1}.kg^{-1})	47 ± 2.45
isometric handgrip strength (N)	527.5 ± 21.4
isometric adductor strength (N)	647.7 ± 61.4

0.8% [16]. 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 [17]. Dintenfass' "Tk" index of erythrocyte rigidity was calculated [18]. RBC aggregation was assessed with the Myrenne aggregometer [19] 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/η) ratio, an index of oxygen supply to tissues, was calculated according to Chien [20] and Stoltz [21], 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 [22,23]. 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 is the relative surface area above the curve calculated over the first 10 seconds. This device measures also disaggregation thresholds, by submitting blood to a succession of shear rates from 600 s^{-1} 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 radiolabelled 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 detected.

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 detected.

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% ($n = 9$).

2.3. Statistics

Values are given as mean ± SEM. Linear and non linear correlations were calculated with a home-made software using least squares fitting.

3. Results

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

Table 2
Laboratory measurements in the 39 subjects of the study (mean ± SEM)

creatine kinase (U/l)	413 ± 56.8
ammonia (μg/l)	68.9 ± 4.4
fibrinogen (g/l)	2.34 ± 0.06
serum zinc (mg/l)	0.92 ± 0.031
IGF1 (nmol/l)	34 ± 1.9
IGFBP1 (ng/l)	15.2 ± 4.3
IGFBP3 (ng/ml)	3.6 ± 0.19
IGF1/IGFBP3 (ng/ml)	9.4 ± 0.6

Table 3
Hemorheologic parameters (mean ± SEM)

ferritin (ng/ml)	75.53 ± 7.3
hematocrit (%)	42.27 ± 0.3
h/η ($mPa^{-1}.s^{-1}$)	14.3 ± 0.19
blood viscosity η_b (mPa.s)	2.97 ± 0.04
η_b at corrected hct 45%	3.13 ± 0.04
plasma viscosity η_p (mPa.s)	1.37 ± 0.01
erythrocyte rigidity "Tk"	0.62 ± 0.01
erythrocyte aggregation "M"	4.5 ± 0.36
erythrocyte aggregation "M1"	9.7 ± 0.43
aggregation kinetics "TA"	3.21 ± 0.28
aggregation kinetics "S_{10}"	22.15 ± 0.9
aggregation kinetics "S_{60}"	39.7 ± 0.86
disaggregation γ_S (s^{-1})	66.8 ± 4.2
disaggregation γ_D (s^{-1})	60.7 ± 6.7

3.1. Relationships between GH-IGF status and blood rheology

IGF-1 was correlated positively with blood viscosity at native hematocrit ($r = 0.546$, $p = 0.000326$, Fig. 1) and at corrected hematocrit 0.45 ($r = 0.546$, $p = 0.000394$) and negatively with h/η ratio ($r = -0.49$, $p = 0.00154$). There was a borderline correlation between IGF1 and "Tk" ($r = 0.312$, $p = 0.05$, Fig. 2). The ratio IGF1/IGFBP3 was correlated with two aggregation indices: Myrenne "M" ($r = 0.485$, $p = 0.014$) and S_{60} ($r = 0.396$, $p = 0.494$). GH peak value was correlated with extracellular water volume ($r = 0.393$, $p = 0.0237$).

3.2. Relationships among body water, fluid loss, performance status and blood rheology

Isometric handgrip strength was correlated with the percentage of extracellular water in total body water ($r = 0.432$, $p = 0.02$). W_{170} was negatively correlated with the ratio between water loss during exercise and extracellular water ($r = -0.529$, $p = 0.0517$, Fig. 3). W_{170} was also negatively correlated

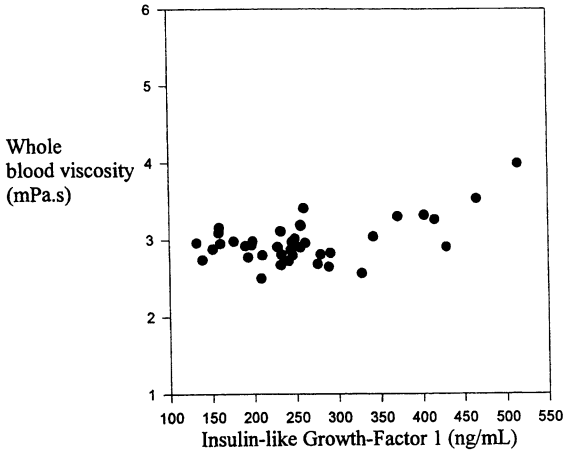

Fig. 1. Correlation between IGF-1 and blood viscosity at native hematocrit ($r = 0.546$, $p = 0.000326$).

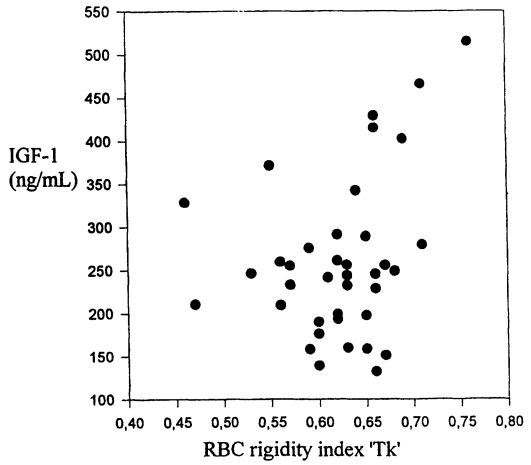

Fig. 2. Correlation between IGF1 and red cell viscometric index of rigidity "Tk" ($r = 0.312$, $p = 0.05$).

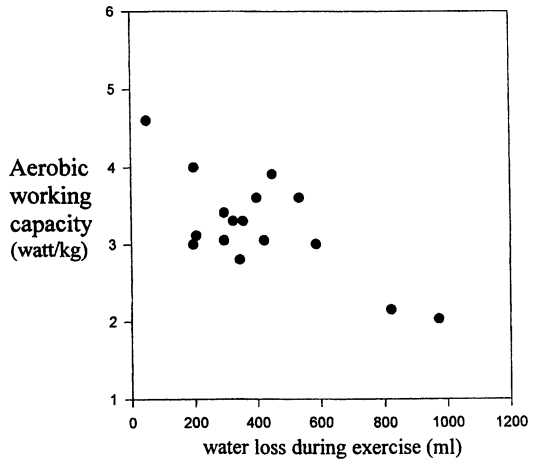

Fig. 3. Correlation between the aerobic working capacity W_{170} and the ratio between water loss during exercise and extracellular water ($r = -0.529$, $p = 0.0517$).

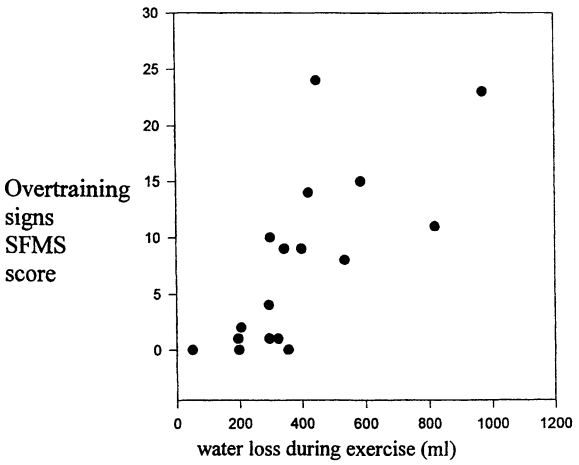

Fig. 4. Correlation between the score of signs of overtraining on the SFMS questionnaire and the volume of water loss ($r = 0.728$, $p = 0.0009$).

with hematocrit ($r = -0.341$, $p = 0.039$). The score of signs of overtraining was correlated with water loss, expressed either as a crude volume ($r = 0.725$, $p = 0.003$, Fig. 4) or as the ratio between water loss during exercise and extracellular water ($r = -0.719$, $p = 0.003$). Water loss expressed as a volume was correlated with the "S_{60}" aggregation index ($r = 0.5844$, $p = 0.036$, Fig. 5).

4. Discussion

While it is well known that the GH–IGF axis is a major regulator of body composition [24], including the body water stores [25], its involvement in fluid homeostasis and blood rheology in sportsmen remains poorly known. In preceding papers, we reported that the magnitude of the exercise-induced GH response was an independent statistical determinant of both the water loss and the postexercise rise in plasma viscosity [26], and that GH deficient adults exhibited some hemorheologic alterations that appeared to

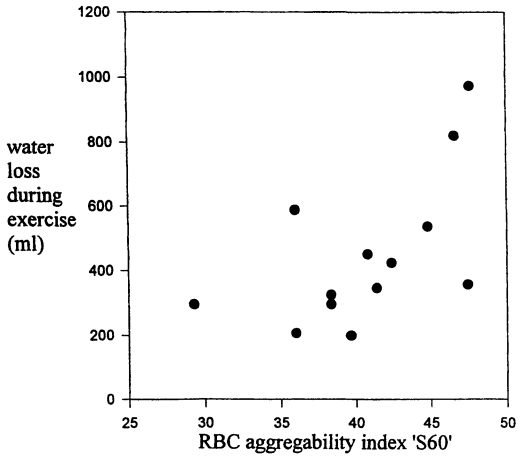

Fig. 5. Correlation between water loss expressed as a volume and the "S_{60}" RBC aggregation index ($r = 0.5844$, $p = 0.036$).

be explained by the specific body composition pattern of these patients rather than the hormonal defect itself [27].

This study aimed at elucidating whether the GH-IGF status in highly trained sportsmen is related to body fluid status, exercise water loss, and blood rheology. Results presented above show some interesting correlations between the GH–IGF-1 status and blood rheology. First, several indexes of the degree of body hydration (including bioimpedance parameters and hematocrit) indicate that hydration is correlated with fitness as assessed by various approaches (W_{170}, isometric handgrip strength, overtraining score). Second, water loss during exercise appears to be inversely related to fitness, and to correlate with pre-exercise values of red cell aggregability. Third, circulating IGF-1 is positively correlated with blood viscosity (expressed either at native hematocrit or at corrected hematocrit 0.45), suggesting that it may unfavourably affect blood rheology.

4.1. Body water, growth hormone, blood rheology and fitness

There is some literature demonstrating that GH and IGFs are important hormones of water homeostasis [25], as further demonstrated by water retention in GH-treated patients [28]. In this study, we observe a correlation between GH peak value and extracellular water volume which is consistent with our previous observations [26]. On the whole, it appears that subjects who release more GH have more extracellular water, a condition which can be expected to be beneficial for exercise hemodynamics [29] and may improve sweating capacity, a major factor in exercise thermoregulation [2,26]. Interestingly, not only aerobic capacity is related to increased extracellular water stores, but also isometric handgrip strength. This latter parameter is correlated with the percentage of extracellular water in total body water, further indicating the close relationship that has been repeatedly described [29] between training-induced "autohemodilution" [9] and fitness. A negative correlation between W_{170} and hematocrit is also found, consistent with many previous studies [9,30]. While there is a general belief in athletes that the more they increase their hematocrit, the more they will be fit, there is exactly the opposite picture in physiological conditions [9,30], due to a higher water content in extracellular fluid compartments in trained people.

4.2. Water loss, blood rheology and fitness

Water loss is the key mechanism for exercise thermoregulation [2,26] and is thus improved in trained people [2], parallel with an increase in GH secretion [26]. We previously hypothesized, based on both experimental data and literature, that there is a physiological relationship between these two parameters. Thus, higher GH production, together with higher sweating capacity, could be both important mechanisms improving exercise tolerance. However, one could assume that excess water loss during exercise may be, on the contrary, a limiting factor that could decrease performance. Consistent with this last hypothesis, we described a moderate rise in hematocrit in overtrained people [31], a higher plasma viscosity in unfit iron depleted athletes [32], and a higher occurrence of overtraining and iron deficiency states in athletes whose hematocrit is in the highest quintile [30]. In this respect, data shown here are interesting. Water loss during exercise appears to be inversely related to fitness (Fig. 3), and to correlate with pre-exercise values of red cell aggregability (Fig. 5) while the score of signs of overtraining is correlated with water loss (Fig. 4). These data are not in disagreement with the literature indicating the association of fitness with improved sweating capacity. They rather underline, in our opinion, that water loss may be beneficial only to a certain extent and that when it overpasses a physiological limit, it becomes, on the other way about, deleterious. Some recent literature on overtraining may somehow support that assumption, describing overtraining as an "Addison-like" disease [33] and more generally fatigue states as a disorder of water-regulating hormones [34]. However, there seems to be very little information in literature on the boundaries between beneficial and deleterious effects on water loss in athletes. We think that this subject, which may be related to blood rheology, requires a further study.

4.3. Blood rheology and the GH–IGF axis

Consistent with our initial working hypothesis, we find relationships between circulating levels of hormones of the GH–IGF-I axis and blood rheology. The first one is a correlation between the GH post-exercise peak value and the extracellular water volume already discussed above. On the whole, GH responsiveness appears to be associated with expanded extracellular volume and sweating capacity, which are both interrelated and both improve body's ability to exercise [2,26].

Another finding concerning relationships between the GH–IGF-I axis and blood rheology is the unexpected positive correlation between circulating IGF-1 and blood viscosity. While the correlation fits better with a linear law, its shape (Fig. 1) leads to think that viscosity increases only when IGF-I reaches the upper range of physiological values. This findings lead to assume that when IGF-I values are within the upper quintile (which is defined in our laboratory >340 ng/ml) IGF-I may unfavourably affect blood rheology. Since this correlation persists when viscosity is corrected for hematocrit it is not likely to be explained by fluid shifts as the relationships we previously described between overtraining and viscosity [30,31]. Red cell rheology is more likely to be the factor of viscosity related to IGF-I, given the correlations between IGF-I and "Tk" and between the ratio IGF1/IGFBP3 and red cell aggregability. On the whole, these correlations suggest that values of IGF-I within the upper quintile are associated with an impairment of blood fluidity, possibly due to an effect of IGF-I on red cell deformability and aggregability. We are not aware of previous reports on the hemorheologic effects of IGF-I, a potent growth factor which exerts many actions in the body [1,2]. By contrast, a large literature can be found on erythrocyte IGF-I receptors [6], which appear to have a physiological stimulatory action on erythropoiesis [7]. Since the ratio between young and old red cells and the amount of reticulocytes markedly modify the rheological properties of red cells [18,20], most of this effect could be related to shifts in young/old RBC ratio.

However, a direct impact of IGF-I on RBC rheological properties, as already reported for other hormones like insulin [35–37], glucagon [38] or epinephrine [39] has also to be investigated.

In conclusion, these data further support our hypothesis that the GH–IGF-I axis is a regulator of blood rheology, in physiological conditions such as regular training in athletes. More precisely, IGF-I appears to impair blood fluidity when its values reach the upper quintile of the physiological range. The mechanism as well as the relevance of this process remain to be clarified.

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