Nutrition as a determinant of blood rheology and fibrinogen in athletes

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Abstract. Blood rheology is influenced by metabolism and nutrition. We investigated this issue in 41 elite athletes exercising 13 ± 0.9 hr/wk (mean age: 23.9 ± 0.67 yr; mean $V_{O_2 \, max}$: 52.6 ± 2.3 ml/min/kg body weight) with a standardised nutritional questionnaire suitable for sports medicine. Calorie intake (% of recommended intake) was negatively correlated with the RBC disaggregability threshold ($r = -0.505$, $p = 0.01$). There were negative correlations between fibrinogen and protein intake (% of the total caloric intake $r = -0.787$, $p = 0.0008$; amount in g/kg/day $r = -0.597$, $p = 0.03$). Accordingly, the RBC disaggregability threshold was also correlated negatively with protein intake ($r = -0.508$, $p = 0.05$). Lipid intake (g/kg/day) was negatively correlated with the RBC disaggregability threshold ($r = -0.564$, $p = 0.03$) and positively to the hematocrit/viscosity ratio ($r = 0.531$, $p = 0.03$). Carbohydrate intake (g/kg/day) was positively correlated with whole blood viscosity ($r = 0.517$, $p = 0.04$) and negatively to the hematocrit/viscosity ratio ($r = -0.4863$, $p = 0.05$). In addition fibrinogen was negatively correlated with hematocrit ($r = -0.4129$, $p = 0.036$) and positively with a host of aggregation parameters ($p < 0.001$). Therefore fibrinogen levels and red cell rheology exhibit correlations with the nutritional status in athletes. Low protein intake appears to be associated with (mildly) raised fibrinogen and aggregability, and low calorie intake is associated with lower RBC disaggregability.

Keywords: Rheology, nutrition, athletes, fibrinogen, aggregability, protein intake, calorie intake

1. Introduction

Several studies have shown that blood rheology is influenced by metabolic factors, alterations in body composition and body fluid shifts [1]. However, nutritional factors exert their own effect on these processes and may influence hemorheologic changes in elite athletes [2,3]. In order to investigate this issue, we aimed at clarifying the relationships between blood rheology and nutritional status by comparing hemorheological parameters (hematocrit, viscosity, red blood cell disaggregability threshold and fibrinogen) with nutritional intake characteristics (calorie, protein, lipid and carbohydrate intake).

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2. Methods

2.1. Subjects

Subjects used in this study were 41 elite athletes: 37 footballers, 2 karate professionals, and 2 endurance runners (mean age: 23.97 ± 0.67 yr; mean weight: 75.38 ± 1.30 kg; mean height: 177 ± 0.95 cm; BMI: 23 ± 0.73 kg/m^2; mean $V_{O_2 \, max}$: 52.6 ± 2.3 ml/min/kg body weight). They practised exercise during 13 ± 0.9 hr training/wk. The subjects were checked to be on good health and were free of medication.

2.2. Standardised nutritional questionnaire

The athlete alimentation habit was taken by a self-administered questionnaire during a dietary assessment [4,5].

Our questionnaire is based on Monnier et al.'s one [6]. A specific validation of this questionnaire in athletes has been previously published [4]. It consists in evaluating the protein amount and the "extra" calories taken each day.

Protein intake is evaluated according to the following formula:

Meat (g/d)/5 + fish (g/d)/6 + eggs (number/d) × 12.5 + milk (ml/d) × 0.023

    + yoghurt (number/d) × 3.5 + cream cheese (number of parts/d) × 7

    + cheese (number of parts/d) × 7 + bread (g/d) × 0.1 + 10 g supposed to be unavoidable.

"Extra" calories correspond to: (1) 300 kcal/d if the subject is prone to nibbling, (2) $50 \times N$ with $N =$ number of parts of delicatessen, pizza, cakes eaten per week, (3) $200 \times n$ with $n =$ number of meals taken at restaurant per week, and (4) $50 \times L$ with $L =$ number of liters of soda that have been drunk each day.

These data allow to determine the calorie intake according to the following equation:

Calorie intake (kcal/d) = protein intake (g/d) × 24 + extras + alcohol (l/d) × 300.

Lipid intake may be also evaluated according to the following formula:

Lipid intake (g/d) = [fish + meat] (g/d) × 0.1 + eggs (number/d) × 6 + milk (l/d) × 0.3

    + cheese (0.25 g/100 g) + butter (84 g/100 g) + oil (100 g/g) + extras × 15.

Carbohydrates intake is deduced according to the formula:

Calorie intake − (calories from lipid intake + calories from protein intake

    + calories from alcohol intake).

2.3. Laboratory measurements

Samples for hemorheological measurements (7 ml) were drawn with potassium EDTA as the antico-agulant in a vacuum tube Vacutainer [7]. Viscometric measurements were done at very high shear rate $(1,000 \text{ s}^{-1})$ with a falling ball viscometer (MT 90 Medicatest, F-68280 Saint Benoît) [8,9]. Accuracy of the measurements was regularly controlled with the Carrimed Rheometer 'CS' (purchased from Rheo, 91120 Palaiseau, France) [10]. The precision of this method ranges between 0.6 and 0.8% [11]. We measured with this device apparent viscosity of whole blood at native hematocrit, plasma viscosity, and blood viscosity at corrected hematocrit 45% according to the equation of Quemada [12]:

$$\eta_{\text{blood}} = \eta_{\text{pl}} \times \left(1 - \frac{1}{2}k \times \text{Hct}\right)^{-2},$$

where η_{blood} is the whole blood viscosity, η_{pl} is the plasma viscosity, k is the index of erythrocyte rigidity of Quemada and Hct is the native hematocrit.

Hematocrit was measured with microcentrifuge. The hematocrit/viscosity (Hct/η) ratio, an index of oxygen supply to tissues, was calculated according to Chien [13] and Stoltz [14], with hematocrit (as percentage) divided by viscosity at high shear rate determined as described above.

The Dintenfass "Tk" index of erythrocyte rigidity was calculated [15,16] according the following formula:

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

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

RBC aggregation was measured using two well-standardised methods. The first one was the Myrenne aggregometer [17] 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 second one was the SEFAM aggregometer which is based upon the experiments of Mills [18,19] on cell disaggregation behaviour in shear flow. 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. The SEFAM agregometer allows to measure the total disaggregation threshold (γ_s). This 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.

Fibrinogen was measured with the method of Von Clauss.

2.4. Statistics

Values are presented as mean $\pm$ standard error (SE). The relationship between hemorheological para-meters (Hct, Hct/η, fibrinogen and RBC disaggregability threshold (γ_S) and nutritional intake (protein, lipid, carbohydrate and calorie intake) were explored.

We used the software package Statview (Jandel Corporation, San Rafael, USA).

In a first step, a stepwise analysis was performed. In a second step, we chose the better model that rely the correlation found in first step. Correlations were also performed by classical Pearson analysis. The relationship between different variables and the choice of the best model were determined on the
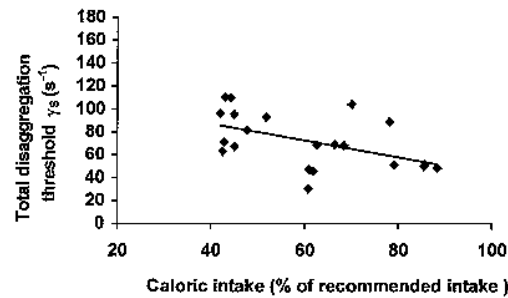
Fig. 1. Negative correlation between caloric intake expressed in percentage of recommended intake (%) and red blood cell (RBC) disaggregability threshold γs (s^{-1}) ($r = -0.505$, $p = 0.01$).

basis of the correlation coefficient value and the statistical significance. Significance level was defined as $p \leqslant 0.05$.

3. Results

3.1. Calorie intake

Calorie intake (% of recommended intake) was correlated with red cell rigidity index Tk ($r = 0.544$, $p = 0.02$) and negatively correlated with total disaggregation threshold γs ($r = -0.505$, $p = 0.01$) (Fig. 1). The best correlations were found with linear relationships corresponding to the following equations:

$$Tk = 0.4644 + 0.0022 \text{ Calorie intake } (\%),$$

$$\gamma s = 116.81 - 0.74 \text{ Calorie intake } (\%).$$

3.2. Protein intake

In the whole group of 41 subjects, we found negative correlations between fibrinogen and protein intake (expressed as % of the total calorie intake: $r = -0.787$, $p = 0.0008$; expressed as amount in g/kg/day: $r = -0.597$, $p = 0.03$). Accordingly, total disaggregation threshold, γs, was also correlated negatively with protein intake expressed as amount in g/kg/day ($r = -0.512$; $p = 0.05$). These correlations are presented in Figs 2(a) and 2(b), respectively. The best correlations were found with linear relationships corresponding to the following equations:

$$\text{Fibrinogen} = 5.1986 - 0.2286 \text{ Protein intake } (\%),$$

$$\text{Fibrinogen} = 3.4086 - 0.6999 \text{ Protein intake } (g/kg/day),$$

$$\gamma s = 108.91 - 41.171 \text{ Protein intake } (g/kg/day).$$

In addition fibrinogen was negatively correlated with Hct ($r = -0.4129$, $p = 0.036$) and positively with γs ($r = 0.537$, $p = 0.01$), aggregation index at 10 s "S10" ($r = 0.67$, $p = 0.01$) and aggregation index at 60 s "S60" ($r = 0.723$, $p < 0.001$).
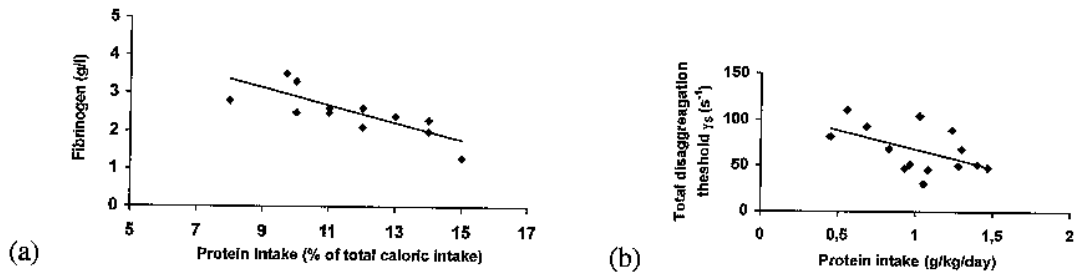
(a)　　　　　　　　　　　　　　　　　　　　　　　(b)

Fig. 2. Negative correlations between (a) the protein intake expressed in percentage of daily nutritional intake (%) and fibrinogen (g/l) ($r = -0.787$, $p = 0.0008$) and (b) the protein intake expressed in g/kg/day and RBC disaggregability threshold, γs (s^{-1}) ($r = -0.597$, $p = 0.03$).
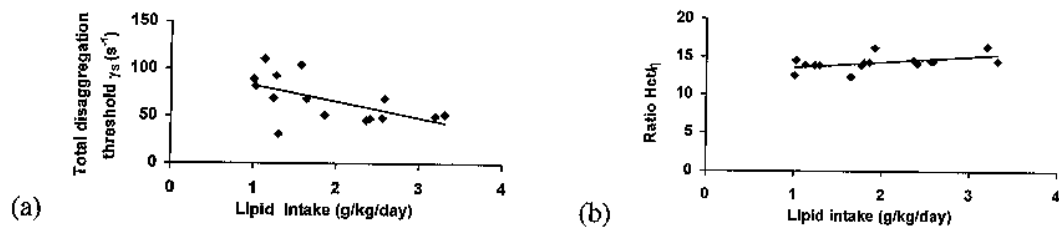

(a)　　　　　　　　　　　　　　　　　　　　　　　(b)

Fig. 3. (a) Negative correlation between the lipid intake (g/kg/day) and RBC disaggregability threshold γs (s^{-1}) ($r = -0.564$, $p = 0.03$), and (b) positive correlation between the lipid intake (g/kg/day) and hematocrit/blood viscosity ratio (Hct/η) ($r = 0.531$, $p = 0.03$).

By contrast, protein intake was not significantly correlated with the overtraining score (expressed as % of the total calorie intake: $r = -0.271$, $p = 0.277$; expressed as amount in g/kg/day: $r = -0.413$, $p = 0.07$). This score exhibited a borderline correlation ($r = 0.39$, $p = 0.05$) with the total disaggregation threshold, γs.

3.3. Lipid intake

Lipid intake (g/kg/day) was negatively correlated with total disaggregation threshold, γs ($r = -0.564$, $p = 0.03$) and positively to Hct/η ($r = 0.531$, $p = 0.03$) according to the following equations:

$$\gamma s = 99.891 - 17.253 \text{ Lipid intake (g/kg/day)},$$

$$\text{Hct}/\eta = 12.834 + 0.7368 \text{ Lipid intake (g/kg/day)}.$$

The correlations are presented in Figs 3(a) and 3(b), respectively. Lipid intake was not correlated with η_{blood} (expressed as % of the total calorie intake: $r = -4.402$, $p = 0.109$; expressed as amount in g/kg/day: $r = -0.335$, $p = 0.204$).

3.4. Carbohydrate intake

First of all, carbohydrate intake (g/kg/d) exhibited a highly significant negative correlation with lipid intake ($r = 0.976$, $p < 10^{-12}$). Carbohydrate intake (g/kg/day) was positively correlated with η_{blood} ($r = 0.517$, $p = 0.04$) and negatively to the Hct/η ($r = -0.487$, $p = 0.05$). The correlations are
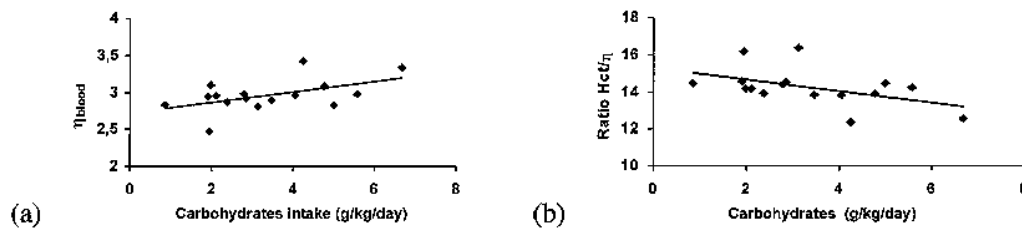
(a)   (b)

Fig. 4. (a) Positive correlation between the carbohydrates intake (g/kg/day) and whole blood viscosity (η_{blood}) ($r = 0.517$, $p = 0.04$), and (b) Negative correlation between the carbohydrate intake (g/kg/day) and hematocrit/blood viscosity ratio (Hct/η) ($r = -0.4869$, $p = 0.05$).

presented in Figs 4(a) and 4(b), respectively. They follow the equations written below:

$$\eta_{blood} = 2.724 + 0.071 \text{ Carbohydrate intake (g/kg/day)},$$

$$\text{Hct}/\eta = 15.319 - 0.314 \text{ Carbohydrate intake (g/kg/day)}.$$

4. Discussion

This study shows that the nutritional habits of athletes as assessed by a standardised questionnaire are associated with alterations in blood rheology.

In this study we used a standardised self-administered questionnaire which is based upon the evaluation of protein intake and was primarily designed for obese patients. We previously studied the validity of this questionnaire in athletes, and reported a satisfactory concordance between it and a more extensive nutritional assessment. Actually, the questionnaire was even more reliable in athletes than in patients and thus allowed, in routine condition, a simple and accurate assessment of calorie and protein intake [4,5]. Obviously, some more specific information such as the balance between saturated and unsaturated lipids, as well as rapid vs slow carbohydrates, can not be accurately measured with this quite simplified tool. By contrast, we think that the robustness of the method allows a satisfactory evaluation of both protein and calorie daily intake in athletes.

Increasing lipid intake seems to be associated with an increase in RBC disaggregability. This finding is in agreement with a number of studies showing that circulating blood lipids increase erythrocyte aggregability [20,21]. Interestingly, however, the Hct/η, a classical proposed index of the positive effect of blood fluidity on O_2 transfer to tissues [13,14], exhibits a positive correlation with lipid intake. Since most of the alimentary lipids in our country located on the Mediterranean seaside, consist of unsaturated fatty acids, this relationship may indicate a beneficial effect of these lipids in athletes on blood viscosity at high shear rate, as previously demonstrated by the team of Guezennec [22,23] with (n-3) unsaturated fatty acids, which actually improved the aerobic working capacity of the subjects due to an increase in red cell deformability. Since the alimentary questionnaire employed here is unable to define which kind of lipids were responsible for this relationship, this issue will require further studies.

It should be stressed that we also find a negative correlation between the Hct/η and carbohydrate intake. In fact, this correlation is likely to indirectly mirror correlation of this parameter with lipid intake, since it is well known that increasing carbohydrates in the daily nutritional intake automatically results in a lower lipid intake. Accordingly, a partial correlation analysis eliminates this latter correlation and only selects the preceding one.

Another correlation is found between carbohydrate intake and η_{blood}. In this case, it can not be explained by the lipid intake since it fails to be correlated with η_{blood}. Actually, we have no clear explanation for this correlation since viscosity factors considered individually are not correlated with carbohydrates. Similarly, the positive correlation we find between total calories and red cell rigidity, Tk, has no clear explanation, although it may be assumed to reflect to some extent an effect of nibbling and extra-calories, with presumably an excessive intake of saturated fat. Clearly, our standardised questionnaire is unable to detect a clear explanation of this finding.

By contrast, we would want to point out two interesting negative correlations: the correlation between total calories and the RBC disaggregability threshold, γ_S, and the correlation between protein intake and both fibrinogen and RBC disaggregability.

The first one is likely to indicate that low calorie intake is associated with lower RBC disaggregability: the total disaggregation threshold increases when calorie intake decreases. This finding is interesting to analyse in connection with the nutritional theory of overtraining [24]. Presumably, insufficient calorie intake may be associated with some degree of failure of free-radical scavenging mechanisms and some degree of mild inflammation that may induce some subtle red cell damage which is not detected with whole blood viscosity or Myrenne aggregometry. However, a large body of literature suggests that such alterations of red cell aggregation may modify blood distribution and O_2 supply to tissues, at least at rest [25].

Even more interestingly we also evidence that when athletes do not eat enough proteins, their fibrinogen is increased and their red cell disaggregability is decreased. Not surprisingly, fibrinogen and RBC disaggregability are well correlated, although a r value of 0.537 indicates that disaggregability is not fully explained by fibrinogen and may also rely upon intrinsic erythrocyte factors. Data [26,27] on the overtraining syndrome indicate that an insufficient protein intake may impair the processes of muscle repair during recovery, resulting in a rise in pro-inflammatory cytokines [28] including those that are well known to increase plasma fibrinogen levels. Interestingly, we find a correlation between the overtraining score and the RBC disaggregability threshold, γ_S, a parameter of red cell aggregation.

On the whole, whether these relationships between nutritional status and blood rheology in athletes are relevant by their own is unclear. However, these correlations further confirm the strong influence of nutrition on athlete's physiological parameters. In addition, they are in agreement with our previous assumption of blood rheology being a marker of athletes' physiological status which is itself, obviously, correlated with fitness.

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