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Increased blood viscosity in iron-depleted elite athletes

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Abstract. Since iron deficiency is associated with abnormal erythrocyte rheology, we investigated relationships between plasma ferritin and blood rheology in 36 male elite sportsmen (age: 22.38 ± 0.9 years). On the whole, ferritin was negatively correlated with blood viscosity ($r = -0.36$, $p < 0.05$). When 23 subjects with low ferritin levels suggesting mild iron deficiency were compared with 13 matched sportsmen with normal ferritin levels, iron-deficient sportsmen were shown to have a higher blood viscosity at 1000 s^{-1} (3.17 ± 0.09 vs. 2.85 ± 0.06 mPa s, $p < 0.05$), explained by a higher plasma viscosity (1.38 ± 0.02 vs. 1.31 ± 0.02 mPa s, $p < 0.05$), while hematocrit and RBC rigidity index T_k were similar in the two groups. RBC aggregability index M (4.59 ± 0.58 vs. 2.95 ± 0.43 mPa s, $p < 0.05$) and M_1 (8.46 ± 0.58 vs. 6.07 ± 0.55, $p < 0.01$) were higher in iron-deficient subjects. Serum zinc was lower in iron-deficient sportsmen (0.73 ± 0.02 vs. 0.83 ± 0.02 mg/l, $p < 0.01$), but the score of early signs of overtraining was higher in this group (10.84 ± 1.61 vs. 4.08 ± 1.11, $p < 0.01$). These data suggest that mild iron deficiency as commonly seen in athletes, before anemia occurs, is associated with an increase in plasma viscosity and RBC aggregation, together with an increased subjective feeling of exercise overload.

Keywords: Plasma ferritin, blood viscosity, plasma viscosity, RBC aggregation, sportsmen, overtraining, serum zinc

1. Introduction

Iron is probably the most studied and the best known trace element. This interest is largely explained by the frequency of iron deficient states and by the possibility of treating them with iron supplementations. Almost 30–40% of the total body iron is stored under the form of ferritin and hemosiderin, while a lower amount is stored as transferrin [1]. Therefore, serum ferritin is a reliable marker of iron stores. Although a high ferritin value cannot rule out the existence of an iron deficiency, a low ferritin value is highly specific of a deficiency [2,3].

Such iron-deficient states are particularly frequent in highly trained athletes, and even more in women [4]. The mechanism of these deficiencies is complex: hemolysis resulting from mechanical damage on red cells [5–7], higher iron loss in urine, faeces, or sweat [6,8–10], decrease in iron digestive absorption [11], some disturbances in erythropoiesis [9], or inadequate intake [12,13]. In the case of "sports anemia" the effects of this deficiency on performance are well known: there is a reduction in exercise performance and a decrease in working capacity [14,15], with a higher accumulation of lactate into blood and a lower VO_2 max [16,17]. Relationships between iron deficiency in trained subjects and either erythrocyte metabolism, oxygen consumption, or blood lactate have been extensively studied [18–23].

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However, whether iron deficiency is associated with hemorheological changes is poorly known. Experimental studies in iron-deficient rats have evidenced a lower erythrocyte flexibility that seemed to be related, at least in part, to a lower hemoglobin content of erythrocytes [24]. We are not aware of similar findings in humans. However, we recently reported that borderline deficiencies in another trace element, zinc, are associated with a decrease in red cell flexibility [25], while oral zinc supplementation increases red cell flexibility and decreases red cell aggregability [26].

Therefore, we investigated relationships between iron status (as reflected by plasma ferritin) and blood rheology in sportsmen.

2. Subjects and methods

We studied blood rheology at rest in 36 male elite sportsmen (national level in football, volley and karate) (age 22.38 ± 0.9 years; weight 80.54 ± 1.23 kg; height 185.36 ± 2.11 cm). All subjects answered a standardized questionnaire developed by the French consensus group on overtraining of the *Société Française de Médecine du Sport* (SFMS) in order to quantify the early clinical symptoms of the overtraining syndrome [27]. This questionnaire includes 58 items: the number of positive items gives a numerical "score" that helps to evaluate the degree of exercise overload in sportsmen submitted to a heavy training program.

Blood samples were drawn after an overnight fast in order to measure plasma ferritin, hemoglobin concentration, hematocrit, mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), hemorheological parameters and serum zinc.

Plasma ferritin was measured by the solid-phase two-site immunoradiometric assay kit FER-CTRIA. Assay sensitivity, defined as the amount significantly different from zero with a probability of 95%, is 1 ng/ml. With this assay, normal values for men range between 75 and 300 ng/ml.

Blood samples for hemorheological measurements (7 ml) were obtained with a large bore needle (Luer adaptor Venoject, set into the catheter) to avoid shear damage to erythrocytes. A vaccum tube was used for blood withdrawal, with potassium EDTA as the anticoagulant. Viscometric measurements were done at very high shear rate ($\gamma = 1000\ s^{-1}$) with a falling ball viscometer (MT 90 Medicatest, F-86280 Saint Benoit) [28,29]. Temperature of blood during measurements was 37°C. Accuracy of the measurements was regularly controlled with the Carrimed Rheometer CS (purchased from Rhéo, 91120 Palaiseau, France) [30]. The coefficient of variation of this method ranges between 0.6 and 0.8% [31]. 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 ($\eta_b = \eta_{pl} \times (1-(1/2k) \times h)^{-2}$, where η_b is blood viscosity, η_{pl} plasma viscosity, h the hematocrit and k a shear-dependent intrinsic viscosity of the red cells according to Quemada) [32]. Hematocrit was measured with microcentrifuge. Red cell deformability was estimated by the index of erythrocyte rigidity T_k which was calculated according to the equation of Dintenfass from blood viscosity (η_b), hematocrit (h) and plasma viscosity (η_{pl}) as follows: $T_k = (\eta_r^{-0.4} - 1) \times (\eta_r^{-0.4} \times h)^{-1}$, where η_r is relative blood viscosity η_b/η_{pl} [33,34]. Red blood cell aggregation was assessed at native hematocrit with the Myrenne aggregometer [35] which gives two indices of RBC aggregation: M (aggregation during stasis after disaggregation at 600 s^{-1}) and M_1 (facilitated aggregation at low shear rate after disaggregation).

Serum zinc was measured by flame atomic absorption spectrophotometry (FAAS, model 2380, Perkin-Elmer). The lower limit of sensitivity of this method is 0.0125 mg/l. Its coefficient of variation (CV) ranges between 4 and 7%.

3. Statistics

Values are presented as mean ± standard error on the mean. Correlations were tested by linear regression analysis. Comparisons were performed with Mann–Whitney test. Significance level was defined as $p < 0.05$.

4. Results

In the whole group of 36 subjects, we found correlations between plasma ferritin and the following parameters: whole blood viscosity at native hematocrit ($r = -0.36$, $p = 0.03$) (Fig. 1), serum zinc ($r = 0.47$, $p = 0.007$) (Fig. 2), and the score of early signs of overtraining ($r = -0.56$, $p = 0.002$) (Fig. 3).

We found also correlations between serum zinc and plasma viscosity ($r = -0.52$, $p = 0.0025$) (Fig. 4), the score of early signs of overtraining ($r = -0.59$, $p = 0.001$) (Fig. 5).

According to plasma ferritin level, subjects were divided into two subgroups: low plasma ferritin group and normal plasma ferritin group ($42.13 \pm 4.12 < 75$ ng/ml, $n = 23$, vs. $108.08 \pm 8.29 > 75$ ng/ml,

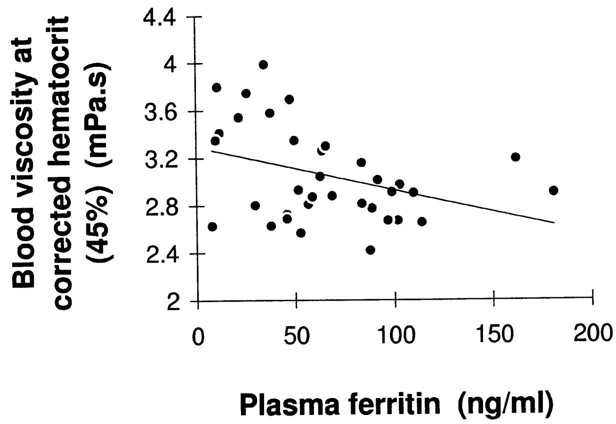

Fig. 1. Correlation between plasma ferritin and whole blood viscosity at native hematocrit ($r = -0.36$, $p = 0.03$).

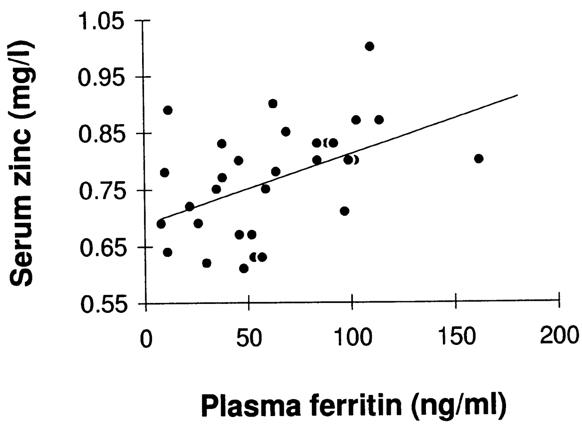

Fig. 2. Correlation between plasma ferritin and serum zinc ($r = 0.47$, $p = 0.007$).

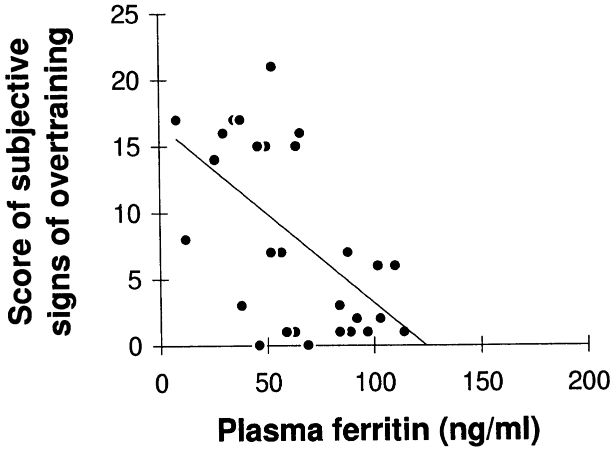

Fig. 3. Correlation between plasma ferritin and the score of early signs of overtraining ($r = -0.56$, $p = 0.002$).

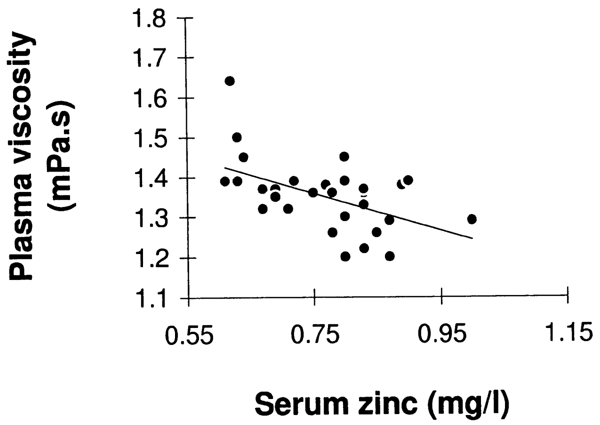

Fig. 4. Correlation between serum zinc and plasma viscosity ($r = -0.52$, $p = 0.0025$).

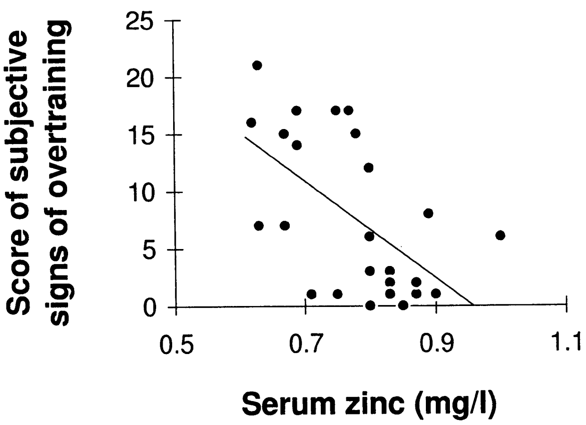

Fig. 5. Correlation between serum zinc and the score of early signs of overtraining ($r = -0.59$, $p = 0.001$).

Table 1
Anthropometric characteristics in two groups

	Low plasma ferritin	Normal plasma ferritin
Age (year)	20.93 ± 1.02	24.92 ± 1.55 *
Weight (kg)	81.76 ± 1.48	78.38 ± 2.14
Height (cm)	188.57 ± 1.57	182.31 ± 1.47 *
BMI (kg/m^2)	23.02 ± 0.42	23.49 ± 0.31

$^*p < 0.05$.

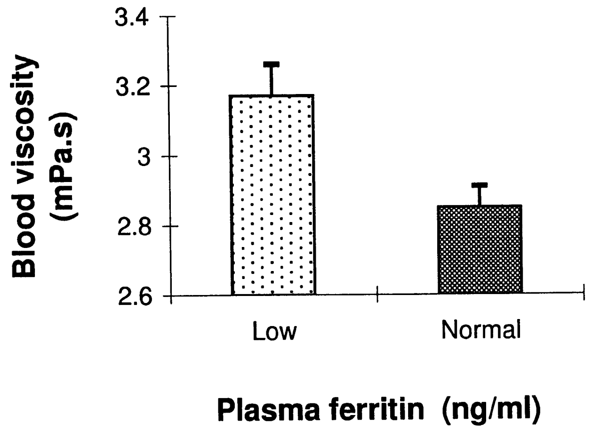

Fig. 6. Comparison of blood viscosity between the two groups showing that it was higher in low plasma ferritin group ($p = 0.018$).

$n = 13$; $p < 10^{-4}$). Age, weight, height and body mass index (BMI) for the two groups are shown in Table 1.

In the low plasma ferritin group, the whole hemoglobin concentration (13.92 ± 0.14 g/dl), as well as hematocrit (42.39 ± 0.36%), MCHC (29.65 ± 0.38 pg) and MCV (86.42 ± 0.9 fl), were all within the normal range, i.e., there was no anemia.

When the subgroup of 23 subjects with low plasma ferritin was compared with the 13 matched sportsmen with normal plasma ferritin, iron-deficient subjects were shown to have a higher blood viscosity at corrected hematocrit (45%) (3.17 ± 0.09 vs. 2.85 ± 0.06 mPa s, $p = 0.018$) (Fig. 6), a higher plasma viscosity (1.38 ± 0.02 vs. 1.31 ± 0.02 mPa s, $p = 0.029$) (Fig. 7), and a higher RBC aggregability index M (4.59 ± 0.58 vs. 2.95 ± 0.43, $p = 0.037$) (Fig. 8) and M_1 (8.46 ± 0.58 vs. 6.07 ± 0.55, $p = 0.009$) (Fig. 9). Hematocrit (42.39 ± 0.36 vs. 41.08 ± 0.74%) and RBC rigidity index T_k (0.66 ± 0.02 vs. 0.65 ± 0.01) were similar in the two groups. Serum zinc was lower in low plasma ferritin group (0.73 ± 0.02 vs. 0.83 ± 0.02 mg/l, $p = 0.003$) (Fig. 10). The score of early signs of overtraining was higher in this group (10.84 ± 1.61 vs. 4.08 ± 1.11, $p = 0.004$) (Fig. 11).

5. Discussion

This study on ferritin and rheology in sportsmen was undertaken on the basis of previous experimental works [24,36] which evidenced hemorheologic alterations in iron-deficient rats. Our results show that sportsmen with low plasma ferritin exhibit a higher blood viscosity, a higher plasma viscosity, and a

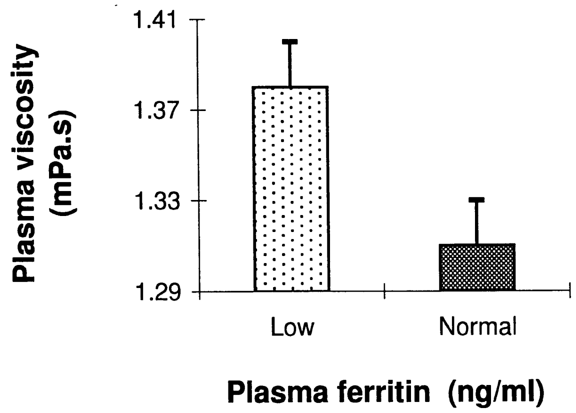

Fig. 7. Comparison of plasma viscosity between the two groups showing that it was higher in low plasma ferritin group ($p = 0.029$).

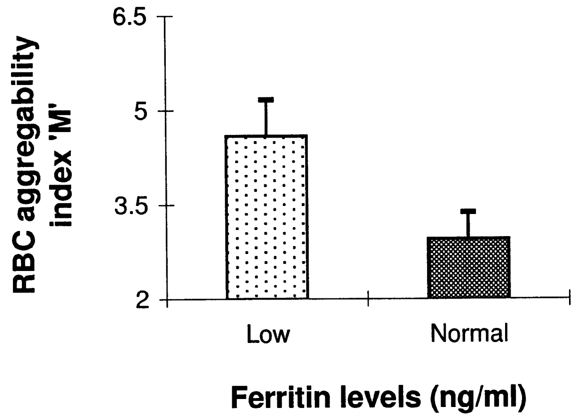

Fig. 8. Comparison of RBC aggregability index M between the two groups showing that it was higher in the low plasma ferritin group ($p = 0.037$).

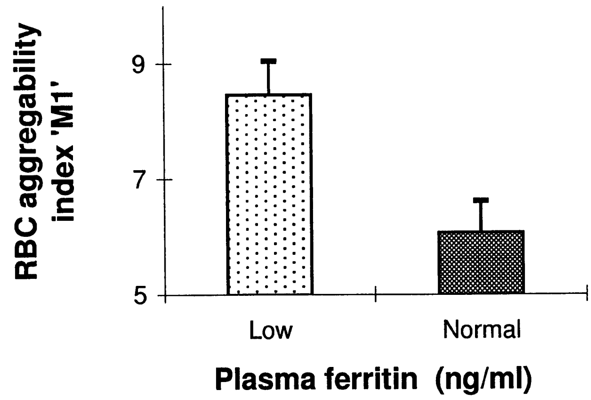

Fig. 9. Comparison of RBC aggregability index M_1 between the two groups showing that it was higher in low plasma ferritin group ($p = 0.009$).

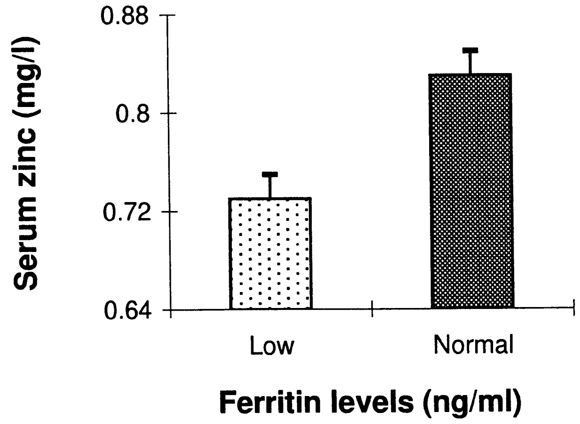

Fig. 10. Comparison of serum zinc between the two groups showing that it was lower in the low plasma ferritin group ($p = 0.003$).

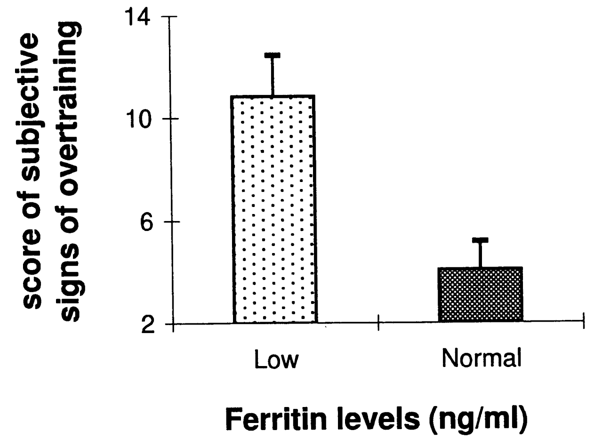

Fig. 11. Comparison of the score of early subjective signs of overtraining between the two groups showing that it was higher in the low plasma ferritin group ($p = 0.004$).

higher red cell aggregabilitity when compared to sportsmen with normal plasma ferritin. By contrast, we find no difference in either hematocrit or erythrocyte rigidity between these two subgroups.

Concerning the matching of the two subgroups, it can be seen in Table 1 that there is a little difference in age and height between the two subgroups. However, the range of age was narrow (18–33 years) and weight and body mass index were not different, so that age and height were not likely to influence our results.

On the other hand, it should be pointed out that our subjects with low ferritin are not anemic, as reflected by their values of hemoglobin, MCHC and MCV. Such an isolated, low value of ferritin, as frequently observed in highly trained individuals, probably reflects an early stage of iron deficiency [2,3].

Low values of ferritin are frequent in individuals submitted to a heavy training protocol [37–41], but several authors suggest that this is not due to exercise itself [42–44], while others explain this by an increase in plasma volume after exercise [45,46]. Although some authors reported that mild iron deficiency may reduce performance even before the occurrence of anemia [5,6,19,34,46–48], this point remains controversial [21–23,47–49], as well as the question of whether these sportsmen should be treated with iron supplements [50] or not [48,51]. Our subjects, with low plasma ferritin, had no significant impairment in performance, but their score of early signs of overtraining is significantly higher. This score has been proposed by a consensus meeting of French Sports Physicians in order to detect early situations leading to the overtraining syndrome [27]. It is correlated with some biological markers of this syndrome [52,53].

This latter finding is not surprising since iron deficiency is one of the biological parameters that have been proposed as markers of the overtraining syndrome [27]. Presumably, excess work load reduces iron stores and thus results in low ferritin and tiredness. Heavy training protocols have been reported to reduce iron intestinal absorption [11]. One of the explanations is that the physiological increase in iron absorption when iron stores are low [54] is impaired by exercise and thus can only occur when subjects remain at rest for a while [11]. In addition, both epinephrine and acidosis blunt red cell production, while old erythrocytes are more fragile and are prone to excessive hemolysis [55,56]. On the other hand, most sportsmen have an insufficient intake of iron and zinc, which are necessary for oxygen handling, mitochondrial electron transport, and wound healing: alteration of any of these processes may impair fitness [13]. Thus, consistent with clinical reports, moderate iron deficiencies are likely to reduce exercise performance and to delay recovery from sports injuries [16,20,57–59]. We think that the association of low ferritin and high overtraining score is largely explained by such mechanisms.

Our findings of a higher viscosity in subjects with low ferritin is consistent with previous works in experimentally iron-depleted rats showing an impairment of blood filterability [36] and erythrocyte flexibility [24]. In the latter experiment, the low deformability of erythrocytes was assumed to result from a decrease in intracellular hemoglobin content. Since hemoglobin and MCHC are not modified in our subjects, it is not surprising to observe no change in erythrocyte deformability. By contrast, plasma viscosity and erythrocyte aggregability are both increased in our hypoferritinemic sportsmen. We have no clear explanation for this specific pattern which is likely to result mainly from plasma protein modifications, that remain to be investigated in a further study.

Interestingly, we find a strong association in this study between iron and zinc status. Sportsmen are frequently deficient in one of these two minerals, or both. In previous studies, we showed that zinc-deficient sportsmen had a higher erythrocyte rigidity and a lower exercise performance [25], while oral zinc supplementation improved blood rheology and exercise tolerance [26]. In fact, the effects of deficiency in these two minerals on blood rheology could be synergistic. However, the hemorheologic pattern of hypoferritinemic subject (high plasma viscosity and high aggregation) is not the same than that of hypozincemic subjects (increased erythrocyte rigidity). While a direct *in vitro* [60,61] and *in vivo* [25] effect of zinc on blood rheology has been demonstrated, little is known on a possible direct effect of iron stores (or circulating ferritin itself?) on blood rheology.

On the whole, these data suggest that mild iron deficiency as commonly seen in athletes, before anemia occurs, is associated with an increase in plasma viscosity and RBC aggregation, together with an increased subjective feeling of exercise overload. We hypothesize that all these biological signs could be early markers of a situation leading to overtraining syndrome and impaired performance.

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