



# Plasma Volume Variations in Professional Soccer Players: Difference Between Pre- and Competitive Season

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## Abstract

**Background:** Variations in plasma volume and hematological parameters occur before and after training in soccer players. However, there are no reports on changes in hematological parameters resulting from a half-season of training in professional soccer players.

**Objectives:** To investigate the effects of training load on plasma volume variations in elite soccer players.

**Methods:** Twenty soccer players from the 1st French division soccer league (Ligue 1) were included in the study. The training load was evaluated using the rating of perceived exertion (RPE, 10- Borg scale) after each training session and each match. Anthropometrics characteristics, hematocrit (Ht), hemoglobin (Hb) and plasma volume variations (PVV) were assessed at 3 different times: T1: Baseline (before the first week of pre-season), T2: At the end of pre-season (after 8 weeks of training) and T3: At the end of the first competitive period (after 26 weeks of training and at halfway of the competitive season).

**Results:** Values of Ht and lean body mass (LBM) increased during the first competitive period from T1 to T3 (Ht:  $\Delta\uparrow 8\%$ ,  $P = 0.037$ , effect sizes (ES) = 1.0; LBM:  $\Delta\uparrow 4\%$ ,  $P = 0.041$ , ES = 0.83) and from T2 to T3 (LBM: ( $\Delta\uparrow 2\%$ ;  $P = 0.05$ ; ES = 0.77). Moreover, PVV decreased from T2 to T3 ( $P = 0.002$ , ES = 0.5) and from T1 to T3 ( $P < 0.05$ ; ES = 1.26). There were no differences in Hb at T1, T2 or T3.

**Conclusions:** Changes in body weights, PVV and Ht during preparatory and competitive phases were affected by the training load and competitive play.

**Keywords:** Hematological Parameters, Anthropometrics Characteristics, Training Load, Elite Soccer Players

## 1. Background

It is well documented by coaches and researchers that pre-season training phase in soccer game is essential to develop physical ability in preparation for the competition season phase (1, 2). Among these physical abilities, Bangsbo (3) showed that over 90% of physiological characteristics of a soccer game are sustained by the aerobic metabolism. Also, Dill and Costill (4) observed an increase in  $VO_{2max}$  after workout endurance allows improved soccer players performance. So, endurance training presents the important co-determinants of football performance, by enhance physical performance and plasma volume (PV) (5-7).

In order to optimize physical performance, previous

studies are interested to control level of PV among soccer players in response to the training (8-10). Recently, the systematic literature review of Zouhal et al. (9) suggests that long-term exercise training can also induce a significant increase of PV in healthy untrained individuals. More specify, Moussa et al. (11) observed that oxygen transport and  $VO_{2max}$  are linked to blood volume and hemoglobin (Hb) concentration. Plasma is the main transporter of nutrients and oxygen to the active muscles. The variation of PV and hematological parameters play a key determinate in the regulation of body fluids in response to physical exertion during training loads (12) or exercise (13). Indeed, a high level of Hb and hematocrit (Ht) is generally associated with an increase in the oxygen transport capacity in the blood and their viscosity respectively (9, 14, 15). The kinetics of

variation of these hematological parameters can be partly explaining the variation of the training load adopted. Consequently, it can be seen that the variation in PV observed after training could affect aerobic performance. In fact, recent studies have shown that the improvement of PV promote an increase in aerobic exercise performance (8, 15). In addition, Selby and Eichner (15) proved that the increase in PV, which can reach up to 20% in subjects highly trained in endurance, improved performance by an increase in the systolic blood volume, an improvement in thermoregulation, and a decrease in blood viscosity.

To our knowledge, few studies are interested in the development of PV among soccer players in response to the soccer season. However, previous studies showed that physical training can improve PV, seems influenced by gender (9), environment (16), intensity, frequency, duration and mode of physical exercise (15, 17-20). Concerning this last factor, the variation in training level depends on the training phases (pre- and competitive season), competition calendar and number of the matches played.

Therefore, the aim of this study was to examine the effect of a half season on the PV variations in professional soccer players. Based on earlier studies (21-23). We hypothesized that the competition phase leads to a significant decrease in BMI compared to the preparatory phase (hypothesis 1). Also we hypothesized that competition phase would cause a significant decrease of PV, Hb, Ht compared to preparatory phase (hypothesis 2). However, the regulation of the training load and the competition volume would not have a significant effect on the variation of mean corpuscular hemoglobin concentration (MCHC) (hypothesis 3).

## 2. Methods

### 2.1. Participants

Twenty elite soccer players aged  $20.3 \pm 1.5$  years old ( $70.9 \pm 2.1$  kg weight;  $1.79 \pm 2.1$  m height;  $11.4 \pm 0.7\%$  body fat;  $62.7 \pm 2$  kg lean body mass (LBM)), all members from 1st French division soccer league, participated in the current study. Players were given information regarding the experimental protocol of the study before signing a written consent form. The study was conducted in accordance with the Declaration of Helsinki and was approved from the Ethics Committee of the University of Rennes 2, France (EC-UR2/1597) before the beginning of subject recruitment. All participants were in good health with no chronic injuries (> 3 days) during the six months of the study. To minimize changes in anthropometric and hematological parameters related to diet, players were asked to follow a

balanced diet (10 kcal/kg, 55% of which came from carbohydrates, 33% from lipids and 12% from proteins, as determined by an experienced nutritionist). Moreover, players were asked to follow a habitual hydration state according to their normal drinking behavior.

### 2.2. Experimental Protocol

#### 2.2.1. Training Program

The evaluation period takes place during the preparation period (from June 30 to August 20) and the competition period (from August 21 to December 21). Three periods of blood samples were collected: (1) before the preparation phase (T1); (2) just after the preparation phase; (3) just after the competition phase (T3).

#### 2.2.2. Training Load Assessment

The difficulty of the perceived effort (RPE) was assessed, using the Borg scale, at the end of training or competition. The perception of effort was calculated according to Foster et al. (23) in arbitrary units (AU). RPE on the Borg scale was multiplied with effective duration (min) of a single training session.

#### 2.2.3. Anthropometric Measurements and Blood Sampling

Blood samples were taken three times a year. The first took place on June 30 when training resumed. The latter on August 20, at the start of the competition period. And finally the last samples are taken on December 21 at the end of the first part of the competition.

For each period, the anthropometric assessment (age, height, body mass, % body fat) was made and blood samples were taken to obtain the Hb values and the Ht level. For anthropometric measurements, body mass was measured to the nearest 0.1 kg on an electronic scale with participants wearing light clothing and walking barefoot (Kern, MFB 150K100). A measuring tape fixed to the wall was used to determine height to the nearest 0.5 cm. Also, four skinfolds and a Harpenden caliper were used to calculate body fat percentage (24). By subtracting the fat mass from the body mass, the fat free mass was calculated.

For blood sampling, blood samples (10-mL) were systematically taken on an empty stomach, in the morning after  $8 \pm 0.5$  hours night sleeping, with a normalized semi-recumbent position, by the same nurse, in the antecubital vein. The venous blood samples collected were immediately placed in ice and then centrifuged for 10 minutes at 1500 g ( $4^{\circ}\text{C}$ ). The decanted plasma is aliquoté then frozen at  $-80^{\circ}\text{C}$  until the days of the assays. Hemoglobin is calculated by the HemoQ analyzer.

The measurement of Ht was carried out from the blood samples taken (25).

The PV variation ( $\Delta PV$ ) was calculated using the formula of Strauss et al. (26):

$$\Delta VP (\%) = \frac{Hgb1}{Hgb2} \times \frac{(1 - Ht2)}{(1 - Ht1)} \times 100 \quad (1)$$

Ht1 = hematocrit during T1; Ht2 = hematocrit during T2 and T3.

### 2.3. Statistical Analyses

Data were expressed as means and standard deviations (SD). Normality of data was assessed and confirmed using the Kolmogorov-Smirnov test. The one way ANOVA test was performed to compare morphological characteristics, and hematological characteristics. When significant differences were observed, Holm-Sidak post hoc tests were used. Cohen's d effect sizes (ES) were calculated to quantify meaningful differences in the data with demarcations of trivial ( $< 0.2$ ), small ( $0.2 - 0.59$ ), medium ( $0.60 - 1.19$ ), large ( $1.2 - 1.99$ ), and very large ( $\geq 2.0$ ). We plotted deltas ( $\Delta$ ) changes between periods on hematological and anthropometric parameters. The correlation coefficients ( $r$ ) between the parameters, in each period, were evaluated using the Pearson test. The level of significance was set at  $P < 0.05$ . All analyses were carried out using SigmaStat.

## 3. Results

### 3.1. Anthropometrics

Changes in anthropometrics are illustrated in Table 1. Result showed a significant increase in body mass at the end of the competition period (T3:  $\Delta 2\%$ ; ES = 0.46 - 0.48) compared to T1 and T2. This is explained by a significant increase in LBM at T2 ( $\Delta 2\%$ ; ES = 0.54) and T3 ( $\Delta 4\%$ ; ES = 1.3) compared to T1. Also, results proved a significant decrease in Body fat percentage at T2 ( $\Delta 15\%$ ; ES = 2.79) and at T3 ( $\Delta 21\%$ ; ES = 3.43) compared to T1.

### 3.2. Hematological Characteristics

The Hb level does not vary during the season. Conversely, training is accompanied by a significant increase in Ht in T3 compared to T1. The Ht seems to increase in T2 (ES = 0.47), however this result is not significant ( $P > 0.05$ ). The average corpuscular Hb concentration is significantly lower in T3 ( $P < 0.05$ ; ES = 2.03) compared to T2. Throughout the season, Hb, Ht and MCHC values match standards among footballers. Table 1 summarizes data of all analyzed hematological parameters.

**Table 1.** Effects of the Period of Season on Soccer Players' Anthropometrics Characteristics and Hematological Parameters<sup>a, b</sup>

Hematological Parameters	T1	T2	T3
Height (cm)	179.5 ± 2.1	179.5 ± 2.1	179.5 ± 2.1
Body mass (kg)	70.9 ± 2.4	70.8 ± 2.5	72.0 ± 2.4 <sup>c, d</sup>
Body fat percentage (%)	11.4 ± 0.7	9.7 ± 0.5	9.0 ± 0.7 <sup>c</sup>
Lean body mass (kg)	62.7 ± 2.0	63.8 ± 2.1	65.4 ± 2.1 <sup>c, d</sup>
Hemoglobin (g/dL)	14.6 ± 0.4	14.3 ± 0.3	14.6 ± 0.3
Hematocrit (%)	42.6 ± 1.2	43.1 ± 0.9	45.9 ± 0.6 <sup>c</sup>
MCHC (pg)	34.5 ± 2.2	33.4 ± 3.8	31.8 ± 1.6 <sup>c</sup>
$\Delta PV$ (%)		+2.2 ± 3.5	-4.7 ± 3.3 <sup>d</sup>

Abbreviations: MCHC, mean corpuscular hemoglobin concentration;  $\Delta PV$ , PV variation.

<sup>a</sup> Values are expressed as mean ± SD.

<sup>b</sup> T1: Before the preparation phase; T2: Before the competition phase; T3: After the competition phase.

<sup>c</sup> Significant difference between T1 and T3 ( $P < 0.05$ ).

<sup>d</sup> Significant difference between T2 and T3 ( $P < 0.05$ ).

### 3.3. Evolution of Training Load During the Season

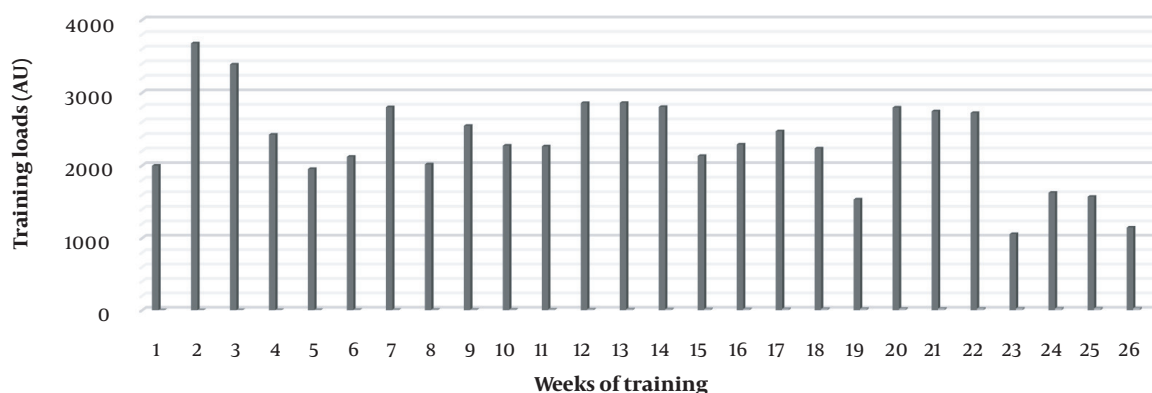
The variation in the average training load per week during the half-season is illustrated in Figure 1. During the preparation period, we observed two weeks (weeks 2 and 3) more intense compared to the whole season. The competition period is characterized by an alternation of more intense periods (weeks 12-13-14 and weeks 20-21-22) and less intense periods (weeks 9-10-11, 15-19 and 23-26). The average training loads per week are  $2547 \pm 738$  AU during the preparation phase and  $2218 \pm 139$  AU during the competition phase.

### 3.4. Relationships Between Hematological Parameters and Anthropometrics Characteristics

Table 2 shows that there were no significant correlations between  $\Delta PV\%$  and Hb of hematological parameters, and anthropometrics characteristics. However, there were significant correlation between Ht, body fat percentage ( $r = -0.871$ ;  $P < 0.01$ ), and LBM ( $r = 0.710$ ;  $P < 0.01$ ). In addition, there were significant correlation between MCHC, body fat percentage ( $r = 0.776$ ;  $P < 0.01$ ), and LBM ( $r = -0.698$ ;  $P < 0.01$ ).

## 4. Discussion

The primary aim of this study was to examine the effect of half season on the PV variations in professional soccer players. The major finding from the current study were that soccer player during preparatory and competition phases increase influence, positively, the anthropometric parameters. In addition, a significant increase of the Ht and Hb concentrations after competition phase



**Figure 1.** Variation in the average training load per week during the season

**Table 2.** Relationship Between Hematological Parameters and Anthropometrics Characteristics <sup>a</sup>

Hamatological Parameters and Period	Height (cm)	Body Mass (kg)	Body Fat Percentage (%)	Lean Body Mass (kg)
<b>Hemoglobin (g/dL)</b>				
T1	0.087	0.136	0.166	0.214
T2	0.088	0.149	0.187	0.233
T3	0.103	0.136	0.165	0.178
<b>Hematocrit (%)</b>				
T1	0.146	0.301	-0.207	0.145
T2	0.137	0.298	-0.320	0.203
T3	0.150	0.318	-0.871 **	0.710 **
<b>MCHC (pg)</b>				
T1	0.188	-0.223	0.277	-0.287
T2	0.142	-0.191	0.451	-0.473
T3	0.091	-0.345	0.776 **	-0.698 **
<b>ΔVP (%)</b>				
T2	0.111	0.106	0.109	0.179
T3	0.098	0.136	0.137	0.165

<sup>a</sup> \*\* Significant relationship (P < 0.01).

compared to the start of preparatory period (T1). However, Hb and PV have significantly decreased during competition phase compared to the start of preparatory period (PV:  $\Delta$ 314%) and T2 (Hb:  $\Delta$ 8%). The observed changes of body fat percentage and LBM (kg) were related to the significant change of Ht and MCHC after the competition phase.

During half season of training which contains the three evaluation phases (before the preparation phase; after the preparation phase; after the competition phase), a significant change was observed in BMI, lean mass and fat mass during T2 and T3 compared to T1. This result supports our hypothesis (hypothesis 1). It seems reasonable to

suggest that training program during competition phase, more than preparation phase, can improve significantly on body compositions and therefore on muscle tone in relation to training program load. In agreement with the current results, Salhi et al. (27) showed that chronic football training has an effect on body composition and hormones associated with the process of physiological regulation of food consumption. In fact, soccer training might alter body composition (body composition, BMI, and BF%) in elite male soccer players by decreasing leptin levels and increasing GLP-1 levels. Other studies, such as Saidi et al. (10), reported that no significant anthropometric parameters

were found after six weeks of training during the match congestion period. These disparities in results could be explained mainly by differences in the evaluation period, as well as factors such as intensity, duration, frequency and the level of expertise of the players.

To our knowledge, no study has studied the relationship between the hematologic status indices with body composition over half season soccer player. However, Jabbour et al. (21) observed that the decrease in PV after intense exercise protocols is greater with an increase of BMI. In addition they showed that this decrease can persist up to 24 h after intense physical exercise in normal subjects.

The regulation of hematological status indices is one of the main determinants of optimal performance in endurance sports such as soccer. Several investigators suggested that endurance training leads to decrease the value of hematologic indices like erythrocytes, Hb, mean corpuscular volume and Ht (28, 29). It was shown that these variations are due to the expansion of the PV (9, 30) from an increase in aldosterone production, a decrease in urodilatin activity and the sensitivity of central baroreceptors located in the oblong medulla (31).

Also, several investigators showed a positive relationship between PV and endurance training (7, 9, 32). Although other studies have shown slight changes in hematological variables in athletes during periods of intensive training compared to basal condition (33). Other investigations have verified a stability of hematological indices during training programs (22).

The current study showed a significant increase in Ht followed by a significant decrease in VP and MCHC at the end of the competition period (T3) compared to the start of the recovery phase (T1) and the start of the competition phase (T2). These results did not support our hypothesis (hypothesis 2). In accordance with the present study, Silva et al. (22) observed a significant decrease in PV at the end of twelve weeks of intense training program in professional football players. Among the uncontrolled variable of the present study, the fact that the players playing in Brazil, which are a hot and humid country, with the hydration of the players were not controlled during this study. Also, Heisterberg et al. (33) observed that 20% decreases in PV in men's aged 19 - 29 years, performing intense exercise.

So, the differences between the present study and those mentioned above may have occurred because of the characteristics of age, soccer training programs (training load; mean weekly volumes) and environmental factors (dehydration).

It is therefore possible that these results come from dehydration. These results are already confirmed by previous studies which have shown that the reduction in PV can be observed in dehydrated athletes (5, 9). However, during

the current study, diet and hydration were controlled. It therefore seems unlikely that they are the source of our results.

As part of our study, we use the training load to represent the intensity, frequency and duration of the exercises.

Indeed, the average training load per week and higher during the preparation period (2547 AU) compared to the competition period (2218 AU) soccer season. No significant increase in PV during the preparation phase can be explained by the fact that the training load during this phase is not large enough. Indeed, the studies of Jeong et al. (1) concerning Norwegian and Korean professional players respectively report average training loads per week of  $3577 \pm 920$  AU and 4343 AU for the preparation phases, 2536 AU and 1703 AU for the competition phases. The training loads for the preparation period obtained during these studies are considerably higher than the current results. One possible explanation is that the increase in training load causes hemodilution, attesting to improved  $VO_{2max}$  (9, 32).

We can therefore explain that the variation in PV is sensitive to the intensity, frequency, duration and mode of physical exercise adopted during training or competition.

The present study observed that Hb, MCHC and  $\Delta PV$  (%) were high in the basal state, then decreased during the preparation phase (T1; T2) and more significantly after the competition phase (T3) where the training load and the volumes of competition is important for professional soccer players. However, a progressively significant increase of Ht was reported from T1 to T3 ( $P < 0.05$ ). These results did not support our hypothesis (hypothesis 3). Several studies showed that the improvement of training charge especially during competitive phase induced a significant increase of Ht and decrease of MCHC (25, 33). Indeed, Heisterberg et al. (33) revealed no changes in Hb during all season period, but a decrease in MCHC was observed at competitive phase compared with the previous phases. Also, a significant increase of Ht over the competitive phase. Heisterberg et al. (33) observed that the decrease of Hb (not significant) and MCHC causes a potential increase in physical performance supported by a significant increase in LBM and decrease in fat body mass. However, the intense training period with the frequent matches per week can highly change the variation of Ht and MCHC in soccer players (33).

On the other hand, a significant decrease of Hb, Ht with no significant change of MCHC was observed after six-week period of congested match play in elite male Tunisian soccer players (19). Also, Meyer and Meister, (34) showed a significant decrease of Ht over the season in professional soccer players of Germany.

These results suggested that intensive physical stress affects PV and hematological parameters, which may nega-

tively influence players' physical performance. Also, these variations produced the appearance of sports anemia is often perceived as a first sign of overcoming or overtraining (19).

So, to better control physical performance, the regulation of training and the competitive workload with hematological parameters will be necessary.

#### 4.1. Conclusions

In conclusion, this study showed that a 26-week football training session significantly decreases the PV. Our study also highlights that the training load impacts the variation in PV. Indeed, a high training load seems necessary to increase and maintain the PV. In addition, an insufficient training load appears to result in a decrease in PV. However, we must be careful with the results obtained. Indeed, the PV is subject to many factors and it is very difficult to take them into account as a whole. In our study, the hydration and nutrition of the players were controlled but it remains difficult to impose a diet and hydration identical to all. In addition, the PV is subject to large inter and intra individual variations which can impact the results. It seems important to take them into account in the future. Finally, additional studies seem necessary in order to deepen the effect of the training load on the PV.

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#### Footnotes

**Authors' Contribution:** AB, and HZ conceived and designed research. NJ, FR, FR, EL, and HZ conducted experiments and analyzed data. AB, NJ, and HZ wrote the manuscript. IL, AB, NJ, FR, and HZ revised the manuscript. All authors read and approved the manuscript.

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**Ethical Approval:** The study was conducted in accordance with the Declaration of Helsinki and was approved

from the Ethics Committee of the University of Rennes 2, France (EC-UR2/1597) before the beginning of subject recruitment.

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