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The Effect of Pre-season Training and Early Competitive Period on Haematological, Skeletal Muscle and Physiological Markers in Starters and Non-starters Greek Professional Soccer Players

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Abstract

Metabolic responses to training in soccer depend on the combined physiological stress of pre-season training and official games. The purpose of the present study was to monitor the metabolic changes during the pre-season and first competitive mesocycle by measuring haematological and physiological parameters in soccer players with and without playing action. Twenty-one professional players, split into starters (n=10) and non-starters (n=11) having played in >75% and <25% of the total game time (7 games), respectively, participated in the study. Blood samples were collected at the start of the preparation (July), before the first official game (September) and at the end of the first competitive mesocycle (November), and analyzed for haematocrit, haemoglobin, urea, creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), iron, ferritin, free testosterone and cortisol. VO2max and running velocity at 4 mM lactate (V4) were measured in July and September. Heamoglobin and haematocrit increased during the pre-season (13.98 vs. 14.29 g/dL, p=.024 and 41.51 vs. 42.55 %, p=.005; respectively) and rose further during the early competitive season (14.68 g/dL, p=.001 and 43.87 %, p<.001; respectively). Urea, CK and AST increased (p<.05), while ferritin decreased (p<.05) during the pre-season. Testosterone increased (p<.05) during the competitive mesocycle, while there was no change in ALT and cortisol. Starters had lower urea (31.77 vs. 36.90 mg/dL, p=.016) and iron (77.7 vs. 92.8 μg/dL, p=.029) but higher ferritin (p<.05), VO2max and V4 compared to non-starters (56.6 vs. 53.9 ml O2/kg/min, p<.001, and 13.4 vs. 12.7 km/h, p=.02; respectively). In conclusion, during the pre-season there was evidence of catabolism that reversed to anabolism during the first competitive mesocycle and starters had higher physical fitness parameters compared to non-starters.

Keywords: *soccer, overtraining, haematological markers, skeletal muscle biomarkers, physiological markers*

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Ερευνητική

Η Επίδραση της Περιόδου Προετοιμασίας και της Αρχικής Αγωνιστικής Περιόδου σε Αιματολογικούς, Φυσιολογικούς και Μυϊκούς δείκτες σε Βασικούς και Αναπληρωματικούς Έλληνες Επαγγελματίες Ποδοσφαιριστές

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Περίληψη

Οι μεταβολικές προσαρμογές στην προπόνηση ποδοσφαίρου εξαρτώνται από τον συνδυασμό των φυσιολογικών ερεθισμάτων της προ-αγωνιστικής και της αρχικής αγωνιστικής περιόδου. Σκοπός της παρούσας μελέτης ήταν να καταγράψει τις μεταβολικές αλλαγές κατά την διάρκεια της περιόδου προετοιμασίας και της αρχικής αγωνιστικής περιόδου σε βασικούς και αναπληρωματικούς ποδοσφαιριστές. Εικοσι-ένα επαγγελματίες ποδοσφαιριστές χωρισμένοι σε βασικούς (n=10) και αναπληρωματικούς (n=11) με χρόνο συμμετοχής >75% and <25% (7 αγώνες), συμμετείχαν στην έρευνα. Δείγματα φλεβικού αίματος ελήφθησαν στην αρχή της προετοιμασίας (Ιούλιος),, πριν τον πρώτο κανονικό αγώνα (Σεπτέμβριος) και στο τέλος του πρώτου αγωνιστικού μικρόκυκλου (Νοέμβριος) και αναλύθηκαν για αιματοκρίτη, αιμοσφαιρίνη, ουρία, κρεατινοκινάση, ασπαρτάμη αμινοτρανσφεράση, αλανίνη αμινοτρανσφεράση, σίδηρο, τεστοστερόνη και κορτιζόλη. Η μέγιστη πρόσληψη οξυγόνου και η ταχύτητα στα 4mM γαλακτικού μετρήθηκαν τον Ιούλιο και τον Σεπτέμβριο. Η αιμοσφαιρίνη και ο αιματοκρίτης ήταν αυξημένοι και στις δυο συνεχόμενες μετρήσεις τον Ιούλιο και τον Σεπτέμβριο (13.98 vs. 14.29 g/dL, p=.024 and 41.51 vs. 42.55 %, p=.005; (14.68 g/dL, p=.001 and 43.87 %, p<.001; αντίστοιχα). Η ουρία, κρεατινοκινάση, ασπαρτάμη αμινοτρανσφεράση αυξήθηκαν, ενώ η φεριτίνη μειώθηκε. Η τεστοστερόνη αυξήθηκε κατά την αγωνιστική περίοδο ενώ η κορτιζόλη και η αλανίνη αμινοτρανσφεράση ήταν σταθερές. Οι βασικοί ποδοσφαιριστές είχαν χαμηλότερη ουρία (31.77 vs. 36.90 mg/dL, p=.016) και σίδηρο (77.7 vs. 92.8 μg/dL, p=.029) αλλά ψηλότερη φεριτίνη σε σχέση με τους αναπληρωματικούς. Στους βασικούς ποδοσφαιριστές η μέγιστη πρόσληψη οξυγόνου και η ταχύτητα στα 4mM γαλακτικού ήταν μεγαλύτερες σε σχέση με τους αναπληρωματικούς (56.6 vs. 53.9 ml O2/kg/min, p<0.001, και 13.4 vs. 12.7 km/h, p=.02; αντίστοιχα). Συμπερασματικά, υπήρχαν δείκτες καταβολισμού κατά την διάρκεια της προετοιμασία οι οποίοι αντιστράφηκαν κατά την αγωνιστική περίοδο, συνιστώντας αναβολισμό.

Λέξεις κλειδιά: *ποδόσφαιρο, υπερβολική προπόνηση, αιματολογικοί δείκτες, μυϊκοί δείκτες, φυσιολογικοί δείκτες*

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Introduction

The soccer season is commonly planned in three distinct periods: the pre-season, in-season, and transition periods (Silva et al., 2016). The ''pre-season'' period is characterized by a high frequency of training sessions, which focus on fitness rebuilding following the transition period. During this period, players are typically exposed to friendly games and are subjected to rapid increases in training load that contribute to substantially increasing the physiological stress of the pre-season period that can be more intense than in-season training (Jeong et al., 2011). In contrast, the focus of ''in-season'' training is frequently on the maintenance of specific capacities developed during pre-season (Bangsbo, 1994a; Reilly, 2007). The habitual activity of soccer players during the competitive season entails a cycle of training, taper, competition and recovery over a period of one week (Reilly, 2007).

Feelings of fatigue and acute reductions in performance is a consequence of the normal training process. However, when the balance between training stress and recovery is disproportionate overreaching and, subsequently, overtraining may develop (Koutedakis et al., 1990). Overreaching and overtraining are parts of the same pathophysiological spectrum but the time needed to recover from the overtraining syndrome is much longer (Carfagno & Hendrix, 2014). Monitoring hormonal and biochemical indices of muscle damage are useful in evaluating the occurrence of overreaching and/or overtraining (Halson & Jeukendrup, 2004).

Considering that football (soccer) players are exposed to significant training loads that can provoke overreaching during the pre-season, it is critical to monitor the adaptive process in order to prevent further aggravation of homeostasis that may advance overreaching into long term overtraining. Furthermore, the potentially greater competitive demands placed on the actual starters (S) compared to non-starters (NS) in a soccer match may result in different adaptive responses/recovery during the early competitive season. Previous investigations of haematological parameters during the pre-season have reported both a decrease (Ostojic & Ahmetovic, 2009) and an increase (Malcovati et al., 2003; Silva et al., 2008) in haemoglobin and haematocrit. Temporal hormonal responses to a competitive soccer season have been previously reported (Kraemer et al., 2004). Testosterone concentrations were significantly higher (~29%) at the end of the competitive season compared to baseline values (one week before the competitive season). In contrast, there were no significant changes in plasma cortisol concentrations during the course of the season. However, Kraemer et al. (2004) did not evaluate hormonal responses during the pre-season and the transition to the competitive season.

Therefore, the purpose of this study was to investigate/ monitor the changes in haematological, hormonal and physiological parameters in Greek soccer players with and without playing action during the pre-season and the transition to the first competitive mesocycle of a soccer season.

Methods

Subjects

Twenty-one (n=21) professional soccer players (Age: 26.2 ± 5.6 yrs; height: 1.80 ± 0.01 m; weight: 77.7 ± 6.1 kg) from the same football team competing in the second national division during the 2013-14 competitive season, split into Starters (n=10; having played in >75%) and Non-Starters (n=11; having played in <25 of total game time), volunteered for the study, following written informed consent approved by the University Ethics Committee. All the players had been playing at professional level for at least 3 yrs (range 3-14 yrs).

Experimental design

The players visited the laboratory for blood sampling in 3 occasions: at the beginning (July) and the end of the pre-season period (September, 8 wks), and at the end of the subsequent first competitive mesocycle (November, 8 wks). Physiological assessment was also performed in the first 2 visits.

Exercise training program

Pre-season: During the pre-season the players completed in total 84 training sessions and participated in eight "friendly" matches. The training sessions were focused on the development of: aerobic capacity (25 sessions), strength (8), speed (8), neuromuscular coordination (8), specific soccer endurance (5) and skill/game tactics (28).

First competitive mesocycle: The team participated in 7 official matches. Every weekly microcycle consisted of one day-off following the match, which was either on Saturday or Sunday, one twice-a day training session following the day-off and then one-a day training sessions until the match (Table 1).

Table 1. Weekly microcycle during the early competitive mesocycle.

Day	Training/competitive session
Sunday	Official Match
Monday	Day-off for the players that participated in the match Match simulations in small field (2 vs. 2 up to 5 vs. 5) for the players that did not participate in the official match
Tuesday	Day-off
Wednesday	Morning: Strength training with or without plyometric and speed training (total duration ≤60 min) Afternoon: Match simulations in small field (2 vs. 2 up to 4 vs. 4) or speed endurance (20 min. total). Tactical training (defensive and offensive plans)
Thursday	Tactical training in ½ field. Match simulations in ¾ field
Friday	Speed training. Match simulations (medium – high intensity, 20-30 min)
Saturday	Reaction speed. Match simulations in ½ field (15 min)
Sunday	Official match

Haematological parameters: In each laboratory visit in the morning following 12 hrs fasting, blood samples (10 ml) were drawn from the antecubital vein, using a 20-gauge disposable needle equipped with a Vacutainer tube holder (Becton Dickinson, Franklin Lakes, NJ) with the subject in a seated position. Each sample was divided in two aliquots. In one aliquot EDTA was added to prevent clotting and it was analysed within one hour for haematocrit, haemoglobin, urea, creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and iron, while in the other aliquot blood was allowed to clot at room temperature and subsequently centrifuged (1500g, 4oC, 15 minutes). The resulting serum was placed into separate microcentrifuge Eppendorf tubes in multiple aliquots and frozen at –70^{o}C for later measurement of ferritin, free testosterone and cortisol. Haematocrit and haemoglobin were measured with a haematology analyser (K-1000, Sysmex Norderstedt, Germany). Urea was measured with a microscopy analyser (iQ200 Beckman-Coulter, Brea, Ca., USA) that has an error of measurement of 3.0%. CK, AST, ALT and iron were measured with a clinical chemistry analyzer (AU400, OLYMPUS CO., Tokyo, Japan; inter-assay coefficient of variation, 5.3%). Ferritin was measured with an immunoassay system analyser (ADVIA Centaur CP; Siemens Healthcare Diagnostics, Deerfield, IL, USA; inter- and intra-assay coefficient of variation, 4.3% and 2.7%, respectively). Testosterone concentration was determined using a solid-phase, competitive chemiluminescent enzyme immunoassay (Immulite 2500, Siemens, Germany). Cortisol was measured using an electrochemiluminescence immunoassay (Roche, Basel, Switzerland). Intra- and inter-assay coefficients of variation were 5.1% and 11.7% for testosterone, and 5.2% and 7.4% for cortisol, respectively.

Physiological assessment

Maximal oxygen uptake (VO2max) and velocity at 4mM lactate (V4): VO2max was measured twice (July and September). During warm-up the players walked for 3 min at a speed of their choice and run for 5 min at moderate pace (8 km/h) on the treadmill (Technogym Runrace 1200, Italy). Immediately following warm-up, the players commenced a progressive run with the initial treadmill speed set at 10 km/h that increased, by 2 km/h every 3 min for 12min, and after that by 1 km/h every min until volitional exhaustion. At the end of each stage the players stepped off the treadmill to allow finger capillary blood sampling for lactate measurement (Accutrend Lactate, Roche Diagnostics, Germany), and then continued to the next stage within 20-30 s. Heart rate (HR) was continuously measured with a HR monitor (Team Polar, Polar Electro Oy, Kempele, Finland) and VO2 was measured by open-circuit spirometry (averaged every 30 s) with the use of an automated online pulmonary gas exchange system via breath-by-breath analysis (Vmax29, Sensormedics, NewYork, USA). The criteria used for the determination of VO2max where:

1) plateau of VO2 (increase <2.1 ml/min/kg)
2) respiratory exchange ratio > 1.1

3) HR ± 5% of predicted HRmax
4) blood lactate > 8 mM

In every determination at least 3 out of 4 criteria were satisfied. V4 was calculated by linear interpolation from the velocities at blood lactate concentrations before and after 4mM (Heck et al., 1985).

Statistical analyses

Data are presented as mean ± SD. A one-sample Kolmogorov-Smirnov test was used to determine data normality. A two-way (time x group; July, September, November x starters, non-starters) for repeated measures analysis of variance (ANOVA) was used to identify significant interactions. When a significant interaction was observed, a Tukey post hoc test with Bonferroni correction was used to identify the points of difference. Significance was accepted at $p < 0.05$. All analyses were performed with Statistica v11.0, StatSoft.

Results

Starters vs. Non-starters

Haemoglobin remained stable in the starters, while in the non-starters increased continuously (Fig. 1, upper). There was a significant interaction between time and group ($F=2.92$, $p=.067$). Haematocrit in the starters remained stable in September, while in November was increased compared to July ($p=.013$). In the contrary, the haematocrit of the non-starters was increased both in September ($p=0.007$) and November ($p<.001$), compared to July (Fig. 1, lower). There was also a significant interaction between time and group ($F=3.58$, $p=0.038$). Starters had lower iron compared with the non-starters (77.7 vs. 92.8 μg/dL, $p=.029$). Starters had significantly higher ferritin compared with non-starters (118.57 vs. 81.91 ng/mL, $p=.033$) and values in both September and November were lower than those in July (97.81 and 95.72 vs. 107.21 ng/mL, $p=.005$ and $p<.001$, respectively). Also, starters had higher VO2max and V4 compared with the non-starters (56.6 ± 1.7 vs. 53.9 ± 1.6 ml O2/kg, $p<0.001$ and 13.4 ± 0.4 vs. 12.7 ± 0.4 km/h, $p=.02$; respectively) in July.

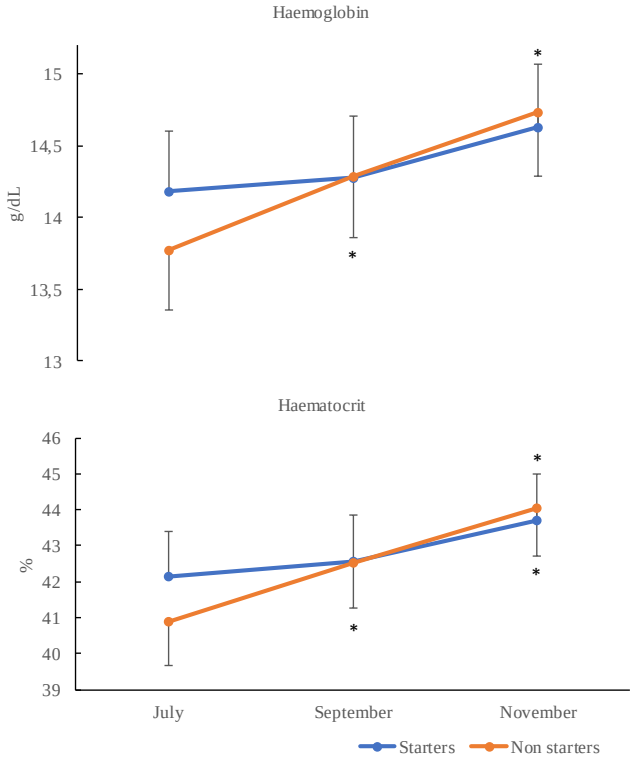

Figure 1. Haemoglobin (upper) and haematocrit (lower) of starters (n=10) and non-starters (n=11) in July, September and November. Data are mean (± SD); * denotes significant difference from July; ($p<0.05$).

All players

There were no differences between the two groups in any parameter other than in haemoglobin and haematocrit in July (baseline), hence the data are presented combined as a total sample. Thus, haemoglobin increased during the pre-season (13.98 vs. 14.29 g/dL, p=.024) and rose further during the early competitive season (14.68 g/dL, p=.001). Urea and CK were significantly higher in September compared with November (35.50 vs. 33.20 mg/dL p=.016 and 410 vs. 242 U/L, p=.006; respectively), while there was a non-significant trend for values in November to be higher than those in July. AST in September was higher compared with July (32.8 vs. 27.7 U/L, p=.014), while there was no difference in November compared with July (29.6 vs. 27.7 U/L, p=.44, Fig. 2, upper). There was no change in ALT during the experimental period. Free testosterone in November was significantly higher from both values in July and September (21.17 vs. 17.79 and 18.17 nmol/L, p<.001 and p<.001, respectively; Fig. 2, lower), while cortisol remained stable throughout the experimental period. VO2max was higher in September than July (57.3 vs. 53.3 ml O2/kg, p<.001) and V4 was higher in September compared with July (13.5 vs. 12.5 km/h, p<.001, Fig. 3).

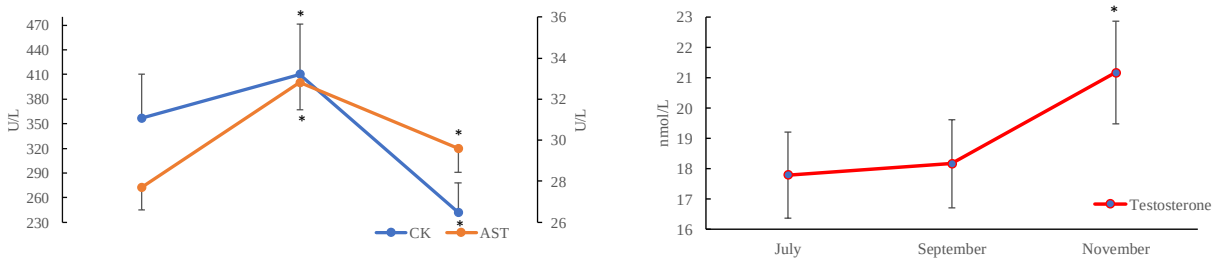

Figure 2. Markers of muscle damage (upper; CK, creatine kinase; AST, aspartate aminotransferase) and anabolism (lower; testosterone) in July, September and November. Data are mean ± SD, n=21; * denotes significant difference from July and September; p<0.05.

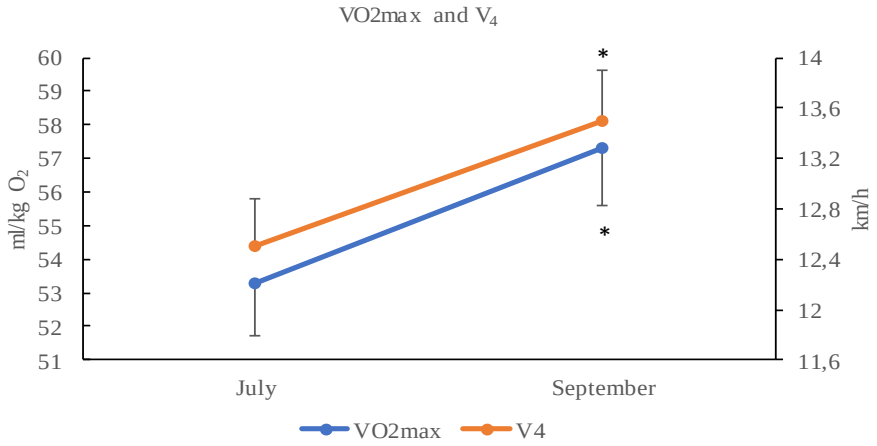

Figure. 3. Maximal oxygen uptake (VO2max) and velocity at 4 mM lactate (V4) in July and September. Data are mean ± SD, n=21; * denotes significant difference from July; p<0.05.

Discussion

The purpose of the present study was to monitor the adaptive changes during the pre-season and first competitive mesocycle by measuring haematological and physiological parameters in soccer players with and without playing action. We found that the pre-season was characterized by increases in muscular damage indices (i.e., urea, CPK, AST) and reduced iron reserves (ferritin) compared to the early competitive mesocycle. Interestingly,

there were no differences between starters and non-starters in the adaptive responses to pre-season training and early competitive mesocycle.

Even though there is a consensus about the decrease in haematocrit, haemoglobin concentration, and red blood cell count (erythrocytes) induced by the endurance training (Convertino, 1991; Schumacher et al., 2002), nevertheless, increases in hematocrit, hemoglobin concentration in soccer players following a period of intense training have also been observed (Silva et al., 2008). The unexpected increases in haemoglobin and haematocrit observed in the present study can be manifestations of two possible factors: first, an increase in erythropoiesis (i.e., increased total erythrocytes), or plasma volume changes. Since plasma volume decreases proportionally to the hypohydration level (Sawka, Montain & Latzka, 1996) and plasma volume reduction is also commonly observed in dehydrated athletes (Dill & Costill, 1974) one possible explanation of our findings of increased haemoglobin and haematocrit could be dehydration. Furthermore, the increases in haemoglobin and haematocrit observed herein are reflected in the significant increases in VO2max and V4 during the pre-season. Considering that an increase in hematocrit can both provoke a reduction in physical fitness, due to an increase in blood viscosity (Brun et al. 1998), and benefit physical fitness, due to the associated enhanced oxygen delivery, the increased fitness parameters in our study is most likely the net balance of these two competing mechanisms.

The higher group average CK than normal physiological range (470 vs. 50-270 U/L) noted in September is reflective of the training overload in the previous pre-season preparation period of 8 wks. The period of July-September in our study included the largest volume of endurance and strength training, in agreement with previous reports (Reilly, 2007; Jeong et al., 2011). CK release in the extracellular muscle space is linearly related with the intensity and duration of exercise and, therefore, has been used as an index of muscle damage during exercise (Ispirlidis et al., 2008; Yamin et al., 2007; Plebani, 2010) and for evaluation of physiological adaptations to training loads (Brancaccio, 2010; Coutts et al., 2007). In line with the above considerations, more than 70% of the players in our study had elevated CK in September. Additionally, AST and urea peaked in September, confirming the taxing magnitude of the previously applied training load. AST is also used as an index of muscle damage (Banfi et al., 2012) and urea, even though it can increase following elevated protein uptake, is used as an index of catabolism due to acute intolerance of the applied training load (Hartmann & Mester, 2000; Urhausen & Kindermann, 2002). In contrast, in November where volume and type of training were reduced, only 5 out of the 21 players had CK >270 U/L.

The lower ferritin values, in both September and November compared to July, indicate not only that the preparation pre-season provoked a reduction in iron stores, but also that the early competitive season didn't allow for sufficient recovery to the level at the beginning of the pre-season period. Serum ferritin is widely used as index of the body's iron stores and has been related to the training load (Bannister & Hamilton, 1985; Pate et al., 1993; Malcovati et al., 2003; Williford et al., 1993). Considering that increases in hemoglobin and hematocrit (i.e., erythropoietic demand) have been linked to marked decreases in serum ferritin concentration (Malcovati et al., 2003), the low ferritin values observed in our study are justified by the concomitant continuous increases in hemoglobin and hematocrit up to November.

The stable cortisol and testosterone levels combined with the improvements in VO2max and V4 indicate that the players were adapting well to the training load during the pre-season. Decreased testosterone combined with elevated cortisol has been associated with prevailing catabolism attributed to excessive training loads during the pre-season (Kraemer et al., 2004). In addition, we found a testosterone increase during the early competitive season indicating that the players shifted to an anabolic state. Indeed, an elevation in testosterone concentrations can assist in balancing the catabolic effects of cortisol and promote a shift to a systemic anabolic state (Kraemer et al., 2004).

Even though the selection of the players for participation in the matches was based on tactical/skill criteria, nevertheless, the starters had higher physical fitness levels as indicated by VO2max and V4. Our findings are in agreement with Ziogas et al. (2011) suggesting that velocity at lactate threshold can discriminate soccer players of different endurance capacity. Following training during the pre-season both VO2max and V4 increased about 8% when combining both groups. Previous reports have shown improvements in V4 and VO2max during the pre-season ranging from 4.5 to 21.6% (Helgerud et al., 2001). Earlier serial evaluations of aerobic fitness variables during the season, in both starters and non-starters, have shown that VO2max fluctuates little and can remain unchanged, while V4 can continue to improve (Impellizzeri et al., 2006), suggesting that V4 is more informative in the physiological evaluation of top-level soccer players (Paraskevas & Hadjicharalambous, 2018).

It has to be acknowledged that a limitation of the present study is the lack of VO2max and V4 evaluation

following the early competitive mesocycle. Such data would have allowed for an association of the observed shift in anabolic state, as indicated by the hormonal responses, with fitness parameters relevant to soccer performance.

In conclusion, our findings show that even though the training load during the pre-season provoked significant muscle damage, the metabolic stress was well tolerated and during the early competitive mesocycle there was a shift towards anabolism and positive adaptive responses in both starters and non-starters.

Implications for Competitive Sports

Our findings demonstrated that starters had better physical fitness than non-starters at the start of the pre-season and suggest that players should maintain a certain level of fitness during the off-season. Haematological parameters and skeletal muscle biomarkers are useful in monitoring training adaptations during the pre-season and the transition to the competitive season in Greek professional soccer players.

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