Journal of Microbial & Biochemical Technology - Open Access

JMBT/Vol.1 Issue 1

Research Article | OPEN ACCESS Freely available online | doi:10.4172/1948-5948.1000007

Red Cells Responses of Professional Soccer Players Submitted to Specific Training Methods in the Intensity of the Anaerobic Threshold

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Abstract

This study aims to observe the effect of three different methods of training in the red cells of the professional soccer players, from 2nd division in the Rio de Janeiro Championship. The sample was composed by 20 individuals of the masculine gender, being selected of randomized form, the age of the group were of 23,42 ± 6,5 years. The athletes had controlled diet made by a nutritionist, the ambient and their temperature were controlled, they had been submitted to an evaluation the Bruce's protocol (1976), to determine the intensity of the anaerobic threshold for application in the different protocols of training (intervaled, intermittent and of game). Different collections of blood had been carried through: 48h before initiating the study and pre-test/post-test of each protocol of training. The statistical tools used were: Shapiro-Wilk's test and the inferential statistics used were the multi-variance analyzes Anova 3 x 3 with complementary post hoc Scheffé. The value of $p \leq 0,05$ was determinate as the significant level. Alterations had been observed at the moment before and after the tests, for the hematological profile, and a bigger alteration in the protocol of action of the game, demonstrating that it's the method that provides the biggest physical stress.

Keywords: Exercise; Soccer; Blood cells

Introduction

Soccer is a game of high complexity and performance is dependent of physiological demands that are multifactor, which varies form during the match (Silva, 2006; Cédric et al., 2007). In the last few decades the physical condition in soccer is one of the factors that had the best evolution. The knowledge about the physical conditioning has vital importance for the success of a team during the competition (Cunha, 2003).

The aerobic capacity has decisive paper in soccer (Santos and Soares, 2001), because with better oxygen consumption it is possible to get conditions of excellent performance (McMillian et al., 2005). The aerobic metabolism dominates most of the energy release during the match, when considers in the distance covered (Cédric et al., 2007), however the most decisive actions, characterized for time of action with ball and sprints, are covered by resources of the anaerobic metabolism (Cédric et al., 2007; McMillian et al., 2005).

Erythrocytes are responsible for the oxygenation of the entire organism (Araujo et al., 2004). Sub-excellent hematological state has been frequent registered in athletes involved in intense physical activity (Boyadjiev and Taralov, 2000; Biancotti et al., 1992).

Hematologicals parameters as hemoglobin and erythrocytes present high sensitivity to the effect of acute exercise (Fallon et al., 2001; Petibois et al., 2003). During events of endurance, that require fast consumption of oxygen, the red cells' ability to move through the capillaries can limit the performance of the athlete (Smith et al., 1999).

The zone of training in the intensity of the anaerobic threshold (AT) continues being used, although the confused interpretations about the concentrations of this metabolite to the physical exercise (Myers and Ashley, 1997). The sanguineous viscosity and aggregation of the red cells is correlated to red cells and lactate concentration (Varlet-Marie and Burn, 2004).

With this important correlation, this study aims to observe the responses of three specific training methods (intervaled, intermittent and action of the game), in the intensity of the anaerobic threshold, on the hematologicals parameters (red cells), in professional soccer players from 2nd division of the Rio de Janeiro Championship.

Materials and Methods

The sample of the study was constituted of randomized form, with 20 individuals of the masculine gender, professional soccer athletes, with register in the Brazilian Soccer Confederation (CBF) being regularly enrolled in the state championship of Rio de Janeiro of 2nd division.

Informed consent was obtained from each participant with no refusals, and the experimental procedures were executed in ac-

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Received December 21, 2009; **Accepted** December 29, 2009; **Published** December 29, 2009

Citation: Gomes ALM, Seixas-da-Silva IA, Otañez JD, Kanifis F, Romero S, et al. (2009) Red Cells Responses of Professional Soccer Players Submitted to Specific Training Methods in the Intensity of the Anaerobic Threshold. J Microbial Biochem Technol 1: 037-042. doi:10.4172/1948-5948.1000007

cordance with the Declaration of Helsinki (World Medical Association, 2008). The research project for this study was submitted to and approved by the Committee of Ethics in Research Involving Human Beings of the Castelo Branco University – UCB (protocol number: 0012/2007).

With intention to determine the characteristics of the sample and the respective speeds of training it were done evaluations in the Laboratory of Exercise Physiology of the Estácio de Sá University (LAFIEX). The temperature and the humidity of the environment (laboratorial and field) had been measured with the Torricelli mercury barometer with precision in 0,5 mm and 0,1cm Inço-term® (Italy). The body temperature of the athletes was measured with mercury thermometer Torricelli® (Italy).

It were collected anthropometrics measures of body weight and stature, increased of the calculation of the relative fat, which was gotten by the Jackson & Pollock's protocol of 3 skinfold thickness (Jackson and Pollock, 1978), with use, respectively, of one digital balance with resolution of 100g, Filizola®, model PL150 Personal Line (Brazil); professional stadiometer, Sanny® (Brazil) and Lange® Skinfold Caliper (USA), with 1mm of resolution and constant pressure of 10g/mm^2. All the collection points had obeyed the prescribed in the International Standards for Anthropometric Assessment (ISAK) (Marfell-Jones et al., 2006).

The evaluation of the cardiorespiratory capacity was carried through by ergospirometry method, having used protocol in treadmill Inbramed® KT 200 Plus (Brazil), using Bruce's protocol of gradual loads (Bruce, 1973) (American College of Sports Medicine, 2003) and, measured by the VO2000® gas analyzer, (Brazil). The heart rate (HR) was measured using a heart rate monitor Polar® (USA).

Collection of the lactate concentration, in the respective intensities, was made with Yellow Springs 1500 Sport (USA) in which it's procedure of calibration and analyzes of the lactate concentration was carried through each five collections, having as reference a 4 mmol/L lactate concentration, supplied for the equipment.

The above described procedures had been carried through in the following protocol: Bruce adapted test (1993) in the treadmill (American College of Sports Medicine, 2003). The protocol is based on the increase of the intensity and inclination to create one physical stress, where to each load increment a collection of blood was carried through before and after this increment, in which it had duration of 3 minutes, which has ideas experimental conditions for control of the variables (American College of Sports Medicine, 2003). The intensity of the training was the anaerobic threshold (AT), determined by the relation with the maximum oxygen consumption ($\dot{V}O_2max$), the lactate concentration and the HR. This control aimed to determine the intensity of the different strategies carried through in the training methods (ACSM) (American College of Sports Medicine, 2003).

For the training methods had been used: 100 high and long cones, 50 round short ones Plastic® (Brazil), 40 Nike® balls (USA), 6 Casio® HS-100 chronometers (USA), sonorous whistle Sifio® (Switzerland), 50 aluminum props of 2 meters Light Metal® (Brazil), one software to control the rhythms and speeds of training Speed Test CEFISE® (Brazil), another software to control the HR, the intensities of each method of training by telemetry, the ribbons capitation and control of beatings per minute Micromed® (USA).

As form to prepare the athletes for the different experimental protocols, it was made a warm up during 15 minutes, divided in three equals parts, where initially they had carried through 5 minutes stretching different muscular groups, giving emphasis to the inferior members; the 5 posterior minutes was running with low intensity, with 60% the maximum heart rate and the last 5 minutes consisted of individualized movement of control and domain the ball.

To prevent the interference of the nutritional variable it was established a diet, made by a nutritionist, with the data presented for the clinical evaluation and hematologicals parameters and biochemists. The sample had received nutritional prescription (high carbohydrate diet with 60-65% of carbohydrate; 10-15% of proteins and 20-30% of lipids, beyond micronutrients adequacy) during all the experimental protocol.

The protocols had been based on the characteristics of the sport, in which they had established as different strategies of training for soccer players. The duration of the different types of training was equal to the consuming and stresses suffered by the athlete for the main characteristic specifies of the sport, where it has duration of 90 minutes.

The intervaled training contained exercises of displacement with and without ball, with different types of movement, distances that varied of 20 to 400 meters. The recovery intervals were related to: a short distance is equal to short interval, and when the distances increased the intervals also increased, to the intensity of the anaerobic threshold.

The intermittent training is a work method that is characterized for a continuity during the trainings, not existing a period of recovery but speed variations. Around the field twelve lines of cones with space of 5 meters of one for the other and 12 columns of cones with space of 5 meters of one for the other were placed. The training occur of continue form without interval of a work for another, and the difference is in the alteration of the intensity, in the different strategies of movement with and without ball and in the distances of the races that were be able to vary in function of the time, which varied of 30 seconds up to 10 minutes.

For the action of game training, it was made a simulation of one match, with 2 periods of 45 minutes each with interval of 10 minutes between them, without having substitution of any player or position, with equal tactical diagram between the teams. The first 15 minutes the tactical diagram was zone, from 15 minutes to 30 minutes the individual tactical diagram was used and in last the 15 minutes the tactical diagram was zone again, for the two periods of the match.

The blood collection was made in the antecubital vein in the pre test and post test, in EDTA pipes. Immediately after the collection, the blood was centrifuged, being the plasma separate, congealed and stored -70°C, to prevent the loss of volatile material and/or metabolism for the appeared elements. The collections of data had been carried through by Pedra Verde Laboratory (Brazil).

The statistics used for the degree of homogeneity and normality of the sample was the Shapiro-wilk's test, determinative tool for the decision process, and the descriptive treatment was used as reference of the study.

The Anova 3 x 3 with complementary post-hoc test of Scheffé was used, which it identifies in combinatorial and comparative way, where if they give the possible differences revealed for the test of variance (Shimakura, 2005).

The considered level of significance was $p \leq 0,05$, that is, 95% of certainty for the affirmations and/or refusals that the present study comes to denote.

Results

The athletes had as characteristic: age of 23,42 ± 6,5 years-old (average ± standard deviation), weight 71,07 ± 8,6 kg, stature 175,7 ± 5,3 cm, BMI 23,86 ± 2,7 kg/m^2 and percentage of fat 10,42 ± 3.3%.

The red cells had not presented significant alterations in the three types of methods trainings. The erythrocytes had increased in 1,1% in the intervaled method and reduction of 0,9% in the intermittent and 1,1% in the action of the game. The hemoglobin reduced 0,13% and 0,27% in the intervaled and intermittent respectively, however in the action of the game it increased in 1,7%.

The hematocrit that initially presented with average percentile of 45 ± 4,4 increased in 0,1% in intervaled and the reduction of 1,5 and 2,1% in the intermittent and action of the game. The globular volume increased 0.89% in intervalado and action of the game and 0.55% in the intermittent. The mean corpuscular volume (MCV) reduced in the three protocols of training, the mean corpuscular hemoglobin reduced in 1,1% in intervaled and the reduction of 0,93% and 3% in the intermittent and action of the game, respectively. The concentration of mean corpuscular hemoglobin (CMCH) increased in the three protocols of trainings.

In this study it did not have significance in the alterations found in the red cells. The globular counting, the hematocrit and the hemoglobin represent different amounts of red globules that are present in the blood sample. Being considered as values of concentration that allows evaluating the state of normality, anemia or polyglobulia, as well as the hemodilution or the hemoconcentration (Sacher and Mc Pherson, 2002).

In the three protocols of exercise was not characterized presence of iron-deficiency anemia before or after the efforts. The central nervous system, the myocardium, the other muscular mass and the abdominal masses and abdominal visceras are the organs and tissues whose functions consume more oxygen, and therefore with bigger gradient between arterial and venous pO_2, they are the ones that more feel the anemia (Guezennec et al., 2005; Sacher, 2002). It has an increase of the overload of the heart work in the exercise, in anemia situation, that increases the demand of oxygen where occurs tissue hypoxia (Arai, 2006).

It was verified that different alterations in the concentration of these parameters had occurred, alterations that can be disclosed by the increase or reduction of the erythrocyte in function of the increase or reduction of the hemoglobin concentration, as well as, increase or reduction of the hematocrit in function of the increase or reduction of the concentration and/or volume of the erythrocyte or increase or reduction of the plasmatic volume.

This study observes these alterations under effect of different training methods and about the intervaled method it can promote improvements in the capitation of oxygen beyond the possibility of physiological similarity to sport through the use of a soccer ball in the trainings (McMillian et al., 2005; Laursen and Jenkins, 2002). Laursen and Jenkins, (2002) affirm that the intervaled method can promote central adaptation through the improvement in the distribution of oxygen for the muscles in activity and peripheral adaptation in what the best ability of the muscle in activity says respect to produce and to use the ATP.

Discussion

(Mavrommataki et al., 2006) had carried through study with objective to examine the effect of 1$^{1/2}$, 3 and 6 minutes of recovery time on the maintenance of the power and heart rate during exercise of intervaled rows and verified that even so incomplete the intervaled of 6 minutes promoted greater maintenance of the power. We verify that in the end of the exercise it had increase in the erythrocytes, hematocrit, globular volume and CMCH in 1,1%, 0.1%, 0.89% and 0.49% respectively and reduction of 0,1%, 1.2% and 1.1% in the hemoglobin, MCV, MCH, respec-

	Intervaled	Intermittent	Action of the game	Intervaled	Intermittent	Action of the game
	ERYTHROCYTE (millions/mm^3)			MCV (%)		
Pre test	5,02 ± 0,39	5,02 ± 0,39	5,02 ± 0,39	89,53 ± 0,54	89,53 ± 0,54	89,53 ± 0,54
Post test	5,08 ± 0,32	4,97 ± 0,35	4,96 ± 0,26	88,67 ± 0,40	88,37 ± 0,70	89,35 ± 0,29
	HEMOGLOBIN (g/dl)			MCH (%)		
Pre test	14,48 ± 0,35	14,48 ± 0,35	14,48 ± 0,35	28,8 ± 0,32	28,8 ± 0,32	28,8 ± 0,32
Post test	14,46 ± 0,22	14,44 ± 0,29	14,73 ± 0,26	28,48 ± 0,18	29,07 ± 0,40	29,67 ± 0,28
	HEMATOCRIT (%)			CMCH (%)		
Pre test	45 ± 0,99	45 ± 0,99	45 ± 0,99	32,18 ± 0,32	32,18 ± 0,32	32,18 ± 0,32
Post test	45,05 ± 0,77	44,3 ± 0,85	44,05 ± 0,67	32,34 ± 0,28	32,64 ± 0,40	33,39 ± 0,19
	GV U3					
Pre test	0,89 ± 0,005	0,89 ± 0,005	0,89 ± 0,005			
Post test	0,88 ± 0,004	0,89 ± 0,004	0,88 ± 0,004			

For the red cells had not presented differences for *p≤0,05*. GV = globular volume, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, CMCH = concentration of mean corpuscular hemoglobin.

Table 1: Red cells responses of soccer players.

tively, that is, these effect lead to suggest hemoconcentration, even so the athletes have ingested 250ml of liquid to 15 minutes each. After the exercise, occurs significant increase in the levels of the hematocrit and concomitant reduction of the deformability of the erythrocytes. The hemoconcentration is caused by the fluid transference of the blood for the interstitial space (El-Sayed et al., 2005).

According to (Eichner, 1999) the plasmatic volume is reduced in 10% to 20% in acute and vigorous exercises because of the increase of the sanguineous pressure and consequent increase in the muscular compression on veins, which increases the pressure of the liquids inside of the capillaries to active the musculature. The osmotic pressure of tissues increases with the formation of lactic acid and other metabolites in the muscles making the plasma exit of the blood for tissues, what it liberates water and the same one is eliminated by the sweat (Eichner, 1999). In trainings of sprint-intervaled carried through with mice verified increase of proteins of the thermal shock (HSP)(Ogura, 2006) that they are proteins express in the cells of universal form, under conditions of stress, as thermal shock, glucose privation and exposition the inductive agents of oxidative stress (Bessman and Johnson, 1975)].

(Laursen and Jenkins, 2002) affirm that the intervaled trainings promote physiological adaptations in sedentary and active individuals, like the activity of the oxidative enzyme in athlete and adaptations in the activity of the glycolytic enzyme what it contributes for the increase of the performance in athlete through the biggest capacity to against H+ and regulation of the sodium-potassium-exchanging.

Another finding was referring to the reduction of the MCV. The mean corpuscular volume is determined by a frequency in the routine of similar distribution of the size of erythrocytes in normal individuals and patient with a simple population of erythrocytes and directly becomes related the classification of anemias (Araujo et al., 2004; Bessman and Jhonson, 1975).

In the majority of the anemias, the variations of MCV occur parallel to the changes in the weight of the mean corpuscular hemoglobin (MCH) and the alterations in these two indices are correlates (Araujo et al., 2004). These findings corroborate with our results, where found reduction of the MCV and the MCH even so this study has presented chronic responses to the trainings in parallel of olympics athletes.

The CMCH presented increase in this activity, but according to Selby (Selby et al., 1987), this is a hematimetric index that presents limitations in it's interpretation, therefore exactly in cases of deficiency of hemoglobin synthesis, the volume of the erythrocytes diminishes that the CMCH remains steady, however in this study the trainings intervaled took the reduction of the hemoglobin and increase of the CMCH that can have relations to other factors related to the effect of the activity on the form of the erythrocytes.

The exercise can make morphologic changes in the erythrocytes and increase of the fragility resulting in anisocytosis, poiquilocitoses and estomatocitose (Selby et al., 1987). (Hawknis, 2002), affirms that the human erythrocytes when into cytoplasmic alterations in pH are changed of the form of discocytes for estomatócitos (reduction of pH) and burr cells (increase of pH). The gradual decline in the ATP concentration, pH, glucose consumption and enzymatic activity can take the substitution of the form of discocytes for burr cells, estomatócitos and in last period of training esferócitos, and in the last level that the kidnapping of the spleen occurs. Alterations in the membrane of the erythrocytes with the loss of the biconcave morphology and a high CMCH is related to esferocitose. And this tendency to the elevation of CHCM seems to occur in esferocitose being one of the factors the dehydration of the erythrocytes (Chatard et al., 1999; Robinson, 2005).

We cannot affirm on alterations in the form of the erythrocytes, but we verify (Smith et al., 1999) with the objective to determine if the difference in the deformability of the erythrocytes between cyclists and sedentary verified high percentage of erythrocytes of low density and MCV and concludes that the cyclists had presented great quantity of young cells and increase of the erythrocytes' turnover.

The reduction of pH and increase of the lactate capture for the erythrocytes during the exercise can contribute for hemolysis; therefore it can be associated to the increase of the MCV. If changes occur in the environment, the organism will go to develop adequate anatomical and physiological modifications in some systems, even though in that they are not displayed directly and in what it says about the erythrocytes, alterations in pH and level of oxygen promote increase by the volume of the same (Chatard et al., 1999; Robinson, 2005; Karakoc et al., 2005).

In this study we find alterations that had taken the reduction of MCV, fact that showed above. Karadoc et al. (Karakoc et al., 2005) carried through with soccer players the MCV reduced and this fact can also be related to the increase of the sanguineous flow of oxygen.

The intermittent method is characterized by the probable increase of the production of oxidative energy (Silveira and Denadai, 2002). We find in this method not significant reduction of the erythrocytes, hemoglobin, hematocrit and MCV in 0,9%, 0.27%, 1,5% and 1,20% respectively, and occurred increase of 0,55% in the GV, 0.93% in MCH and 3.76% in the CMCH. With relation to the observed reduction, we suggest the occurrence of intravascular hemolysis; although we do not quantify the levels of serum haptoglobin. Eichner, (1999) affirms that hemolysis of the exercise is clinically common and for the fact of being a soft occurrence, rarely debilitates the organism, causing anemia.

Robinson et al., (2005) affirms that intravascular hemolysis occurs for some reasons and the biomechanic stress caused by the impact of the feet to the ground is only one of the aspects considering that inflammatory standards for leukocytes also can be involved. The increase of the fragility of the erythrocytes can be caused by one high concentration of lactic acid and hyperthermia also shows to be the cause of the intracellular water increase and to increase of the fragility of the erythrocytes (Kogawa, 1995).

Hargreaves et al., (2007) had verified that after 4 tests of exercise intercalated by 3 moments of passive recovery followed of recovery in a bicycle with low intensity had for a moment concluded that the intermittent exercise of high intensity results in great reduction of the muscular ATP, phosphocreatine and gly-

cogen, with concomitant increase of the H+, lactate and products of the degradation of the ATP. The same did not occur in the study carried through for Krustrup et al., (2003) where intermittent had as one of the objectives to evaluate the physiological reply of elite soccer players during the yo yo intermittent recovery test and to examine the causing factors of fatigue during the intense exercise repeated and had verified that muscular lactate, pH, glycogen and phosphocreatine had not modified when compared to the beginning of the test concluding that the development of the fatigue during the intense exercise is caused by other factors.

In five sessions of continuous and intermittent exercise, Silveira and Denadai, (2002) had found minor production of lactate and greater time of exhaustion in the intermittent when compared with the continuous, suggesting that the intermittent induces a strong inhibitory effect in the glycolytic way with predominance of the oxidative way. Considering this information above, we can associate that the alterations in the erythrocytes can be related to the hyperthermia. Vimieiro-Gome and Rodrigues, (2001) had found moderate risk for hyperthermia in volleyball players when submitted to training sessions of moderate intensity. Already Godek, Bartolozzi, Godek (Banfi et al., 2006) comparing soccer players with cross country athletes developing intermittent exercise in same ambient conditions, verifies that the high tax of sweating and great daily elimination of sweat explains the fact that soccer players has great percentage of corporal fat and bigger area of corporal surface, but a lesser relation in the area of corporal/mass surface and minor aerobic condition.

The alterations suggest that possible hemolysis can be related to thermal stress. It is not determined the fact that intravascular hemolysis lead turnover of the erythrocytes or mobilization of the heme group for the myoglobin synthesis is a physiological or pathological event (Robinson et al., 2005).

In the action of the game method, reductions in the number of erythrocytes in 1,1% had occurred, in the hematocrit in 2,1%, the GV in 0,89% and the MCV in 0,21%. The increases had been of 1,7%, 3% and 3.76% in the hemoglobin, MCH and CMCH respectively, what it leads to conclude that the game took the alterations in the hemoglobin concentration and the erythrocytes volume even so the results are not significant alterations that can modify the normocytosis and normocromia already found before the research. This result corroborates with study of Banfi et al., (2006) where it had reduction of the erythrocytes and followed hematocrit of a session of rugby, even so these variable have presented values increased in the first session of the departure and attribute this fall to the increase of the physical demand in the second session.

It had occurred reduction in the hematocrit and erythrocytes, but it occurred increase of the hemoglobin. Karakoc et al., (2005) had found significant reduction of the hemoglobin after 90 minutes of soccer game as well as GV and reduction MCV. In another study a significant increase in the hemoglobin concentrations was found after mountain bike season followed by the increase of the hematocrit which had fallen until the end of the test and attribute the expansion of the evidenced plasmatic volume more to the end of the test and that the fluid ingestion was not enough to reduce the dehydration (Vimieiro-Gomes and Rodrigues, 2001). These results lead to believe that this increase in the hemoglobin possibly can be transitory, and had the factors as load and way of the exercise beyond ambient conditions that can leads stress thermal, athletically status and the physical demand, factors that can influence in this response. This increase of the hemoglobin explains the increase of MCH and CMCH, therefore they are concentration measured, once that had reduction of the MCV and GV.

The same was found in the study of Karakoc et al., (2005) (Banfi et al., 2006) and concludes that the reduction of the cellular volume and the globular volume takes the reduction of sanguineous viscosity and is measured of protection against the high taxes of cellular death that occur to the end of one day of training (Sentürk et al., 2005).

The three methods had been carried through in the intensity of the anaerobic threshold and they had not produced significant alterations in the hematologicals parameters, but they had presented differentiated answers. The intervaled method suggested as acute response hemoconcentration because of the increase of the hematocrit, erythrocytes and reduction of the hemoglobin. We suggest that these alterations can be caused by the thermal stress and that the intermittent method has caused not significant hemolysis due the reduction of the erythrocytes, hemoglobin and hematocrit and can have caused to the increase of the body temperature. The same occurred with the action of the game where we possibly find destruction of some erythrocytes. The performance in the action of the game promoted alterations in four of the seven hematologicals variables even so insignificant.

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