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Original Article

Nonconventional and conventional biomarkers evaluated in gold medalists beach volleyball players during the 2015 and 2016 seasons (pre-Olympic and Olympic years)

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Abstract:
Little is known about serum biomarkers in elite beach volleyball (BV) athletes. Aim: To investigate the profile of non-conventional and conventional biomarkers of BV-players in pre-Olympic and Olympic years to optimize the periodization process and performance of these athletes. Design: Case report or Brief Communication. Methods: This prospective and observational study included 2-adult gold-Olympic BV-players during the Pre-Olympic and Olympic Games. The investigation was performed according to the periodization of the training in 'training blocks': A) Initial adaptation (endurance training); B) accumulation of force and transition of force/power (strength training) and C) power (competitive target periods). Along the seasons, strength and power were monitored by a digital dynamometer. Additionally, blood samples were collected at 48h post-match or post–block. The non-conventional (leukocyte ROS production and apoptosis/necrosis indexes) and 48-conventional biomarkers were analyzed. Results: Both serum leukocyte $\bullet O_2^-$ and apoptosis/necrosis indexes of BV-athletes increased in periods of intense exercise activity compared with the periods between training blocks: 'restitution' (7-fold) or 'pre-season' (13-fold). Among all the conventional biomarkers, the results were not consistent with the periodization of the athletes. In relation to anabolic/metabolic metabolism, IGF-1/cortisol was a better profile than the testosterone/cortisol ratio. Conclusion: Our findings suggest that non-conventional markers (leukocyte $\bullet O_2^-$ production and apoptosis/necrosis) associated to IGF-1/cortisol ratio should be considered during the BV seasons to follow the performance of the players.
Keywords: physical exercise, biomarker, oxidative stress, ROS production, apoptosis, overtraining

Introduction

Despite the increased interest in biomarkers in sports, its applicability to volleyball players, the 5th most popular sport in the world, has not yet been much investigated (Kobaças et.al 2016, Mazon et.al 2013). More specifically, little is known about biomarkers in elite-beach volleyball (BV) athletes (Homberg and Papageorgiou 1994). Consequently, these parameters solicited by sport physicians may contribute to misinterpretation culminating with inadequate decisions by the staff in professional BV, as observed in other modalities (Becatti et.al 2017, Lews et al 2016) Thus, it is relevant to know reference values of BV-athletes for a better understanding of training response optimizing the periodization process.

In parallel, cumulative evidences have shown that intensive training periods can trigger reactive oxygen species (ROS) production by cells (Becatti et al 2017, Reid 2016, Vollard et al 2005). Interestingly, while many studies have reported that ROS are involved in muscle damage and chronic fatigue (Becatti et all 2017, Reid 2016) it has also been suggested that low-ROS concentrations play a fundamental protective role in exercise-induced adaptations (Becatti et al 2017). However, the variation of ROS bioavailability that may enhance the performance without compromise adaptive pathways still needs to be elucidated (McLeay et al 2017). Therefore, monitoring ROS levels in BV-athletes could be an innovative tool to try to predict risk of injury or overtraining. Our aim was to quantify the levels of non-conventional and conventional biomarkers in elite BV-athletes during

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the 2015-2016 seasons (pre-Olympic and Olympic years) to optimize the design of their performance monitoring.

Material & methods

Subjects

This prospective and observational study included 2 Olympic BV-players (30 y) realized between January 10, 2015-August 24, 2016 and was approved by the Brazilian-Ethical-Committee for human research *'Plataforma-Brasil'* (#1.540.498). The athletes were informed about the investigation and gave their written informed consent to participate. None of athletes contracted infectious diseases or received any deliberate supplementation/medication that could modify the observed results. It should be noted that only for the purpose of comparison in the strength and power evaluations, we used as reference data unpublished average values of 10 competitive and 10 amateur athletes.

Periodization of training

The periodization was divided into 3-blocks: A) general or introductory adaptation (under endurance training-'pre-season'); B) accumulation of force and transition of force/power (under strength training-'accumulation') and C) power (competitive target periods - most important competitions–'competition'), all of these always interspersed with periods of recovery between the respective blocks. During all blocks, it was aimed the maintenance of high-force and power due to the high number of competitions throughout the pre-Olympic and Olympic cycles.

Evaluation of strength and power

The data of strength and power were monitored by a computerized digital dynamometer (PeakPower-CEFISE®, Nova Odessa-São Paulo-Brazil) connected to a bar. The parameters obtained were absolute power (watts) and maximum strength (Kgf) developed throughout the concentric phase of "half squat" exercise (50Hz).

The one maximum repetition (1RM) test

The BV-athletes warmed up prior to testing by cycling for 10-20min with static stretching and myofascial release. After 5min of rest period, the BV- players were familiarized with each of the resistance machines (PeakPower) by performing 8-10 repetitions of a light load (~50% of predicted 1RM). After more 5min of rest, they performed a load (~80% of estimated 1RM) through the single leg half squatting. After each successful performance, the weight increased until a failed attempt occurred. One minute of resting was given between each attempt and the 1-RM (Kg) was attained within 5 attempts and 5min rest separated each test.

Power Test

The athletes were evaluated in high-speed-half-squat, with emphasis on the execution of the concentric component (high-speed climb), isolating the eccentric component (slow descent with a rapid stop isometry before the concentric phase/climb). After the physical trainer command, athletes were submitted to 6-repetitions of the 'high-speed-half-squat' with 60% of load; 70%-80%-90% of 1RM (pre-determined), interspersed for 5min apart, recording the best relative power produced.

Blood samples

All samples were collected from the antecubital vein (seated position) in EDTA-containing Vacutainer glass tubes (Becton, Dickinson and Co, Franklin Lakes-NJ), 9-10 a.m., 48h post-match/post–block, excluding the influence of acute exhaustive exercise. After, were centrifuged at 2,000 *g* /10min and serum stored at –20°C. All measurements were obtained via automatic biochemical analyzer (AU680, Olympus/Beckman Coulter, Munich-Germany) or hematological analyzer (Coulter LH750, Beckman Coulter, Brea, CA-USA).

Measurement of ROS production in the blood

An aliquot of the blood sample was transported to the laboratory of Translational Physiology-UFES and analyzed within 24 h from the time of collection. The blood ROS samples were quantified by flow cytometry as previously reported (Porto et al 2015).

Measurement of apoptosis/necrosis

Apoptotic/necrotic leukocyte cells were quantified by annexin V-FITC and propidium iodide (PI) double staining using an annexin V-FITC apoptosis as previously reported (Porto et al 2015).

Quality of Analysis

Based on the descriptive pattern of this study in two individuals, no statistical analysis for comparison was performed in a large part of the study. All data were processed using the GraphPadPrism-5 software.

Results

Subjects

General characteristics

The anthropometric characteristics of athletes during the 2015-2016 seasons (pre-Olympic and Olympic years) are summarized in Table 1. Although they exhibit similarities in ethnicity, age, body fat and lean mass, their distinct physical structures (height/weight/body mass index [BMI]) induced them to assume fixed functions during all games (AC: attack and block player and BS: defensive player).

Table 1. Anthropometric characteristics of the elite BV players

Parameters	AC	BS
Race/Ethnicity	White	White
Age (Years)	30	30
Height (cm)	202	185
Weight (Kg)	113.5 (112.4-116.4)	90 (88.8-91.2)
Body mass index (Kg/m^2)	27.9 (26.7-28.2)	26.3 (25.9-26.6)
Body Fat (%)	12 (9.33-15.35)	11 (8.97-12.39)
Lean mass (%)	88 (84-90.6)	89 (87.6-91.3)

Training load measurements

The performance of BV-athletes was strongly associated with strength-power parameters, recorded in each block of periodization. Figure 1 demonstrates successfully the similar profile of strength (A) and power (B) between these elite-athletes during the seasons. As expected, the strength and power indexes increased from the first accumulation period. Successfully, these values remaining stable, appearing all points higher than the competitive (~3 and ~2-fold respectively) and amateur athletes (~10 and ~4-fold, respectively), figure 1.

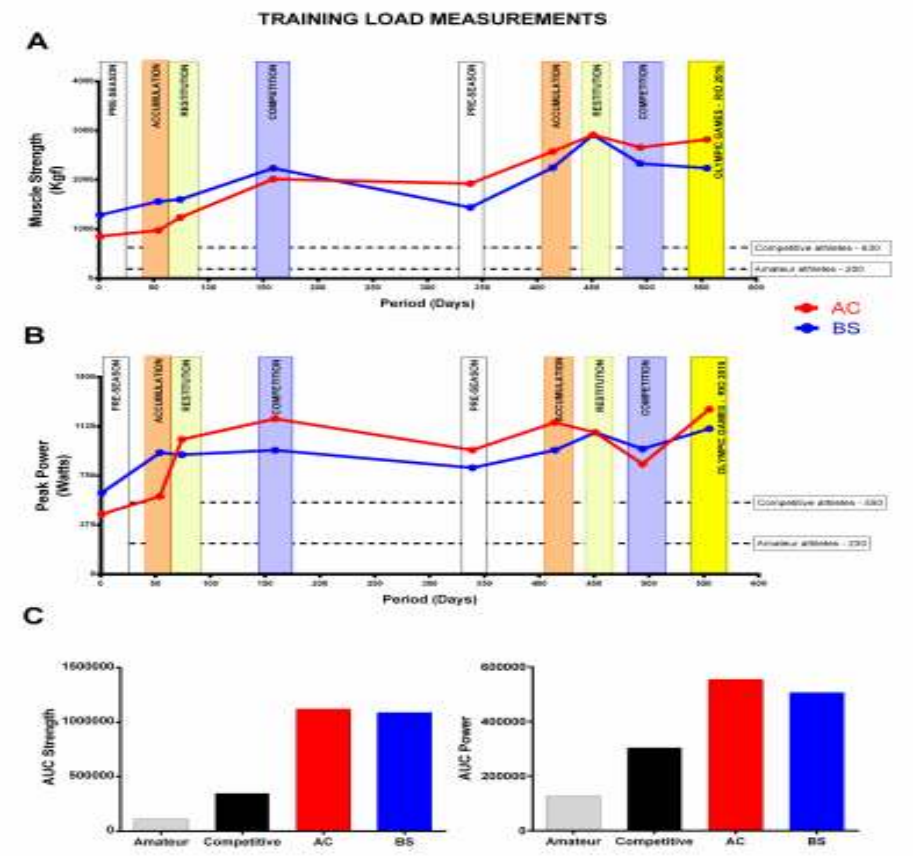

Fig 1. Strength (A) and power (B) profiles evaluated in gold Olympic BV players. As expected, both indexes increased in the first accumulation periods and remaining stable during the 2015 and 2016 seasons. C) Bar graphs showing the area under curve (AUC) of strength and power, in comparison with amateur or competitive athletes

Non-conventional biomarkers: ROS and apoptosis measurements

Figure 2A shows that in both athletes the main increment of ROS bioavailability (only $\bullet O_2^-$) occurred in periods of intense exercise activity ('accumulation' and 'competition' periods) with an increase of ~7- and 13-fold compared with 'restitution' and 'pre-season' periods, respectively. Also, the changes in the rate of apoptosis+necrosis-index followed the same profile observed for $\bullet O_2^-$ production being consistent in both athletes, reaching highest values in periods of competition, figure 2.

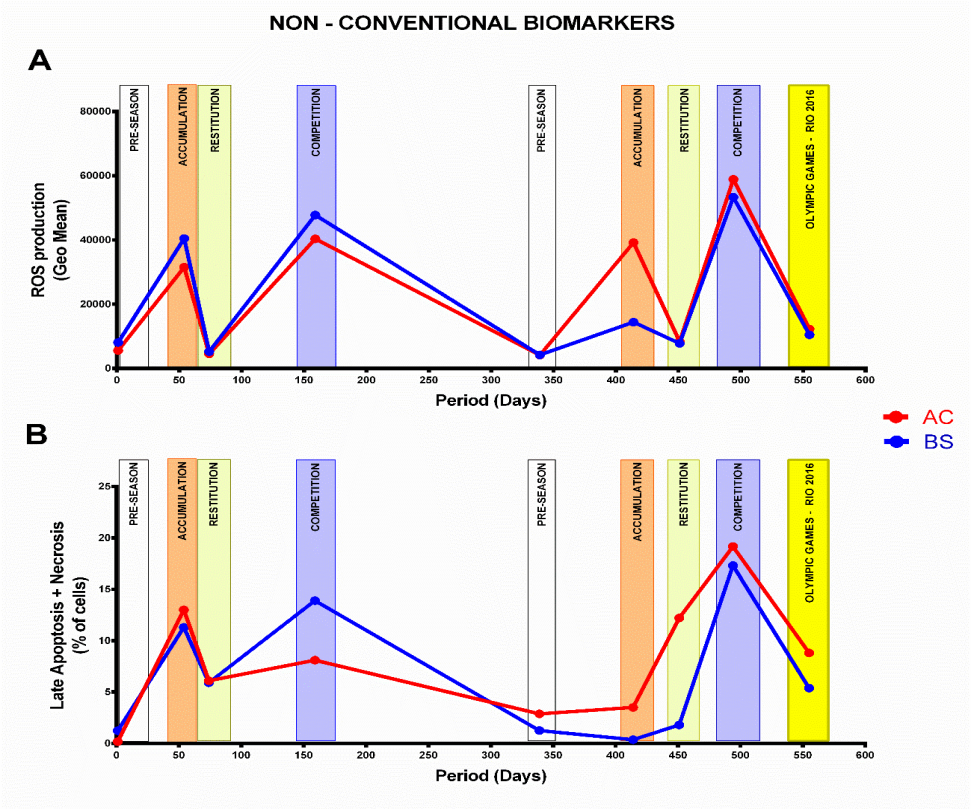

Fig. 2. Evaluation of non-conventional biomarkers through of (A) blood ROS production and (B) late apoptosis/necrosis in gold Olympic BV players during the pre-Olympic and Olympic seasons.

Conventional biomarkers evaluated

During longitudinal study, we monitored a total of 42 conventional biomarkers as potentially relevant. Figure 3A and 3B demonstrated that, in both elite-athletes, the serum lactate and lactate dehydrogenase did not show any relevant changes during the different stages of periodization, revealing values compatible with those found for non-athletes throughout the seasons.

Regarding the serum creatine kinase (CK) (Figure 3C), the BS player showed higher values than the clinical reference in ~95% of the seasons, mainly in periods of intense exercise activity ('accumulation' and 'competition' periods). However, AC showed high values only in ~ 40% of the cycle and without association with periods of intense exercise activity. In Figure 3D, the serum levels of CK-MB (an isoform of CK) were similar between athletes and kept below the maximum clinical reference value during all the cycle.

We also evaluated two hormone-ratios currently explored in sports medicine to evaluate anabolic/catabolic status: testosterone/cortisol (T/C),[10] and insulin growth factor/cortisol (IGF-1/C),[11] (Figures 3E and 3F, respectively). Although the absolute values were not equal, the curves presented a similar profile, indicating a predominance of anabolism in the Olympic phase (2016 - after 350 days) compared to pre-Olympic phase. The remaining 42 parameters are shown in supplementary data related to this article (Supplementary Data - Table 2), figure 3.

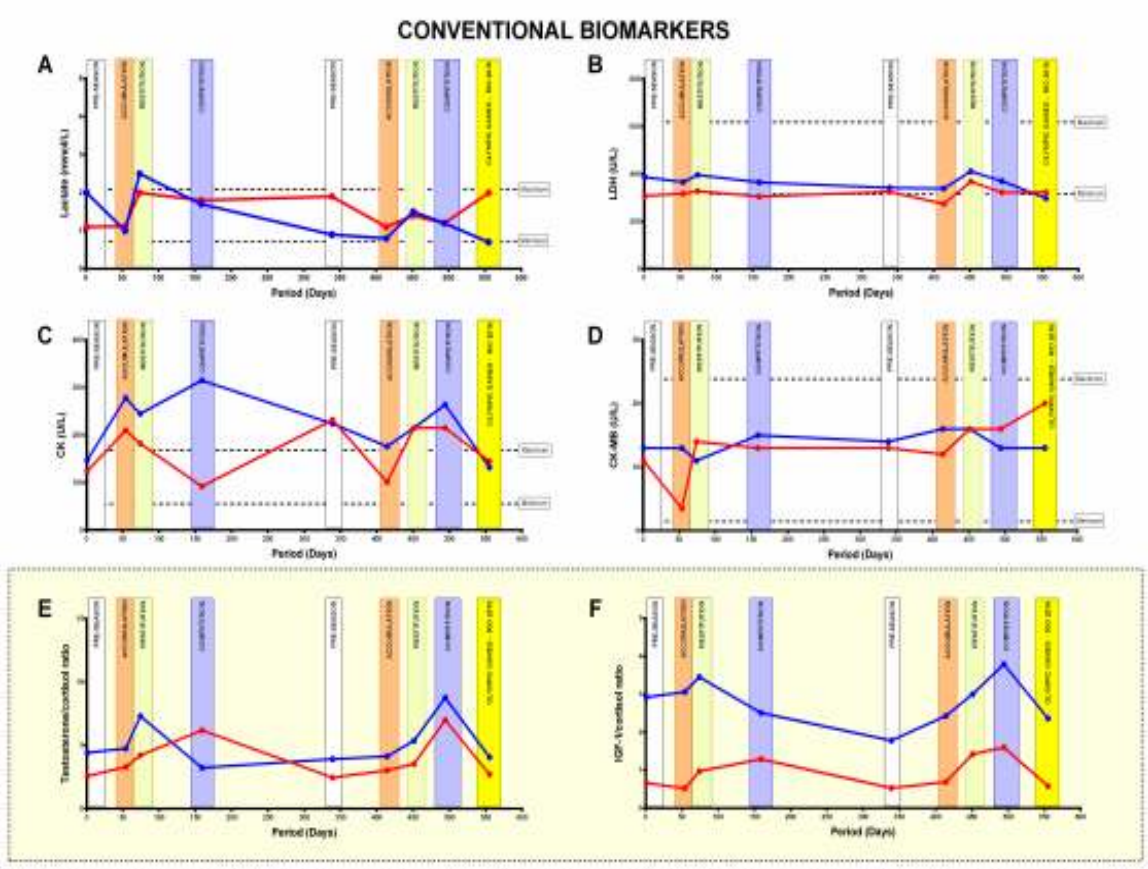

Fig. 3. Measurement of conventional biomarkers in elite BV players during pre-Olympic and Olympic years. A) Analysis of serum lactate and B) lactate dehydrogenase showing a flat profile. C) Evaluation of creatine kinase (CK), demonstrating predominance in BS versus AC while in D) the isoform CK-MB shows similarity between athletes. In yellow box, is demonstrated a similar profile between anabolic/catabolic hormone ratios: E) T/C (testosterone/cortisol) and F) IGF-1/C (insulin growth factor/cortisol).

Discussion

The optimum performance throughout the seasons was achieved through the use of uncommon individualized loads and monitoring with non-conventional biomarkers, reinforcing the link between $\bullet O_2^-$ and cell damage, clearly evidenced by increased cell apoptosis/necrosis in the overreaching periods as suggested by others (Powers and Jackson 2008, Ryu et al 2016).

Regarding the conventional biomarkers, some researchers agree that they may not represent a reliable method to monitoring overreaching/ overtraining in elite-athletes (Meyer and Meister 2011, Ostojic and Ahmetovic 2008). However, information regarding BV-players is still unknown. Thus, our data corroborate evidences observed in other sport modalities with elite-athletes, showing only minor laboratory changes (Becatti et al 2017). Regarding the difference of CK observed among the athletes, this may be supported by inter-individual variations and/or intensity of exercise. It is worth highlighting that in 2016, BS was elected as the best defensive and offensive player, justifying, at least, in part, his fundamental participation in the tournaments that could culminate with increment of CK. Regarding the endocrine biomarkers, our data reinforces that IGF-1/C might be a useful substitute parameter of an imbalance between anabolic/metabolic metabolism compared to T/C, justified by linear production and longer half-life of IGF-1 (Nassib et al 2016).

Although this study was performed by analyzing only 2 athletes (a complete BV team), we had the unprecedented opportunity to investigate the changes in non-conventional and conventional biomarkers in elite BV-players during 2 consecutive seasons (pre-Olympic/Olympic years), culminating in the conquest of Olympic gold-medal in Rio 2016. Therefore, these data might open new strategies of research on sports medicine, especially for optimize the performance monitoring of BV-athletes.

Conclusions

Overall, our findings suggest that increase in leukocyte $\bullet O_2^-$ production and apoptosis/necrosis index (classified as non-conventional markers) associated to IGF-1/cortisol ratio should be considered during the BV

seasons to follow the performance of players, preventing overtraining and injuries, optimizing the monitoring of elite BV-athletes.

Conflicts of interest

The authors declare no conflict of interest.

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Author contributions

Conceived and designed the experiments: HOA, FLA and TMCP. Performed the experiments: HOA and MLP. Analyzed the data: ECV, MPB and TMCP. Contributed reagents/materials/analysis tools: SSM, ECV, BPC and TMCP. Wrote the paper: HOA, MPB, ECV, BPC MCT and TMCP. All authors read and approved the final version of the manuscript.

References

Becatti M, Mannucci A, Barygina V, Mascherini G, Emmi G, Silvestri E, Wright D, Taddei N, Galanti G, Fiorillo C (2017). Redox status alterations during the competitive season in élite soccer players: focus on peripheral leukocyte-derived ROS. *Intern Emerg Med*

Homberg, S. and Papageorgiou, A (1994). Handbook for beach volleyball. Meyer & Meyer Verlag.

Kocabaş R, Namiduru ES, Bagçeci AM, Erenler AK, Karakoç Ö, Örkmez M, Akan M, Erdemli HK, Taysi S, Tarakçioglu M. (2016). The acute effects of interval exercise on oxidative stress and antioxidant status in volleyball players. *J Sports Med Phys Fitness*.

Lewis NA, Newell J, Burden R, Howatson G, Pedlar CR (2016). Critical Difference and Biological Variation in Biomarkers of Oxidative Stress and Nutritional Status in Athletes. *PLoS One*;11(3):e0149927.

Mazon J, Gastaldi A, Di Sacco T, Cozza I, Dutra S, Souza H. (2013). Effects of training periodization on cardiac autonomic modulation and endogenous stress markers in volleyball players. *Scand J Med Sci Sports*;23(1):114-20.

McLeay Y, Stannard S, Houltham S, Starck C (2017). Dietary thiols in exercise: oxidative stress defence, exercise performance, and adaptation. *J Int Soc Sports Nutr*;14:12.

Meyer T, Meister S. (2011). Routine blood parameters in elite soccer players. *Int J Sports Med*;32(11):875-81.

Nassib S, Moalla W, Hammoudi-Nassib S, Chtara M, Hachana Y, Tabka Z, Chamari K, Elloumi M (2016). The IGF-1/cortisol ratio as a useful marker for monitoring training in young boxers. *Biol Sport*; 33(1):15-22.

Ostojic SM, Ahmetovic Z. (2008). Weekly training volume and hematological status in female top-level athletes of different sports. *J Sports Med Phys Fitness*;48(3):398-403.

Papacosta E, Nassis GP, Gleeson M (2015). Effects of acute postexercise chocolate milk consumption during intensive judo training on the recovery of salivary hormones, salivary SIgA, mood state, muscle soreness, and judo-related performance. *Appl Physiol Nutr Metab*;40(11):1116-22.

Porto ML, Rodrigues BP, Menezes TN, Ceschim SL, Casarini DE, Gava AL, Pereira TM, Vasquez EC, Campagnaro BP, Meyrelles SS (2015). Reactive oxygen species contribute to dysfunction of bone marrow hematopoietic stem cells in aged C57BL/6 J mice. *J Biomed Sci*;22:97.

Powers SK, Jackson MJ (2008). Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiol Rev*;88(4):1243-76.

Reid MB (2016). Reactive Oxygen Species as Agents of Fatigue. *Med Sci Sports Exerc*;48(11):2239-2246.

Ryu JH, Paik IY, Woo JH, Shin KO, Cho SY, Roh HT (2016). Impact of different running distances on muscle and lymphocyte DNA damage in amateur marathon runners. *J PhysTher Sci*;28(2):450-5.

Vollaard NB, Shearman JP, Cooper CE (2005). Exercise-induced oxidative stress:myths, realities and physiological relevance. *Sports Med*;35(12):1045-62.