CHAPTER
18

Systematized review study on biochemical biomarkers of overtraining

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ABSTRACT
In sports, the demand for victory increasingly places the professional athlete face to face with the incessant search for both physical, tactical, and strategic performance, since these factors are fundamental to obtaining a victory. However, overtraining can lead to disorders that are harmful to the athlete's health. Objective: systematized review on biochemical biomarkers of overtraining. The PRISMA Checklist was used for development and the PICO strategy for data extraction. Inclusion criteria were articles with primary data published in the last 10 years, in Portuguese, English and Spanish that were directly related to the central objective of the study. Results: After using all the inclusion and exclusion criteria, 11 articles were chosen for the development of the article, which was directly related to the theme of the study. In these articles, four major classes of molecules with potential biomarkers of overtraining were obtained: -related to intermediary metabolism; - related to the organism's redox profile; -with hormonal function, and; -with immune function.

Keywords: Overtraining, Sports Medicine, Sports Science**.**

1 INTRODUCTION

Nowadays it is evident that the employee's delivery to work has been increasing, this has brought important consequences for the health, both mental and physical of these individuals. Not differently, in sports and especially in football, the charge for victory increasingly puts the professional athlete face to face with the incessant search for performance both physical and tactical, and strategic (LEMYRE, P.N.; HALL, H.K.; ROBERTS, G.C. 2008), since these factors are fundamental to obtain a victory. However, when accompanied by a poor recovery, overtraining can lead the athlete to biochemical, physiological and psychological changes responsible for the occurrence of disorders and dysfunctions harmful to the athlete's health (SOUZA et. al., 2010). Given this context, the investigation of possible psychophysiological correlations, as found in Overtraining (Overtraining Syndrome (OT)) can help protect these athletes, especially players of the basic category of professional soccer, since this phase is fundamental for the development of specialized physical skills (BONORINO et. al., (2008); BRANDÃO, (2004); KROLLNER AND KROLLNER, 2018).

Due to the demands for better performance, athletes have presented a higher incidence of burnout and decision-making, especially in competitive periods (Verardi et al. 2014; Verardi et al. 2015). Thus, it is essential to search for stress markers focused on sports, which may or may not have a direct relationship with OT that so terrorize the sport and the health of athletes.

2 METHODOLOGY

However, studies that bring a better understanding of the mental and physical health of athletes are increasingly important, since they can directly help in reducing the psychological exhaustion of athletes, as well as the involvement of injuries. Thus, studies in this area can serve as an indispensable tool for the health and performance of the athlete, as well as for the proper functioning of the team, the club and the sports modality as a whole. Given this, the main objective of this systematized review is to search through the PRISMA method (*Preferred Reporting Items for Systematic Reviews and Meta-analyses),* through the PICO strategy (an acronym for P: population/patients; I: intervention; C: comparison/control; O: outcome), evidence in the literature on biochemical biomarkers of *Overtraining*.

Being a systematic review, the development took place from the Methodological Guidelines for the Elaboration of Systematic Reviews, following the determination of the research question, leading to the selection and identification of the databases available for consideration, as well as the use of inclusion and exclusion criteria known as eligibility criteria, to then start the search for viable research materials, followed by the analysis of the text in search of vital data to elaborate and support

the discussion of the results found, and finally, to be able to present the study carried out. (Brazil, 2012)

According to Donato, & Donato (2019), the systematic review is a method of investigative research, which makes use of scientific evidence found in the systematized literature, in search of correlations with strategic interventions and critical analysis of data, thus allowing, through a comparative bias, the integration between data contained in specific studies, forming a systematization of the evidence around the theme, which allows a greater direction for future productions. To support the study, the updated 2020 PRISMA protocol guideline was used for this review, which has an expanded checklist and details of elements considered fundamental. Elements not in italics are considered "essential" and should be reported in the main report or as supplementary material for all systematic reviews (BMJ, 2021).

To this end, on October 16, 2022, at 9:30 a.m., searches were conducted on the VHL (Virtual Health Library) website (https://bvsalud.org/) through two authors concomitantly, the keywords: Overtraining AND biochemical markers OR biochemical biomarkers. Next, the inclusion and exclusion criteria were applied, including Articles published in the last ten years, in English, Portuguese, and Spanish. Then, only the articles for which the full texts were found and which were part of studies with primary data were considered, excluding review articles and those that obtained secondary data. After reading the full text of the chosen articles (in which it was possible to find the keywords, in the title or abstract), those that did not contemplate the information related to the objective of the present study were excluded. It is worth mentioning that the same process was also carried out on the search site SCIELO (Scientific Electronic Library Online) (https://search.scielo.org/history/?lang=pt). The step-by-step can be seen in Figure 1, which represents the detailed flowchart of the search steps performed in the present study.

Therefore, the methodology was divided into 4 steps to follow the PRISMA protocol, where the first stage included the identification from the searches of the terms in the databases presented, and after collecting the full texts went through the second stage, which is the screening, where the eligibility criteria were applied in the search in the databases. The third stage is followed by the selection of clinical studies with primary results, consisting of the selection made in the screening, and finally, those selected after full reading to eliminate the articles outside the context of the review and confirm the analysis, in addition to eliminating duplicity, the included ones were obtained (Moher, Liberati, Tetzlaff, & Altman, 2015).

After defining the theme to be searched, the searches were initiated from the keywords already described previously in the methodology. Having applied the inclusion and exclusion criteria, only eleven articles were used for the present systematized review, which presented information aimed

directly at the objectives of the present search. It is worth mentioning that the PRISMA methodology was used for the development of this review, as well as the PICO strategy for the search and extraction of the results of the articles.

From the extraction of the data, it is possible to observe numerous very important pieces of information reported by the authors. Therefore, such results will be presented in the chapter below.

3 RESULTS AND DISCUSSION

Joro and his collaborators (2020) divided two groups of athletes, one of whom underwent more strenuous training, while the other performed the same routine workouts for a period between 6 and twelve months. At the end of the experiment, the authors observed decreased irisin values in athletes who submitted to stronger training when compared to athletes who remained in the same training routine during the rest period. However, when submitted to the maximal exercise test in cycle ergometer, the authors observed an increase concomitantly with the values of malonaldehyde followed by a decrease in the antioxidant protection, ORAC (oxygen radical absorption capacity) of the athletes submitted to exhaustive training. Thus, it can be considered that when performing exercises more strenuously, athletes may be affected by a higher oxidative state, thus leading to future injuries if this condition becomes chronic.

In a study with handball athletes Marin and his collaborators (2013) found increased values of TBARs (thiobarbituric acid reactive substances) and carbonylated proteins in athletes when submitted to more exhaustive training, in addition to decreased values of thiols. This may indicate, according to them, an association between overtraining and oxidative stress. The authors also portray having observed an increase in key enzymes of muscle catabolism CK (Creatine Kinase), LDH (Lactate Dehydrogenase), and AST (Aspartate Aminotransferase) in this same condition, thus indicating that strenuous exercise may be associated with an increase in catabolism and possible muscle damage even under resting conditions.

When analyzing some molecules in the saliva of water polo athletes Ferlazzo and his collaborators (2021) observed decreased concentrations of cortisol and testosterone one day after they had played, but, according to them, such values increased the next day (the second day after the game). However, the concentrations of sIgA (secretory immunoglobulin A)/proteins and AOPP (Advanced Protein Oxidation Products)/proteins increased from the first day onwards. For the authors, these values suggest that the athletes were affected by oxidative stress after the game, especially on the second day. In the same study, the authors found decreased values of free testosterone A ratio/cortisol in the morning of the second day after, indicating that there is an important catabolic response after the match, as well as an impairment in the immune response of these athletes.

After analyzing twenty-five soccer athletes compared to physically active individuals under maximal exertion conditions, in maximal anaerobic and maximal aerobic exertion tests), Humińska-Lisowska et al. (2021) observed increased cfDNA (circulating free plasma DNA) values shortly after exercise, followed by decreased values. However, the values found were observed only in athletes under the maximal anaerobic exercise test, which was not found for the participants of the control group. According to the authors, the use of cfDNA as a measure of injury assessment under exercise conditions can be applied only under conditions of maximum effort.

Travis Anderson et al. (2021), aimed their work to analyze the salivary cortisol values of athletes in stressful conditions and healthy individuals soon after waking up and at various times after. According to the authors, cortisol values demonstrated time-per-group interaction for athletes under OT syndrome, however, these values were not observed for sedentary individuals. Thus, they suggest that salivary cortisol analyses may be important as a biomarker of OT in athletes.

Flavio A. Cadegiani and his collaborators (2021) evaluated fifty-one men aged between eighteen and fifty years, of whom fourteen of these were athletes affected by OT syndrome, twenty-five healthy athletes, and 12 healthy sedentary participants. When comparing healthy athletes to those affected by OT syndrome, they observed higher levels of cortisol; ACTH (Adrenocorticotropic Hormone); Gh (Growth Hormone); IGF-1 (Insulin-like Growth Factor 1 or Somatomedin C); testosterone; thyronine, estradiol, lactate, CK (Creatine Kinase), total NUC (Nocturnal Urinary Catecholamines) and nocturnal urinary dopamine. In contrast, the total testosterone, neutrophils, and testosterone/estradiol ratio were lower in the OT group. When compared to healthy sedentary individuals, athletes in OT condition had lower levels of low lactate, hematocrit, lymphocytes, and eosinophils, but higher creatinine levels (creatinine levels became similar after adjusting for muscle mass) and CK and a higher platelet/lymphocyte ratio. Thus, the authors conclude that OT syndrome directly affects the immune, musculoskeletal, and adrenergic systems, in addition to promoting increased aromatase activity and inflammatory changes.

In a previous study also developed by Flavio A. Cadegiani and his collaborators (2019), it can be observed that decreased values of neutrophils and testosterone were lower in athletes affected by OT syndrome when compared to healthy athletes and sedentary individuals. However, the values of CK, lactate, estradiol, total catecholamines and dopamine were higher while the testosterone/estradiol ratio and lymphocyte concentration showed decreased values in athletes with OT when compared to sedentary individuals and healthy athletes.

Ionas Papassotiriou and his collaborators (2017) aimed to evaluate the biochemical indices of Greco-Roman wrestling and freestyle athletes during typical pre-season training. To this end, the authors evaluated some biochemical variables before and after the routine training of the athletes. Thus,

the authors observed increased lactate and glucose values of the athletes of the Greco-Roman wrestling groups when freestyle. However, when compared, the lactate values of freestyle athletes were higher than those observed in Greco-Roman wrestling athletes in the rest period, showing that the experience factor interferes importantly. According to the authors, thirteen athletes presented high concentrations of creatinine in their urine, however, these values do not reach extreme concentrations (> 26.5 mmol/L). Concerning proteinuria, according to the authors, no athlete presented evidence. Thus, it can be said that the analyses of lactacidemia and glycemia change physical exertion and the experience of the athletes has an influence on the values of lactate at rest.

When evaluating an elite international rowing athlete, Nathan and his collaborators (2016) did not observe significant changes in hematological, biochemical, thyroid function, immunological, vitamin and mineral values. However, when they evaluated redox state biomarkers such as hydroperoxides and plasma antioxidants such as red blood cell glutathione, superoxide dismutase, coenzyme Q10, vitamin E (α- and γ-tocopherol) and carotenoids (lutein, α-carotene, β-carotene), they observed evidence. Thus, according to the authors, interventions in the nutritional profile can be used as a strong strategy in the recovery of athletes.

Tian et al. (2017) while studying incidences of functional OT, nonfunctional OT, and OT syndrome in elite Chinese wrestling wrestlers over eight years observed that one wrestler experienced functional OT on three occasions, two wrestlers on two occasions, and six female wrestlers experienced it on one occasion. Two female wrestlers experienced non-functional OT on three occasions, three wrestlers tried it twice, and eleven wrestlers tried it once. Nine of the thirteen athletes placed in the top three at the world championships with non-functional OT at least once. The two occurrences of OT syndrome occurred in different female wrestlers. The diagnostic sensitivity for non-functional OT was 29%, 33%, 26% and 35% for creatine kinase, hemoglobin, testosterone, and cortisol and 79%, 88%, 90%, and 82% for creatine kinase, hemoglobin, testosterone, and cortisol, respectively. According to the authors, the *post hoc* analysis showed no mean differences in creatine, hemoglobin, testosterone, or cortisol among the athletes, even though they were diagnosed with functional OT and not.

Finally, but extremely important, in a study with elite rugby athletes, Emanuela Galliera et al. (2014) observed increased values of heart failure markers NT-proBNP (N-terminal B-type natriuretic peptide) and GDF-15 (growth factor and cell differentiation-15) at the end of training when compared to pre-workout values. Because of this, the authors suggest that GDF-15 measurement in particular may be a useful tool in monitoring cardiovascular status during training sessions and competition. Thus, it can help prevent the onset of undesirable cardiovascular events.

4 FINAL CONSIDERATIONS

This sub-item has as its central function, to promote a synthesis of the main results found in the excellent studies extracted in this review. After the steps in the exclusion criteria, inclusion and the presentation of the data in the results and discussion, a compilation of the data was developed here to make the exposition of the content as didactic as possible.

To this end, the authors of this review were careful to subdivide the significantly different biomarkers into four major classes: 1- Biomarkers involving the metabolic state, 2- Involving the redox state, 3- Hormonal metabolic control state, and 4- Immune state. As shown in Figure 1.

Regarding metabolic status, high values of LDH, lactate, glycemia, AST, albumin, CK, and creatine can be observed in athletes affected by OT at rest. Indicating strong evidence that strenuous exercise causes an increase in the catabolic state, followed by impaired recovery of these athletes. Thus, it seems efficient to use these molecules as markers of training overload as well as the recuperative process of athletes.

When it comes to the redox state and cell degradation, the values of antioxidant enzymes such as SOD, CAT, GPx, plasma glutathione, Coenzyme Q, and ORAC, are important in the screening of internal antioxidant power, as well as Vit values. A, E, Coenzyme Q, and Hydrocarbons about the defense obtained by the consumption of exogenous components. However, the values that treat a real response of the antioxidant capacity of the organism are the molecules that indicate injury by the oxidative attack, such as TBARs, AOPP, Carbonylated proteins (Carbonyl Groups), MDA and cfDNA (as a general marker of cell injury). Increased values of antioxidant defenses only demonstrate efficacy when accompanied by decreased values of oxidant injury indicators. Thus, such molecules can be used as an effective dosage tool for training overload, as well as in the recovery of the same and also in the prevention of future development of chronic injuries.

Hormone values do not seem to show a pattern. In some studies, the values of cortisol, testosterone, ACTH, Gh, IGF-1, Thyronine, NT-proBNP, GDF15, Total Catecholamines, and Dopamine were increased in athletes with OT after exertion. However, in other studies, it is possible to observe decreased values of irisin and estradiol cortisol in these athletes after exertion and upon waking, respectively. In another study, cortisol and testosterone values did not change among athletes with OT compared to healthy ones. Thus, we believe that more studies are needed in the context of OT syndrome involving hormonal markers of metabolic control.

Finally, decreased values of IgA, Lymphocytes, Eosinophils, and Neutrophils were found in athletes with OT, thus indicating that strenuous exercise may impair the immunology response. This may explain higher occurrences of viral airway infections in athletes affected by OT. Showing how important it is to use these analyses as a tool in the control of athletes' training.

Figure 1. Representative synthesis of biomarkers found in athletes with OT.

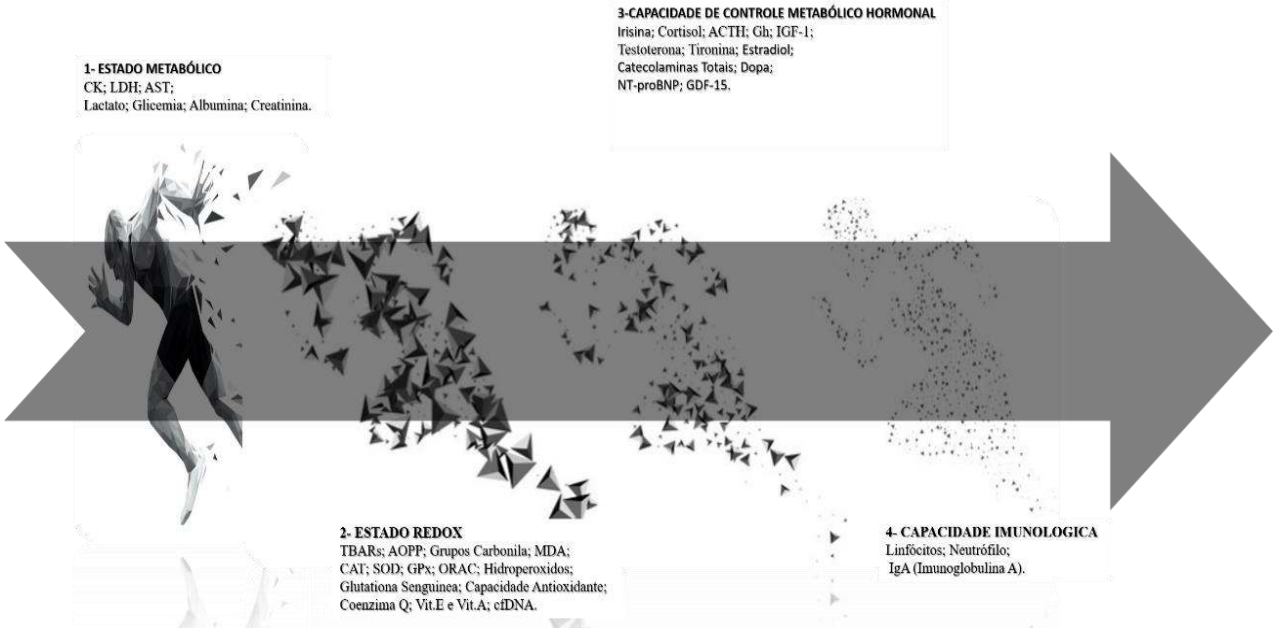

Figure 1: CK (Creatine Kinase; LDH (Lactate Dehydrogenase), AST (Aspartate Amino Transferase); TBARs (thiobarbituric acid reactive substances); AOPP (Advanced Protein Oxidation Products); MDA (Malonaldehyde); CAT (Catalase); SOD (Super Oxide Demutase); GPx (Glutathione Pedoxidase); ORAC (Free Radical Absorption Capacity); Vit. E (Vitamin E or α- and γ-tocopherol); (Vitamin A (Carotenoids); cfDNA (Cell-Free Circulating DNA); ACTH (Adrenocorticotropic Hormone); Gh (Growth Hormone); IGF-1 (Insulin-like Growth Factor 1 or Somatomedin C); Dopa (Dopamine); NT-proBNP (N-terminal B-type natriuretic peptide); GDF-15 (Growth Factor; Cell Differentiation); IgA (Immunoglobulin A).

5 CONCLUSION

Regarding what was found in this systematic review research, it is concluded that there are indeed molecules that can be used as biomarkers of OT. Of these, it can be considered to compose four major classes, those that are part of the intermediate metabolism such as LDH, lactate, glycemia, AST, albumin, CK, and creatine; those involving the redox profile such as SOD, CAT, GPx, plasma glutathione, Coenzyme Q, ORAC, Vit. A, E, Coenzyme Q, Hydrocarbons, TBARs, AOPP, Carbonylated proteins (Carbonyl Groups), MDA, cfDNA, of which make up the class of molecules with endocrine functions such as cortisol, testosterone (these molecules, in particular, does not seem to follow a consensus results profile among the articles), ACTH, Gh, IGF-1, Thyronine, NT-proBNP, GDF15, Total catecholamines and Dopamine, irisin, stadium and finally, the molecules that make up the class of the immune system such as IgA, Lymphocytes, Eosinophils and Neutrophils.

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