MARKED DIFFERENCES IN REDOX STATUS OF PROFESSIONAL SOCCER PLAYERS DEPENDING ON TRAINING TYPES

Authors: Andrea Bolner[1], Carlo Berizzi[2], Sujem Benedetto[3], Roberto Vano[3], Rocco Micciolo[4], Andrea Muscarà[5], Aldo Passelli[6], Ottavio Bosello[7], Giampietro Nordera[1]

1) Oxidative Stress Center (CSOx), Vicenza, Italy, 2) Nutrivector®, Mogliano Veneto - Treviso, Italy, 3) Alagen-io®, Promos Biotech, Udine, Italy,
4) Department of Psichology and Cognitive Sciences, University of Trento, Italy, 5) Friuli Coram srl, Udine, Italy, 6) Udinese Calcio, Udine, Italy, 7) Department of Medicine, University of Verona, Italy.

Introduction

Physical exercise increase metabolic demand, causes muscle damage and inflammation, and induce production of oxygen and nitrogen reactive species (ROS and RNS). Thanks to the involvement in the signalling and epigenetic pathways of these so-called "free radicals" [1-2], aerobic and high intensity training can enhance the activity of various antioxidant enzymes and glutathione redox status [3, 4]. Nevertheless, a prolonged imbalance between training load and recovery may overwhelm the antioxidant defences and helps to generate a permanent loss of the physiological adaptive response, with or without associated clinical signs [5], and to establish a chronic pro-inflammatory status named "ox-inflammation" [6, 7]. Aim of our study was to measure some ox-inflammation biomarkers in a team of soccer players to identify relationships between redox-balance, nutrition, workloads, training types and athletic performances. While training programs are difficult to modify because often implemented independently by the coach, we wanted to evaluate whether a careful nutritional control could allow, on its own, to protect the redox homeostasis and possibly to prevent dangerous imbalance conditions.

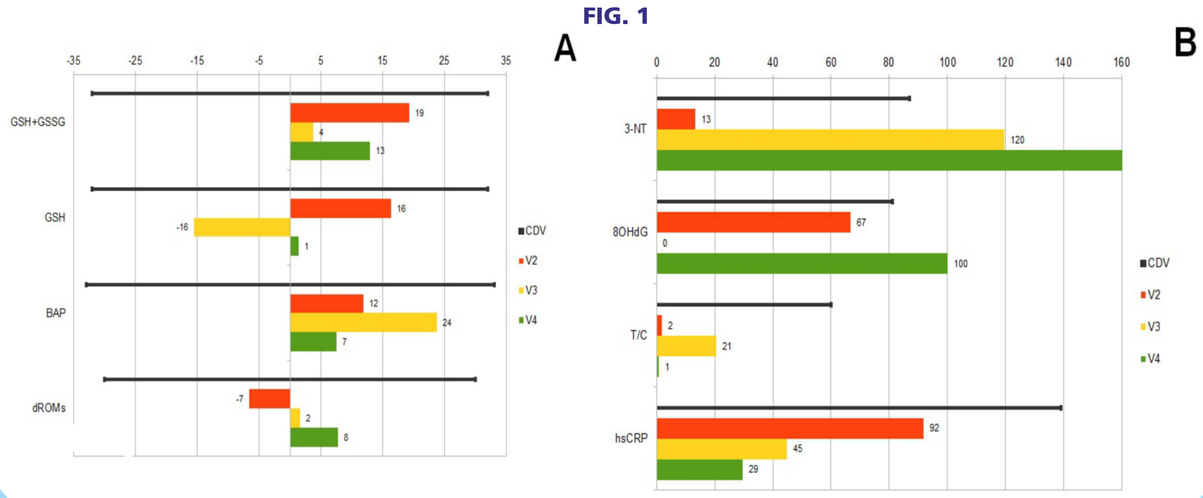
Methods

34 soccer players of a team playing in the Italian A series championship were evaluated every 2 months from pre- until end-season (visits V0-V4). At each time, together with a wide panel of routine biochemical tests and other physio-metabolic evaluations, some relevant plasmatic ox-inflammation biomarkers were analyzed, mostly with HPLC methods: interleukin-6 (IL-6), high-sensitivity C-reactive protein (hsCRP), total peroxides (dROMs), total antioxidant barrier (BAP), total (GSH+GSSG) and reduced (GSH) glutathione and coenzyme Q10, as components of the antioxidant endogenous barrier, vitamins A, E, beta carotene and lycopene, as components of the antioxidant exogenous barrier, 3-nitrotyrosine (3-NT) and 8-hydroxy-deoxyguanosine (8-OHdG), as marker of irreversible nitration and oxidative damage on nucleobases. During all the season, the nutritional program was customized for each athlete to ensure adequate micro- and macro-nutrients supply. The training workloads were measured using GPS based tracking technology.

Results

Until V2, a favourably increase of glutathione redox status and no evidence of oxidative damages were observed (Table1, Fig.1). Few days before V2, due to unexpected choices of the sports club, the team coach has been replaced and the initial training program was suddenly changed, with a sharp increase of high intensity training (Fig. 3). After change, significant decreases for glutathione and exponential increases for 3-NT were observed (+ 1274% at V4 vs. baseline, $p<0.001$). The hsCRP levels, already doubled between V1 and V2, remained high in V3 and were only weakly decreased in V4. The anabolic balance testosterone/cortisol (T/C), almost unchanged between V1 and V2, increased in not significant way at V3 and returned to baseline at V4, showing lack of sensitivity to performance losses. Unlike T/C, indeed, the worsening of the redox state has overlapped, with a certain coincidence of time, to a sharp deterioration in match-performances of the team in the second half of season.

FIG. 1



TAB. 1

	units	V1	V2	V3	V4
GSH+GSSG	umol/L	838 ± 138	**1000 ± 112***	870 ± 176	**947 ± 137***
GSH	umol/L	647 ± 108	**752± 90***	**546 ± 134***	656 ± 111
GSH ratio	%	77± 5	75 ± 6	**63 ± 9***	**69 ± 8***
BAP	uEq/L	2239± 291	2505 ± 770	**2770± 307***	2406 ± 239
dROMs	Ucarr	307± 41	287± 52	312± 44	331 ± 51
Q10	ug/L	345 ± 89	**292 ± 73***	351± 54	368 ± 76
Vitamin A	ug/L	470± 230	410 ± 130	**610 ± 170***	460 ± 240
Vitamin E	ug/dL	1320± 310	1440± 370	1370 ± 270	**1240 ± 200***
beta Carotene	ug/L	587 ± 413	**490 ± 247***	522± 244	514 ± 238
Lycopene	ug/L	319± 124	351 ± 124	**384 ± 108***	311 ± 113
3-NT	ug/L	21± 21	24± 24	47 ± 49	**292 ± 132***
8OHdG	ug/g creat	3 ± 1	8 ± 18	15± 6	16 ± 7
T/C*10^3	ratio	28± 8	27± 12	34± 8	28 ± 7
hsCRP	ug/dL	72 ± 103	**137± 88***	104± 102	93 ± 49
IL-6	ng/L	2.2± 0.6	2.2± 0.5	2.5± 1.0	2.2 ± 0.9
Gred Index	score	7.2 ± 3.0	**6.3± 1.5***	**3.6± 1.4***	**4.6 ± 1.3***
EnLevel	score	-130± 23	-146 ± 22	**-98 ± 27***	-124 ± 24

Table 1. Descriptive statistics for ox-inflammation biomarkers measured from visit V1 to V4. The values significantly changed vs. V1 (Tukey's test, $p<0.001$) are marked with asterisk (*).

Discussion

A right workload and training type produce free radicals that physiologically promote both the synthesis and the reduction of glutathione, enhance the endogenous antioxidant power and promote a reducing environment that supports the adaptive response to training. Imbalances between ROS production and antioxidant response lead to an ox-inflammation status in which, vicious circles produce inflammatory cytokines and free radicals that cause further oxidation and inflammation. The consequent impairment of the antioxidant barrier leads, at first, to the appearance of early signs of overstrain (e.g. muscular fatigue and injuries), and later irreversible oxidative alterations on macromolecules at different cellular levels. The measurement of redox damage biomarkers were useful to assess the extent of redox imbalance and the link with performances, and could be a useful tool to evaluate possible risks to athlete's health. Particularly, it is to remind the involvements of 3-NT in the pathogenesis of the aseptic vascular inflammation and its implication with sport related sudden deaths [9, 10].

Plotting glutathione energy level (En Level) vs. estimated glutathione reductase activity (Gred Index) [Fig. 2], the redox balance alterations occurred in the second half of the championship were underlined, allowing to better understand the events occurred in vivo [11].

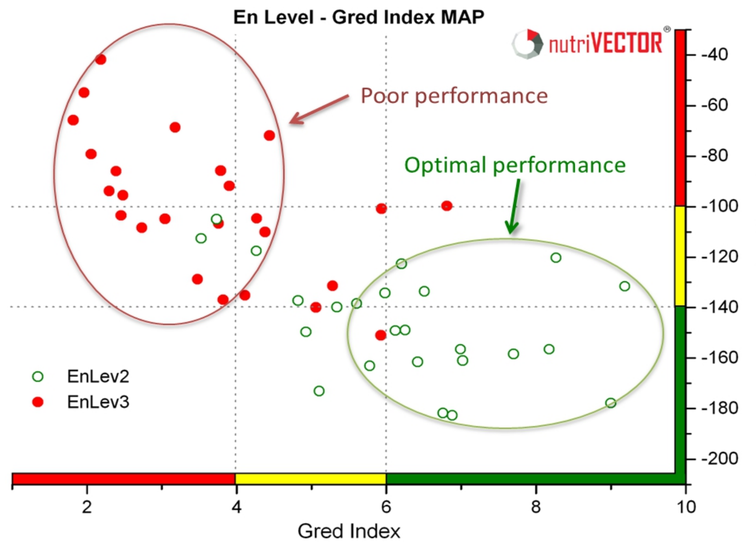


Fig. 2. The En Level/Gred Index Map: at visit V2, in conditions of equilibrium, the values of glutathione energy level (En Level) and estimated glutathione reductase activity (Gred Index) were close to the origin of axes (green circles) and the soccer team won many matches. The opposite happens in redox imbalance conditions (visit V3) when the soccer team got a long list of consecutive lost games. In such a situation the values in the map moved in the opposite direction to that of the origin of axes (red circles).

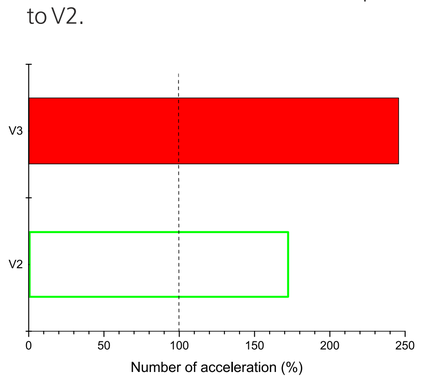


Fig. 3. Average of weekly accelerations (above 3 m·s-2) expressed as percentage relative to a typical Italian official match (marked with black dashed line) [8].
At V3 the increase was +42% compared to V2.

Conclusions

Prolonged increase of high intensity training, without sufficient recovery, seems to have altered the soccer player's ox-inflammation balance. The nutritional intervention was partially able to counteract the alteration but, the appearance of molecular oxidative damage highlighted that probably, it was much more conditioned by physical overstrain. A custom balancing of training load, recovery and nutritional support, is therefore crucial. The measurement of some ox-inflammation markers, as hsCRP, GSH and 3-NT, may be helpful to evaluate the state of physical fitness and early recognize the approaching of the over-training threshold.

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