

Non invasive approach for the assessment of oxidative stress after intense Judo training of high level athletes

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Abstract

The exercise-induced oxidative stress has received considerable attention with many original researches published in recent years. Physical activities (aerobic, anaerobic and resistance exercises) evoke an increased production of high oxidative substances (RONS, both oxygen and nitrogen containing radicals), depending mainly on the intensity of the muscle actions.

The combination of anaerobic and aerobic energy systems as observed for Judoka athletes provides more pathways for free-radical generation with respect to single exercise training.

The aim in this study was to investigate the use of non-invasive simple battery for the assessment and monitor of oxidative stress of Judo athletes for a better characterization of the oxidative stress response to both anaerobic and aerobic incremental exercise typical of Judo discipline.

Introduction

The majority of studies in the last two decades have reported an increase in oxidative stress following a period of acute exercise, both aerobic and anaerobic, and this abundance of evidence seems to support the concept of exercise-induced oxidative stress regardless of the mode and volume of exercise (Fisher-Wellman et al., 2009). High levels of free radicals may cause biological damage when there is an overproduction of RONS and a deficiency in antioxidant defense system.

If the stress is too high, structural modifications, damage of lipids, proteins and nucleic acids may occur as a result of high intensity exercise of moderate to long duration. Biomarkers of this oxidative effect can be recognized for a few days.

On the other hand, moderate intensity (less than 50-60% of the aerobic capacity or of the maximal muscle tension) exercises has been shown to exert low stress without oxidative damage impulse in the normally nourished subjects. Regular training and "normal" diet results a proper defense against excess oxidative stress.

Although high levels of free radicals may damage cellular components, low-to-moderate levels of oxidants play multiple regulatory roles as mentioned above.

Judo is an acyclic sport whose performance is determined by a combination of different physical abilities, and an intermittent sport with high-intensity actions.

Considering that a match can last a few seconds or up to eight minutes (5 min of match + 3 min of golden score), the typical time structure in the match is 20-30 s of activity with a 5-10 s interval, during which the athletes spend about 51% of the time trying to perform a good grip, resulting in a high physiological demand on the upper body (Franchini et al. 2007). Time-motion analysis (Sterkowicz et al., 2000) has shown that judo contests are characterized by maximal efforts (100% VO₂) of 10-15s interspaced with recovery periods of sub-maximal strength that involve pushing, pulling and lifting actions. Therefore, both the aerobic and the anaerobic metabolic systems are alternatively stimulated.

Experimental design

Subjects

This preliminary study recruited Caucasian low level trained (recreational) athletes' members of one regional Friuli Venezia Giulia Judo team (ASD DOJO TRIESTE) as indicated in the following table.

Subject	Age (sd)	Body Mass Index
Male (n = 5)	49 (± 3)	26.1
Female (n = 1)	41	24.4
Young male (n = 2)	19 (± 0)	20.7

The high level athletes, indeed, are members of two North East teams and the data are represented in the two following tables:

Team 2	Age (sd)	Years of Judo training	Body Mass Index
Male (n = 8)	18 (± 2)	9 (± 3)	23.5 (±3.3)
Female (n = 4)	17 (± 2)	7 (± 4)	21.9 (±4.6)

Team 1	Age (sd)	Years of Judo training	Body Mass Index
Male (n = 4)	17 (± 1.5)	9 (± 1.5)	22.9 (± 2.4)
Female (n = 1)	20	15	22.5

Methods

1. Creatinine concentration was estimated from each urine sample by the Jaffé (1886) alkaline picrate method.

2. FRAP assay assess the *in vitro* total antioxidant capacity using the method of Benzie and Strain (1996) which measures reducing ability of biological fluids correlate to antioxidant power.

3. AGEs (Advanced Glycation End-products) are formed as result of free radical reactions with proteins and were determined using the fluorescence method by Campos and coworkers (2012) on urine samples at excitation and emission wavelength of 370nm and 440 nm, respectively.

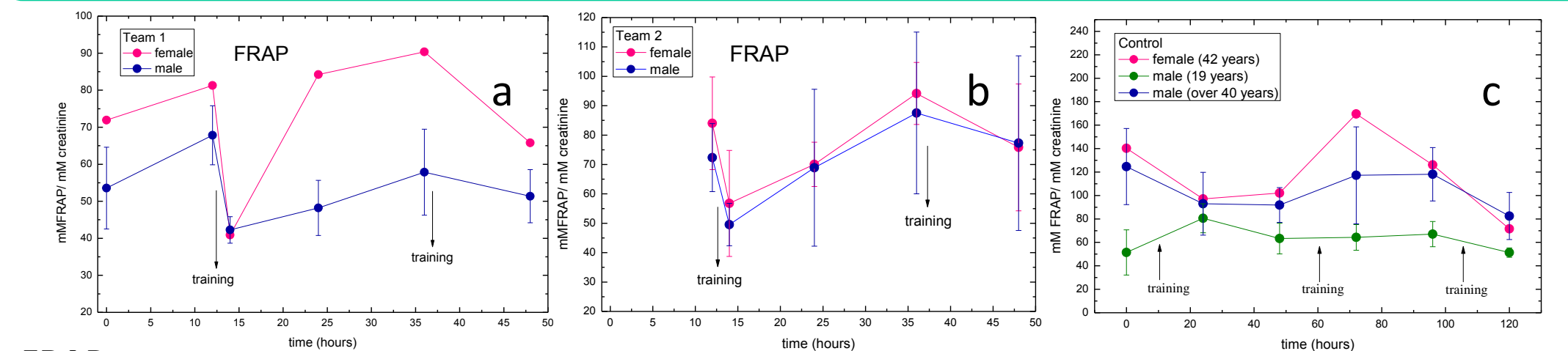
4. MDA (Malondialdehyde) was determined using the TBARS method (Thiobarbituric Acid Reactive Substances) based on the reaction of by-products of lipid peroxidation (LPO), as the malondialdehyde, with thiobarbituric acid (TBA) to yield a red compound.

Urine sample derivatization was carried out following the method described by Agarwal and coworkers (2002) and the MDA product was determined on a Dionex HPLC apparatus equipped with a UV-visible detector AD25 and with a fluorescence detector GF2000. The injection volume was 25 µL and the column a LiChrospher RP-18 (5 µm, 250 mm, Agilent).

References

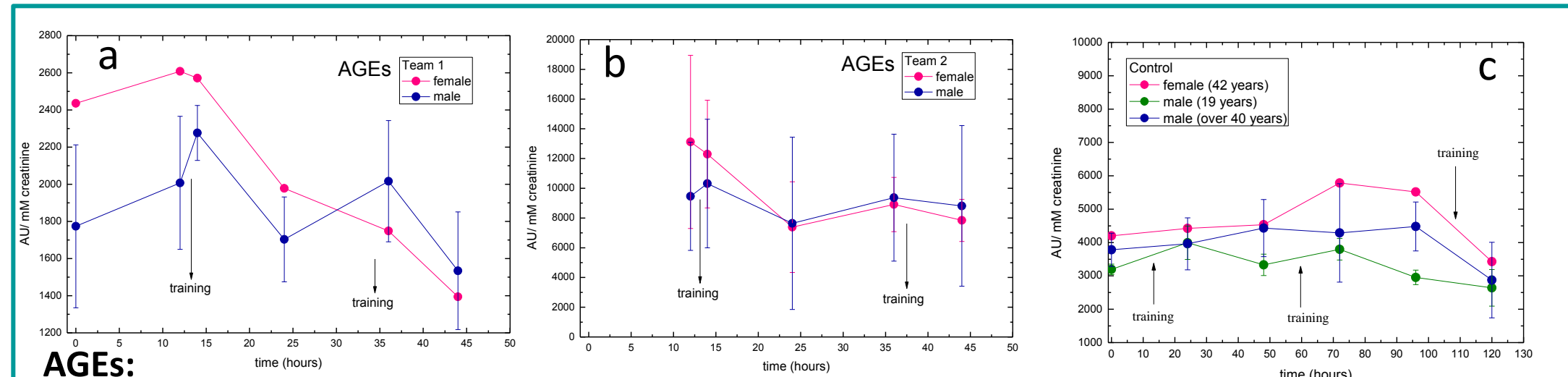
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Results and Discussion



FRAP:

Urinary *in vitro* antioxidant capacity was evaluated by FRAP method. Values for high level (HL) athletes are overall lower (Figure a and b) with respect to those for low level (LL) athletes (control samples, Figure c) in the whole period of investigation. Noteworthy after training sessions the FRAP value decreases rapidly and in few hours returned to the initial value. These effect may be ascribed to a well adapted metabolism to high level training exercises which is able to produce minor amounts of uric acid as antioxidant agent. Some differences have been observed between male and female HL athletes where the female FRAP values are higher than those of male athletes over the whole range of time considered.



AGEs:

In Figure a and b, AGEs levels are very similar in trend as a response to the high level training sessions among HL athletes, but Team 2 shows markedly higher values than those relative to Team 1 athletes due to the more intensive training protocols used by Team 2 in preparation to the Italian Championship 2014.

In comparison to the LL athletes (Figure c) a higher variability of AGEs was observed for HL athletes suggesting a strong dependence of end-products by the intensity and volume of training session.

MDA:

LL athletes (recreational Judo course, 4 hours/week) have been monitored in order to assess the week variability of stress oxidative biomarkers and set background values (baseline) for future uses taking them into account as control samples.

In Figure a, the MDA concentration in urine samples of three typologies of athletes before and after 12 hours of a moderate training session are presented. MDA levels were significantly different both as starting values and after 12 hours post-training recovery with the major variation for young athletes (11 years in average) in comparison to a male and female adult athletes. This is mainly due to the still low degree of adaptation of young organisms to respond to oxidative stress. In other words, there is not yet pronounced the upregulation in the body's antioxidant defense system as derived from an adaptation to regular exercise training.

We found significantly lower increase of levels of MDA in women than in men mainly due to antioxidant properties of the female sex hormone estrogen.

Figure b shows MDA values of HL athletes with respect to those of LL athletes during a week of training. For HL athletes after the training session there is sharp increase of MDA concentration while the LL athletes shows a marked decrease followed by an sharp increase after 12 hours as a consequence of a MDA accumulation in the urine which is cleared after several hours. On the contrary, the antioxidant metabolic system of HL athletes are able to clear urine MDA level in few hours due to the efficient adaptation and a good fitness status.

Conclusions

1. An increased antioxidant capacity may not necessarily be a desirable condition if it reflects a response to increased oxidative stress. Similarly, a decrease of antioxidant capacity may not necessarily be an undesirable condition if the measurement reflects decreased production of reactive species;
2. In a biochemical study it is recommended to use a battery of tests, including different assays for total antioxidant capacity and different assays for oxidative stress markers;
3. Mixed training discipline like Judo may provoke an increase of oxidative stress if the inefficiency of the antioxidant system in response to the supplementary production of free-radicals during exercise is present. Nevertheless, if the training programs are sufficiently long and intense to trigger a consequent adaptive response of the antioxidant system, a decrease of oxidative stress occurs. In our study, in fact, LL athletes show a longer recovery time for RONS clearance;
4. Based on this finding, it may be observed that regular Judo exercise can serve as a stimulus for the enhancement in endogenous antioxidant defense of judoka as a result of their regular and strenuous exercise.

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