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Oxidative status alteration during aerobic-dominant mixed and anaerobic-dominant mixed effort in judokas

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ABSTRACT

This study aimed to depict the oxidative status variation in judokas during aerobic-dominant mixed effort (AeDME) and anaerobic-dominant mixed effort (AnDME). It is to be expected that the sporting commitment of Judo is a stimulus of oxidative stress leading to the recruitment of antioxidant responses. Blood samples were collected from 17 athletes at rest, immediately after a training session (AeDME) and after a 5-min bout (AnDME). AeDME and AnDME caused significant increases in malondialdehyde (MDA) ($p < 0.01$ and $p < 0.001$ respectively) and glutathione (GSH) ($p = 0.018$ and $p < 0.001$ respectively). Blood thiol concentrations decreased following AeDME and AnDME ($p < 0.001$) whilst catalase decreased significantly after AnDME ($p = 0.026$) only. Uric acid increased significantly after AnDME than after AeDME ($p = 0.047$) while, conversely, total bilirubin was higher after AnDME than after AeDME ($p = 0.02$). We may ultimately summarize that AeDME and AnDME caused oxidative stress, higher in AnDME, and some antioxidant response slightly higher in AnDME compared to AeDME. In sports, monitoring of oxidative stress status is recommended as part of the training regimen.

1. Introduction

Although the positive health effects of sports have been widely demonstrated, exercise can cause intense physical stress [1]. Judo is a sport that alters the oxidative state and in which the metabolism can vary greatly between training sessions and competitions [2–4]. Oxidative cell damage can result from the excessive production of reactive oxygen species (ROS) [1]. Oxidative stress is characterized

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by an increase in the production of free radicals that exceeds the physiological capacity of the body to eliminate and/or inactivate them via the antioxidant defense mechanism [5]. When the production of ROS exceeds the defensive capacity of the organism, whether enzymatic [antioxidant superoxide dismutase (SOD), catalase and glutathione peroxidase] or non-enzymatic (vitamins A, C, E and uric acid), these ROS oxidize the constituents of cells [6]. The evaluation of oxidative stress is determined by measuring free radicals, ROS and natural antioxidants [7]. The literature reports very variable and sometimes contradictory results; thus, it seems that no method is favored to certify the measurement of oxidative stress and the simultaneous use of several methods is privileged [8].

In the literature, most of the available studies address sports with an aerobic metabolic requirement [1,9]. However, investigations carried out on anaerobic sports to determine oxidative status are rather limited and report widely discrepant results [1]. Some of these revealed increased oxidation of glutathione following anaerobic exercise [10], while others found an increase in lipid hydroperoxides only without an increase in 3,4-Methylenedioxyamphetamine (MDA) after both exhaustive aerobic and isometric exercise [11], which requires an appropriate election of biomarkers.

El Abed et al. [12], found an increase in SOD without change in plasma glutathione peroxidase following anaerobic exercise in judokas. Other studies have shown that a single anaerobic exercise causes a significant change in catalase, SOD and glutathione peroxidase [12].

Studies that compared the effect of aerobic and anaerobic exercise on lipid peroxidation have found similar responses or a higher increase after anaerobic exercise [11,13]. Ammar et al. [10], have compared aerobic exercise (30 min wingate test) and anaerobic exercise (30s wingate test), and have found a significant increase in MDA following both exercises at t 0 min. After 5 min the MDA following aerobic exercise became significantly higher than after anaerobic exercise. However, they have found a significant difference between the two exercises at t 0 min for SOD, glutathione peroxidase and glutathione reductase [10].

For mixed exercise, the research is quite limited compared to the number of studies done on aerobic and anaerobic exercise. Indeed, Ammar et al. [10], have shown that mixed exercise did not cause a significant increase in MDA or glutathione reductase. However, they have found a significant increase in SOD and glutathione peroxidase [10]. In fact, most often, works on resistance exercises have addressed a rather small amount of muscle [14]. Additionally, most works that have assessed oxidative status in mixed sports have used anaerobic and/or aerobic exercise [15]. Thus, to our knowledge, the use of mixed metabolic demand exercise with variation in metabolism dominance from one exercise to another to measure the change in oxidative status has not yet been investigated.

However, in judo combat, judokas uses practically all of their skeletal muscles to perform a defensive or attacking gesture. In some situations, isometric, eccentric and/or concentric muscle contractions are performed. Indeed, judo is a resistance effort with a mixed metabolic requirement that calls upon the aerobic and anaerobic metabolisms with the dominance of one or the other, depending on the requirements of the situation. In addition, the metabolic demand during the practice of judo varies remarkably. Judo training is based on intermittent resistance exercise with mixed metabolic demand, generally with the dominance of aerobic metabolism. However, during combat, anaerobic metabolism is strongly predominant [16].

Oxidative and antioxidant response in sports with a difference in metabolic demands between training and competition lacks investigation. Our goal was to depict the oxidative status variation during a mixed effort with aerobic dominance AeDME (weekly judo training session) and during a mixed effort with anaerobic dominance AnDME (judo bout). The second objective was to compare the variation in oxidative status between the two types of effort (AeDME and AnDME).

2. Materials and methods

2.1. Study design

To depict the oxidative and anti-oxidative responses following a mixed exercise with aerobic dominance AeDME (weekly training session) and one with anaerobic distinction, AnDME (judo bout), a pretest-posttest study was conducted on Tunisian judokas. In addition, we compared the variation in oxidative status between the two types of effort. The 3-step investigation was performed with a senior technician from the biochemical analysis laboratory and an anesthesiologist.

The blood samples were taken at three time points under similar conditions in the club's training room after 7:00 p.m. (Fig. 1).

Samples were taken from all athletes at nearly the same time to reduce the effect of diurnal variation. The first blood sample was taken at rest. The second was taken immediately after a weekly 90-min training session, under usual training conditions. Then we asked the judokas to stop all training for 3 days. On the fourth day, each judoka had a 5-min bout in which all competition rules were observed except for the bout to be stopped after ippon. Each judoka had to reach 5 min of the bout and the winner is the athlete who collects the most points. The third blood sample was taken immediately after the encounter (Fig. 1).

Fig. 1. Experimental protocol.

2.2. Participants

Seventeen judokas belonging to a local Tunisian club participated in the study. Only judokas who continuously trained regularly for at least 6 months were included in this study. The training session consisted of three phases: 1. warm-up, 2. activities specifically related to judo and 2. cool-down. The warm-up consisted of running in all directions (warming up the upper and lower body), falls in all directions, gymnastic movements (forward and backward rolls, cartwheels …); the judo's activities are mainly dedicated to dynamic components (learning techniques, recovery …) than to static work (muscle strengthening, mini-matches …), with a predominance of aerobic work. These were made up of an orderly sequence of gripping techniques, movement in all directions - off-balancing, passive, semi-active techniques, and the combination of several techniques interspersed with stretching and recovery periods (Uchi Komi, Nage Komi, Yaku Soku Geiko, …, mini-application match). The cool-down consisted mainly of stretching. Written consent of all the judokas and the authorization of the Research Ethics Committee of the Habib Bourguiba University Hospital Center in Sfaxhad been previously obtained (Committee for the Protection of Persons in the South (CPPSUD); reference: CPP SUD N°0070/2018). All anthropometric data and physical examinations of the judokas were recorded at the start of the protocol (Table 1). A clinical examination was carried out at the cardiology department of the Hedi Chaker Hospital in Sfax. Weight was determined directly using a scale. Height was measured using a measuring rod. Heart rate was read from an electrocardiogram at rest.

Judokas did not take any medication or supplements for at least a month before this study and during the study.

2.3. Malondialdehyde (MDA) assay

MDA is one of the end products formed during the free radical-mediated decomposition of polyunsaturated fatty acids (PUFAs). The assay was performed according to the method of Ohkawa et al., [17]. The assay is based on the formation of a colored pigment absorbing at 530 nm between MDA and thiobarbituric acid (TBA) in an acidic and hot medium (100 °C). Put 500 μl of plasma and 500 μl of phosphate buffer for the reagent blank. Add 2.5 ml of TCA (10 %) for each sample and the reagent blank. Incubate 15 min at 95 °C in the water bath (Note: Tubes should be well sealed with parafilm to inhibit vaporization). Centrifuge at 3000 rpm for 10 min. Remove 1 ml of the supernatant. Add 0.5 ml of TBA solution (0.67 %). Incubate 15 min at 95 °C in the water bath (Note: the mixture may turn pink if a pro-oxidative status is present in the sample). Place samples on ice to stop the reaction. Read with a spectrophotometer at 532 nm. Finally, the concentration ([MDA] (nmol/ml)) is calculated using a calibration curve (MDA/OD).

2.4. Glutathione GSH assay

The glutathione assay was carried out according to the method of Weckbecker and Cory [18]. The principle of this assay is based on the measurement of the optical absorbance of 2-nitro-5-mercapturic acid. The latter results from the reduction of 5,5′-dithiobis-2-nitrobenzoic acid (Ellman's reagent, DTNB) by the (-SH) form of glutathione. Deproteinization of the homogenate was essential in order to keep only the specific thiol form of glutathione. The calculation of GSH concentrations was done according to the following formula:

$$[GSH]\ (M\ GSH/mg\ protein) = OD * 0.1 * 0.1525 * 10/12.75 * 0.08 * 0.05 * [Proteins]$$

With:

- D.O., Optical density.
- 0.1, Total volume of the solutions used in the deproteinization (0.08 mL homogenate + 0.02 mL of salicylic acid).
- 0.1525, Total volume of the solutions used in the GSH assay at the supernatant level (0.05 mL supernatant + 0.1 mL Tris +0.0025 mL DTNB).
- 12.75, Absorbance coefficient of the –SH group at 412 nm.
- 0.08, Volume of the homogenate.
- 0.05, Volume of the supernatant

Table 1
Clinical features of judokas.

Characteristics	Mean ± S.D.
Age (year)	18.31 ± 2.02
Height (cm)	174.06 ± 7.03
Weight (kg)	68.62 ± 16.88
HR at rest (beat.min-1)	65.19 ± 11.61
Body mass index (kg/m^2)	22.37 ± 3.67
Fat mass index (%)	14.63 ± 4.77
Body surface (m^2)	1.82 ±. 25

S.D., standard deviation; HR, heart rate.

2.5. Conjugated dienes assay

The primary products of the lipid oxidation containing conjugated double bonds were quantified by UV spectrometry [19]. The lipids were extracted and treated with an organic solvent. A volume of 25 μl of plasma +3 ml chloroform/methanol (2v/1v) (without shaking the tube) was poured into a 15 ml conical tube and centrifuged for 5 min at 3000 rpm. For the vaporization of solvents, 2 ml of supernatant was poured into a glass tube and put in the oven overnight at 45 °C until an extract was formed on the wall of the tube. The extract was dissolved in 1 ml of methanol using the vortex and OD was read at 233 nm.

2.6. Uric acid and total bilirubin assays

Uric acid and total bilirubin, which are considered powerful non-enzymatic antioxidants, were assayed as previously reported [20], with the Cobas 501 Roche automaton (Hoffmann-La Roche SA, Basilea, Switzerland).

2.7. Thiol forms measurement

The serum concentration of sulfhydryl (SH) groups was measured by the method originally described by Elmman [21] and modified by Hu [22]. In this method, the thiols interact with 5,5′-dithiobis- (2-nitrobenzoic acid) (DTNB) acid, forming a highly colored anion with a maximum peak at 412 nm.

2.8. Catalase (CAT) activity

Catalase activity (CAT) was measured at 240 nm using a UV visible spectrophotometer (JENWAY 6105, Labexchange - Die Laborgerätebörse GmbH, Burladingen, Germany) by the change in optical density following the hydrolysis of hydrogen peroxide (H_2O_2) by reacting in 100 mM phosphate buffer for 1 min at pH 7.4 a 200-μl volume of H_2O_2 (13 mM) on 20 μl of plasma, at an incubation temperature of 25 °C.

2.9. Statistical analysis

Statistical data were obtained using SPSS version 23 software (IBM, Armonk, New York, USA). Results are presented as mean ± standard deviation (SD). Normality was tested by the Shapiro-Wilk test in a reduced sample for all biochemical values measured. In the absence of normality, a logarithmic transformation was applied to verify this condition. Then, the means of the measured parameter values were compared using the bidirectional ANOVA test. A value of $p < 0.05$ was considered significant. Tukey post hoc test was used to compare parameters in pairs.

3. Results

3.1. Lipid peroxidation

Analysis of oxidized lipid derivatives showed a significant difference in malondialdehyde (MDA) (Fig. 2, $p < 0.001$) without significant changes in the conjugated diene ($p = 0.195$), among the blood samples taken at rest, after the mixed-dominant effort aerobic AeDME (weekly judo training) or mixed effort anaerobic dominance AnDME (judo match) (Fig. 2).

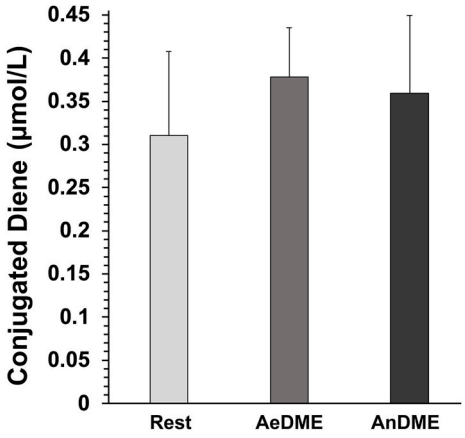

Fig. 2. Blood conjugated diene concentrations (mean ± SD) measured in the three conditions at rest, after aerobic-dominant mixed exercise (AeDME), and after anaerobic-dominant mixed exercise (AnDME). $p = 0.195$, by ANOVA. MDA concentration significantly increased after AeDME and AnDME compared to rest ($p < 0.001$; $p < 0.01$ respectively), with higher concentrations after AnDME ($p < 0.001$) (Fig. 3).

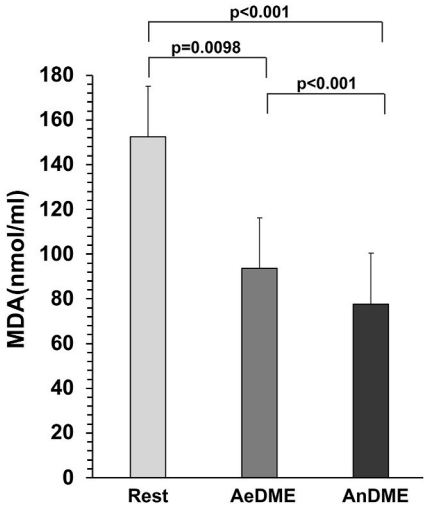

Fig. 3. Blood malondialdehyde (MDA) concentrations (mean ± SD) measured at rest, after aerobic-dominant mixed exercise (AeDME) and anaerobic-dominant mixed exercise (AnDME). $p < 0.001$, by ANOVA; the p values indicated in the figure were obtained by post hoc test comparing parameters in pairs.

3.2. Enzymatic antioxidants

The results showed a significant effect of exercise on catalase values ($p < 0.001$). Notably, post hoc analysis for pairwise sample comparison revealed a significant decrease in catalase after AnDME relative to rest ($p = 0.027$) but not after AeDME ($p = 0.092$) (Fig. 4).

3.3. Non-enzymatic antioxidants

The results showed a significant increase in blood glutathione (GSH) concentrations following the two efforts AeDME and AnDME, compared to the rest (Fig. 5).

Instead, the blood thiol concentrations were significantly decreased following AeDME and AnDME compared to the rest condition ($p < 0.001$, by ANOVA) (Fig. 6).

Uric acid concentration increased significantly after AnDME ($p = 0.0012$), but not after AeDME ($p = 0.366$), with respect to the rest condition (Fig. 7).

Finally, total bilirubin levels increased significantly after AeDME ($p = 0.026$) but not after AnDME ($p = 0.993$) (Fig. 8).

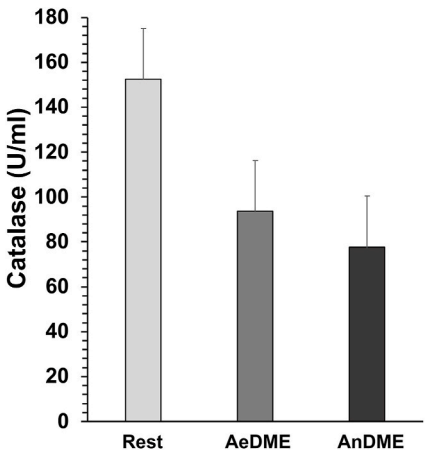

Fig. 4. Blood catalase concentrations (mean ± SD) measured at rest, after aerobic-dominant mixed exercise (AeDME), and after anaerobic-dominant mixed exercise (AnDME). $p = 0.024$, by ANOVA; the p values indicated in the figure were obtained by post hoc test comparing parameters in pairs.

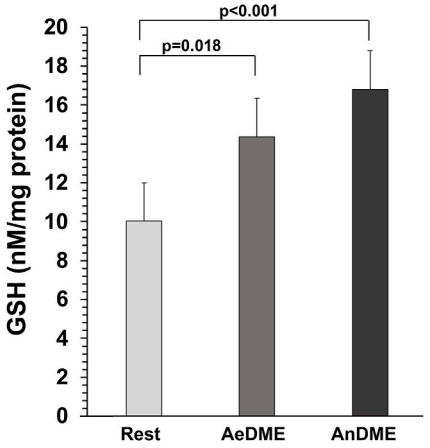

Fig. 5. Blood glutathione (GSH) concentrations (mean ± SD) measured in the three conditions at rest, after aerobic-dominant mixed exercise (AeDME), and after anaerobic-dominant mixed exercise (AnDME). $p < 0.001$, by ANOVA; the p values indicated in the figure were obtained by post hoc test comparing parameters in pairs.

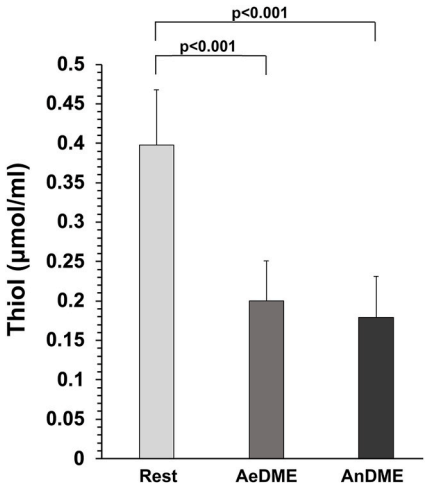

Fig. 6. Blood thiol concentrations (mean ± SD) measured in the three conditions at rest, after aerobic-dominant mixed exercise (AeDME), and after anaerobic-dominant mixed exercise (AnDME). $p < 0.001$, by ANOVA; the p values indicated in the figure were obtained by post hoc test comparing parameters in pairs.

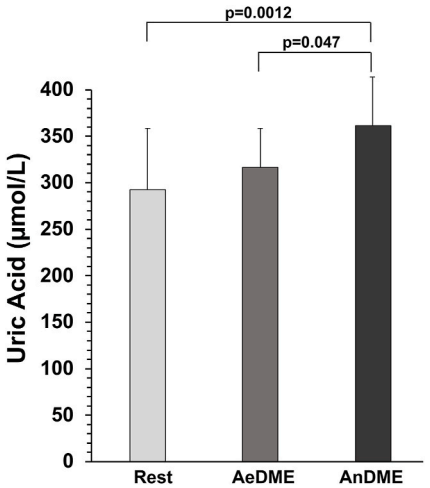

Fig. 7. Blood uric acid concentrations (mean ± SD) measured in the three conditions at rest, after aerobic-dominant mixed exercise (AeDME), and after anaerobic-dominant mixed exercise (AnDME). $p = 0.0016$, by ANOVA; the p values indicated in the figure were obtained by post hoc test comparing parameters in pairs.

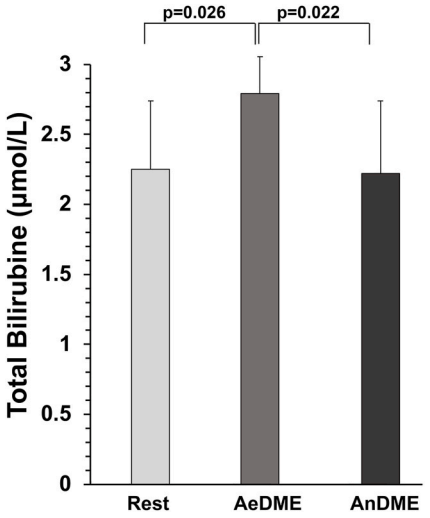

Fig. 8. Blood total bilirubine concentrations (mean ± SD) measured in the three conditions at rest, after aerobic-dominant mixed exercise (AeDME), and after anaerobic-dominant mixed exercise (AnDME). p = 0.011, by ANOVA; the p values indicated in the figure were obtained by post hoc test comparing parameters in pairs.

4. Discussion

Regular exercise increases the production of ROS acting as messenger in signal transduction, thus regulating various cellular functions [23]. However, the imbalance between ROS and antioxidants leads to a disruption of redox control and molecular damage [24]. Oxidative stress induced by exercise is due to increased oxygen consumption (VO2) which causes an increase in oxygen flow of 100 times greater than the resting value in contracting skeletal muscles [25]. The sharp increase in oxygen flux is followed by ROS overproduction [23] and the extent of oxidation is proportional to the modality, intensity, and duration of exercise [26]. On the other hand, stress due to exercise can stimulate beneficial chronic adaptations, such as the enhancement of the antioxidant system [27]. Therefore, it is important to determine the impact of exercise on antioxidant status and oxidative stress in athletes. Sports such as cycling, running, and swimming are the targets of most studies concerning exercise and oxidative stress, whilst such knowledge is lacking in judo. Nevertheless, a previous study reported that judo athletes have higher endogenous antioxidant protection at rest than sedentary subjects and their oxidative stress biomarkers increased in response to acute exercise [28]. Lipid and DNA damage and fatigue induced by such oxidative stress after combat can be decreased by the antioxidant properties of grape juice [29]. The metabolism involved in judo varies greatly between training and combat, thus, we here compared oxidative and antioxidative response during two resistance exercises with mixed metabolic demands: aerobic-dominant mixed effort (AeDME) and anaerobic-dominant mixed effort (AnDME). Both AeDME and AnDME resulted in lipid peroxidation and increased enzymatic and non-enzymatic antioxidant responses; however, combat effort caused higher lipid peroxidation than training. AeDME and AnDME were found to cause lipid peroxidation inferred from an increase in malondialdehyde MDA without significant changes in the conjugated diene. Free radicals (FR) are in fact at the origin of chemical transformations which lead to serious alterations of the lipid structure [30]. MDA is widely used to determine lipid peroxidation [10,28], and AeDME and AnDME caused its increase of about ≈2 times and ≈3 times, respectively. The intensity of both exercises can increase free radicals, which in turn causes oxidative degradation of double bonds of unsaturated fatty acids [30]. An increase in MDA was also reported in judokas [28], after aerobic exercise and in players after rugby or soccer matches [31,32], suggesting that exercise intensity may be a determining factor in free radical production. Accordingly, some studies reported that plasma MDA concentration was significantly higher in the exercise with higher intensity compared to the lower intensity exercise protocol [33]. Exercise has been shown to increase conjugated dienes [34]. However, it has been demonstrated that plasma-conjugated dienes increased after high-intensity interval exercise performed by swimmers and runners, but not after an endurance exercise in marathon runners [31,34]. A significant correlation between stress hormone and lipid oxidation products i.e. conjugated dienes and MDA after exercise was also observed in some studies; it has been suggested that the autoxidation of catecholamines, which generates a superoxide anion radical, could result in the oxidation of lipids [35]. Thus, during short-term resistance exercise, norepinephrine concentration moderately increases, whereas during intense resistance exercise, a more elevated concentration could contribute to oxidative stress. However, after short-term resistance exercise, there are other possible sources of free radicals that cause lipid oxidation [36].

In the present study, we found that conjugated dienes do not increase in response to mixed exercise. This may be due to the type of exercise, or to the increase of plasma antioxidants in response to the oxidative stress of exercise, which could be efficient for inhibiting lipid oxidation. Indeed, an increase in ROS production after exercise is accompanied by an increase in antioxidant responses [10], an occurrence that we also observed in this study regarding GSH, uric acid, and total bilirubin. It is noteworthy to observe that, in the present study, the activity of the catalase enzyme did not increase either after AeDME or AnDME. Rather, a decrease in catalase activity

was measured, in agreement with what was observed in players after basketball or handball matchs [37].

For any exercise to provide health benefits, there should be an optimal level of ROS produced, which can induce favorable adaptations [27], including increased activity of antioxidant enzymes [1]. However, too much ROS may impair antioxidant defense capabilities leading to substantial cellular damage [38]. But, although many studies show an increase in catalase activity [12,13,39], it has no apparent function in serum, being an intracellular enzyme. Therefore, its increased activity after a football match [39] probably indicates increased damage to erythrocyte membranes, which results in its increased leakage into the circulation. To summarize, the consensus is that catalase activity is not increased following training [40]. Many studies show a significant increase in GSH, especially in endurance athletes (cyclists or runners) [41]. We found that plasmatic GSH concentration increase following AeDME and AnDME. GSH acts as a detoxifying agent by removing free radicals and repairing and protecting mitochondrial DNA also helping to regenerate antioxidants vitamin C and vitamin E. High GSH levels increase strength and endurance, shift metabolism from fat production to muscle development, decrease muscle damage, and reduce recovery time [42]. On the other hand, it has also been previously reported that acute aerobic exercise causes a decrease in GSH levels due in part to the inactivation of free radicals [43]. Marin et al. [37] found a decrease in GSH following a handball match; similarly, Zembron-Lacny et al. [44] showed that GSH levels also decreased at the end of the playoff period in professional players. Judokas exercise multiple types of muscle contraction (isometric, eccentric, static, dynamic) and face alteration between ischemia-reperfusion, aerobic-anaerobic pathway and acidosis; probably, the characteristics of mixed effort could induce an increase in GSH concentrations.

The thiols represent a versatile and robust defense system against perturbations caused by oxidative stress. Changes in the thiol status induce the expression of the transcription factors nuclear factor-κB and activator protein-1, which in turn increase the levels cytokines IL-6 and TNF-α, which, in turn, play a role in muscle regeneration and tolerance of ROS-induced muscle damage [44]. However, the effects of exercise on thiol concentrations are contradictory [45].

Most studies focused on the effect of a single high-intensity training regimen or on the use of nutritional supplements, whilst few are the studies on aerobic exercise and its effects on thiols [43,46]. Thus, to our knowledge, this is the first study showing a decrement in thiol level following exercises with differences in metabolic requirements; consistently, training for several weeks reduces thiols in specific tissues [46].

Uric acids are antioxidant generated during the metabolism of purine nucleotides and accounts nearly for 66 % of the total oxygen scavenging activity in the blood serum [43,44], requiring the participation of thiols in its cycle for complete scavenging of such species [47]. In this study, although no significant changes in uric acid concentration were found after AeDM, a significant increase after AnDME was measured, in agreement with what was reported after a handball match and during recovery [31]. On the contrary, Meyer and Meister [48] showed that uric acid levels decreased over an entire season in male German footballers. Uric acid levels increase after intense exercise involving fast-twitch muscle fibers because it increases the antioxidant capacity of whey and reduces oxidative stress during periods of intense exercise. Although high resting uric acid levels play only a short-term role in metabolic responses to exercise, they have been linked to the risk of tendon injuries, poor strength, and disease [49]. Thus, different responses to the two types of exercises could depend on the intensity of the effort and on the ischemia-reperfusion situations; hewer further research on the mechanism is needed. On the other hand, AnDME caused not only higher uric acid levels but also higher MDA and bilirubin levels.

As far as bilirubin is concerned, in biological systems it shows potent antioxidant properties, especially against peroxyl radicals [50]. An increase in total bilirubin was found after AeDME without a significant change after AnDME; therefore, sports practice mixed with aerobic predominance is able to activate bilirubin as an antioxidant response, as previously observed in aerobic training in men. Conversely, participation in aerobic and strength training was positively correlated with plasma bilirubin levels among women [51]. In general, there are few studies regarding bilirubin, and only a limited number of them have demonstrated that bilirubin can be increased with both acute and regular (long-term) resistance exercise [52]. In one study, in which participants were placed in three groups with different exercise volumes for 12 weeks, it was observed that high doses of exercise training are necessary to significantly increase bilirubin levels in previously sedentary postmenopausal women [53]. Exercise that achieves or exceeds the recommended 150 min of moderate to vigorous physical activity per week appears necessary to observe beneficial physiological increases in plasma bilirubin [54]. This is also supported by other studies in which bilirubin increases after 3 months of soccer or rugby training in competitive athletes [55] compared to the general population [56]. There is also evidence that an acute bout of exercise upregulates plasma bilirubin [57]. A maximal exercise test also increased plasma bilirubin among football players [31] and was increased after an ultra-marathon among trained runners [58].

This study has some limitations that should be noted. The first limitation comes from the origin of the sample. The fact that all athletes participated in the same type of training may not encourages us not to generalize the results to athletes representing other workouts and sports. Consistently, the results of the present study cannot be extrapolated to the general population, taking into account that the subjects were only athletes. We believe our research can serve as a basis for further studies enrolling a wider range of participants in order to confirm these findings. In addition, during an official competition, the stress exerted on judokas could be higher than in an unofficial fight (AnDME), as measured in this study. Furthermore, the measurement of other parameters could provide useful further information; for example, it can be assayed glutathione peroxidase in order to evaluate the elimination of hydrogen peroxide and lipid peroxides, or the level of oxidized glutathione (GSSG) as well as the GSH/GSSG ratio to have a more complete picture of the redox state in athletes. Similarly, the assay of plasma concentrations of other nonenzymatic antioxidants (vitamin A, vitamin C, vitamin E) could provide additional information on the effect of exercise modality on antioxidant status. Furthermore, a single blood sample only at the end of exercise may also be a limitation of this study. Therefore, several blood draws are recommended during recovery, which may vary the kinetics of the dosed parameters. Another further limitation of this study is the lack of data on the workload of the training session, or the combat test obtained with a heart rate monitor or with an accelerometer [59].

5. Conclusions

For any exercise to deliver the expected health benefit there should be an optimal level of produced ROS that may induce favorable adaptations, including increased expression of antioxidant enzymes. However, let's not overlook the fact that increasing oxidative stress above the optimal level may compromise health and performance. Both efforts (AeDME and AnDME) caused an increase in free radicals, by increasing the antioxidant response. Nevertheless, anaerobic-dominant mixed exercise **(**AnDME) caused higher lipid peroxidation than AeDME. This confirms the results found in a previous study [12] and suggests that the training practiced by judokas during judo bouts does not meet the beneficial metabolic and physiological requirements. It seems that it is time to think about changing judo training methods to better prepare the judoka for combat.

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Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Research Ethics Committee of the Habib Bourguiba University Hospital Center in Sfaxhad been previously obtained (Committee for the Protection of Persons in the South (CPPSUD); reference: CPP SUD N°0070/2018).

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Data availability statement

The data associated with our study have not been deposited in a publicly available repository. Data will be made available on request.

CRediT authorship contribution statement

Imed Gandouzi: Writing – original draft, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Soufien Fekih:** Investigation. **Okba Selmi:** Investigation, Data curation. **Nasr Chalghaf:** Methodology. **Mouna Turki:** Supervision, Resources, Investigation, Conceptualization. **Fatma Ayedi:** Methodology. **Noomen Guelmami:** Methodology. **Fairouz Azaiez:** Resources. **Nizar Souissi:** Writing – review & editing, Validation, Supervision, Resources, Conceptualization. **Santo Marsigliante:** Writing – review & editing, Supervision. **Antonella Muscella:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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