

Analysis of acute responses from cellular injury biomarkers and oxidative stress in Brazilian jiu-jitsu combat

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Abstract:

This study analyzed the acute effect of Brazilian jiu-jitsu (BJJ) combat on muscle injury and oxidative stress. For this, eight highly-trained male athletes (23.7±3.3 yrs.) were analyzed before and after a 10 min bout. Blood Lactate (LAC) was measured as an indicator of combat intensity. Muscle damage markers were measured creatine kinase (CK) and lactate dehydrogenase (LDH). The levels of Total Antioxidant Capacity (CAOT), catalase activity, and protein carbonylation were measured as oxidative stress markers, $p \leq 0.05$. The main results indicated that there was a difference between pre and post values for the concentration of LAC (0.5 ± 0.2 vs. 1.9 ± 0.9 mmol/L; $p = 0.002$) e CK (66.5 ± 48.1 vs. 80.3 ± 53.1 IU/L; $p = 0.027$). There was no difference for the other measures ($p > 0.05$). Catalase pre-BJJ and LDH post-BJJ had a strong and negative correlation ($r = -0.751$; $p = 0.03$). CAOT and protein carbonylation post-BJJ had a positive and strong correlation ($r = 0.806$; $p = 0.02$). In conclusion, Brazilian jiu-jitsu tends to increase cell damage, especially when related to low pre-combat levels of protective enzymes, such as Catalase.

Key-Words: Martial arts, Muscle Damage, Oxidative Stress, Judo, Sports Nutritional Physiological Phenomena, Athletic Performance.

Introduction

In the last decades, the popularity of Brazilian jiu-jitsu (BJJ) has increased sharply, with practitioners and athletes worldwide. This popularity is due to the growth of mixed martial arts, where the fight on the ground is decisive to the outcome (Andreato et al., 2011; Brandt et al., 2021; Dal Bello et al., 2019; Dos Santos et al., 2019; Fernandes et al., 2018; Mickelsson, 2021, Ratamess, 2011). Chronic training generates a cause-effect relationship in this combat sport, thus improving performance or changing some physiological parameter that determines BJJ (VO^2 , for example) due to a specific intensity and volume of training or competition (Øvretveit, 2018; Øvretveit et al., 2019). Intermittent efforts with dynamical actions characterize BJJ. Therefore, methods to avoid injuries are developed in grappling combat sports (Almeida-Junior et al., 2019, Barreto et al., 2019, Barreto et al., 2021, Miarka et al., 2018).

During the fight, the athlete oscillates between moments of high and low intensities. During high-intensity efforts, intramuscular reserves of ATP are predominantly re-synthesized through phosphocreatine and muscle glycogen (Coswig et al., 2018; Del Vecchio et al., 2018). However, the re-synthetization of ATP through muscle glycogen results in the formation of lactate (Robergs, Ghiasvand, and Parker, 2004). Another biochemical result of high intensity is the formation of free radicals. Intense physical activities have the characteristic of increasing the total oxygen consumption by 20 times more, and muscle tissue that also presents an increase in oxygen uptake in the face of these high-intensity moments. These changes in oxygen metabolism produce free radicals, which are molecules that have an unpaired electron in the last layer (Shalaby et al., 2021). One of the ways to generate this free radical occurs when electrons leak through the mitochondria during aerobic respiration, prostanoid metabolism, catecholamine metabolism, and xanthine oxidase that produces the superoxide anion as a product of the degradation of hypoxanthine to xanthine. Other ways of generating free radicals are through phagocytic cells, the rupture of proteins containing iron, and the oxidation of hemoglobin and myoglobin (Martinović et al., 2010). The release of these free radicals during metabolic processes

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culminates in the action of antioxidant defense mechanisms, regulating the intracellular levels of these reactive species and controlling the occurrence of cellular damage (Tryfidou, McClean, Nikolaidis, & Davison, 2020). The imbalance between these oxidizing and antioxidant agents, with a predominance of oxidizing action and consequent damage, originates the process known as oxidative stress due to the high release of free radicals or an inefficient antioxidant system (Sousa et al., 2017; Tryfidou et al., 2020). Such a process results in the oxidation of biomolecules with loss of biological functions and homeostatic imbalance, causing oxidative damage to cellular and tissue structures and can generate damage to proteins and DNA (Sousa et al., 2017; Tryfidou et al., 2020). Since the optimization of sports performance depends directly on the correct distribution of training loads and the recovery provided to athletes (Campos et al., 2020). The ability to monitor markers of cell damage and oxidative stress is an important aspect of periodization's effectiveness and the prevention of negative effects, such as the drop in yield (Kalkhoven et al., 2021; Simmons, Doma, Sinclair, Connor, and Leicht, 2021). To the best of our information, no research has measured the acute effect of a BJJ combat on oxidative stress markers and whether there is a correlation between performance variables and oxidative stress measures in this combat sport. Therefore, the present study aimed: i) to verify the effects of BJJ combat on the cell damage and oxidative stress parameters, and ii) to correlate cell damage and oxidative stress parameters pre vs. post BJJ combat.

Material & methods

Study design

The present experimental study's purpose is to validate the effects of BJJ combat on the cell damage and oxidative stress parameters, associating cell damage and oxidative stress parameters pre vs. post BJJ combat in high-level athletes. The present research was carried out following the legislation in force and with the rules of the local Ethics Committee.

Participants

Eight male professional BJJ athletes were measured (23.7 ± 3.3 years), brown belt, and black belt graduates. As an inclusion parameter, athletes should have ≥ 18 years, at least six years of BJJ practice, training $\geq$ five times/wk, and; showed experience with BJJ events and competitive rules. Before the experiment, participants attended a briefing meeting and signed an informed consent document to measure that they understood the testing procedures and the risks and benefits associated with the study. No interferences were made in participants' training, nutritional, or hydration status. Following the WMA's Declaration of Helsinki, the local Ethics and Research Committee previously approved this research (nº 1,123,515).

Data collection

The athletes had two meetings with the researchers. First, the athletes were informed about the experimental procedures and study aims, and they received the informed consent form. The participants were instructed not to drink alcohol, any tea, energy drinks, antioxidants, or caffeine 24 hours before collection, sleep 6 to 8 hours the night before collection, and not do a strenuous physical activity the day before. The athletes went through a blood sample collection before performing the combat to check their basal state. At the second meet, all athletes formed pairs, with a body mass difference of no more than 5%. This protocol reproduced the competitive BJJ combat. They carried out uninterrupted combat of 10 minutes on a 300 m² mat and a temperature of 20 °C. The combat started at 12:30 pm; The athletes went through the second venous collection immediately.

Blood collection

Blood samples were collected (8ml) before the exercise session and immediately after. The blood was centrifuged at 1500 x g, four °C for 15 minutes, and the plasma was removed and stored in 2 mL Eppendorf and stored at -80 °C for the analysis.

Evaluation of Cell Damage and Oxidative Stress Parameters

Plasma levels of creatine kinase (CK), lactate and lactate dehydrogenase (LDH) were measured using the Bioclin® commercial kit. (CK NAC kinetic UV – K010, LDH kinetic UV – K014, Lactate kinetic – K084 respectively), following the manufacturer's specifications.

The protocol used for the analysis of the Total Antioxidant Capacity (CAOT) was the inhibition of the self-oxidation of 2,2-diphenyl-1-picrilhidrazi (DPPH) (Janaszewska, and Bartosz, 2002). The catalase enzyme activity was measured as described by Aebi (1984) for quantification of carbonylation by the method called the DNPH alkaline method. Hydroperoxides measurement was made through the FOX method (ferrous oxidation in xylenol orange). The FOX method is based on Fe⁺² (ammoniacal ferrous sulfate) to Fe⁺³ by hydroperoxides in an acidic medium.

Statistical analysis

All statistical analyses were conducted using SPSS 20.0 for Windows. The Kolmogorov-Smirnov test (K-S) was accepted, determining the normal data distribution. Data were presented by mean (M) and standard deviation (SD). For the Student t-test, a double tail was used for paired measurements. Furthermore, Pearson correlation coefficient was conducted to verify the relationship between the variables, using the classification: $r = .7 \leq .9$, strong correlation; $r = .5 \leq .7$, moderate correlation; $r = .3 \leq .5$, weak correlation; $r = .0 \leq .3$, non-correlation. The present research used a significance level of $p \leq .05$.

Results

Statistical analysis indicated a significant difference between moments; Lac pre-BJJ had lower levels than Lac post-BJJ ($p=0.002$), as CK comparisons demonstrated a statistical difference, CK pre-BJJ showed lower values than CK post-BJJ ($p=0.027$). Table 1 shows a descriptive analysis of cell damage and oxidative stress parameters pre and post-BJJ combat.

Table 1. Descriptive and comparative analysis pre vs. post-BJJ combat.

Measures	Results	t-calc.	p-value
Lac pre	0.5±0.2		
Lac post*	1.9±0.9	-4.930	0.002
CK pre	66.5±48.1		
CK post*	80.3±53.1	-2.776	0.027
LDH pre	200.4±40.6		
LDH post	238.9±71.2	-1.349	0.219
DPPH pre	82.0±4.0		
DPPH post	83.1±2.1	-0.874	0.411
FOX pre	0.02±0.0005		
FOX post	0.02±0.0004	0.595	0.571
Catalase pre	0.0002±0.00006		
Catalase post	0.0002±0.00003	1.195	0.271
Protein carbonylated pre	15.4±1.9		
Protein carbonylated post	13.4±4.2	1.162	0.283

Note. Lactate (Lac), creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT), 2,2-diphenyl-1-picrilhidrazi (DPPH).

* $p \leq 0.027$ vs. pre-moment.

Table 2 shows correlations between cell damage and oxidative stress parameters. For the Pearson correlation, Catalase pre-BJJ and LDH post-BJJ had a strong negative correlation ($p = 0.03$). DPPH post and Protein carbonylated post-BJJ had a positive and strong correlation ($p = 0.02$).

Table 2. Person correlations between injury and oxidative stress biomarkers.

		Lac pre	Lac post	CK pre	CK post	LDH pre	LDH post	DPPH pre	DPPH post	FOX pre	FOX post	Catalase pre	Catalase post	Prot. Carb pre
Lac	r	0.53												
post	p	0.18												
CK pre	r	-0.56	0.08											
	p	0.15	0.85											
CK post	r	-0.45	0.18	0.966**										
	p	0.26	0.68	0.001										
LDH pre	r	0.34	0.69	0.08	0.05									
	p	0.41	0.06	0.86	0.9									
LDH post	r	-0.04	0.01	0.23	0.26	0.03								
	p	0.92	0.97	0.58	0.53	0.94								
DPPH pre	r	-0.6	-0.6	0.59	0.61	-0.64	0.1							
	p	.012	0.12	0.13	0.11	0.08	0.8							
DPPH post	r	-0.24	0.29	0.63	0.71	-0.06	-0.32	0.44						
	p	0.57	0.49	0.09	0.05	0.89	0.44	0.28						
FOX pre	r	-0.17	0.19	0.28	0.42	-0.2	0.33	0.28	0.56					
	p	0.69	0.65	0.5	0.31	0.64	0.42	0.51	0.15					
FOX post	r	0.32	0.5	0.05	0.22	0.14	0.41	-0.09	0.36	0.853**				
	p	0.43	0.21	0.9	0.6	0.74	0.31	0.83	0.38	0.01				
Catalase pre	r	-0.05	0.29	0.21	0.12	0.47	-0.751*	-0.19	0.5	-0.2	-0.21			
	p	0.9	0.49	0.63	0.78	0.24	0.03	0.65	0.21	0.64	0.61			
Catalase post	r	0.09	-0.16	0.11	0.23	-0.4	-0.26	0.53	0.63	0.51	0.43	0.14		
	p	0.83	0.7	0.79	0.59	0.32	0.54	0.18	0.09	0.2	0.29	0.75		
Prot carb pre	r	0.25	0.46	-0.29	-0.3	-0.03	-0.22	-0.53	0.12	0.22	0.24	0.13	-0.11	
	p	0.55	0.25	0.49	0.47	0.95	0.61	0.17	0.78	0.6	0.56	0.75	0.79	
Prot carb post	r	-0.39	-0.3	0.52	0.59	-0.71	0.4	0.806*	0.42	0.56	0.27	-0.49	0.46	-0.09
	p	0.34	0.46	0.19	0.12	0.05	0.33	0.02	0.3	0.14	0.52	0.22	0.25	0.83

Note. Lactate (Lac), creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT), 2,2-diphenyl-1-picrilhidrazi (DPPH).

Discussion

The present study verified the effects of BJJ combat on the cell damage and oxidative stress parameters, correlating cell damage and oxidative stress parameters pre vs. post BJJ combat. The main results demonstrated significant differences between pre and post moments; the Lac and CK post-values were higher than pre-values. In addition, catalase pre-BJJ and LDH post-BJJ had a strong and negative correlation, while DPPH post-BJJ and Protein carbonylated post-BJJ had a positive and strong correlation. A concentration of lactate post BJJ, with an average difference of 1.36 mmol/ml for the pre-combat, demonstrates greater participation of the glycolytic pathway during the activity. Once this reaction re-oxidizes the NAD⁺ coenzyme, lactate formation occurs so that the glycolytic pathway continues to work efficiently. Despite the scarce literature on the relationship between Brazilian jiu-jitsu and lactate, understand that such a process occurs during a fight due to the intermittent characteristic of sport (Lopes et al., 2020).

The enzymes CK and LDH are considered by some authors as markers of training intensity since acute exercise promotes an increase in these biomarkers (Ide et al., 2019). In this circumstance, Lima et al. (2017) suggest that high CK and LDH values indicate muscle overload, something present in combat sports. Having Catalase as an antioxidant enzyme means that saltation aims to prevent the accumulation of hydrogen peroxide. Such action is of paramount importance since reactive hydrogen peroxide, based on Fenton-Chaben-Wein actions, tends to generate an increase due to oxidative damage in the cells involved (Jakubczyk et al., 2020). This process means that as Catalase acts as an antioxidant, normally, its values are inversely proportional to those of LDH since cell damage, as a result of the generation of free radicals, is a common mechanism for cell damage and has been implicated in the pathogenesis of a series of disorders, including reperfusion injury after ischemia, as well as a variety of metabolic, toxic or hypoxic conditions (Jakubczyk et al., 2020). With this, we can justify the low values of catalase pre-BJJ when compared to the LDH values post-BJJ. Although the literature relating the variables during the combat of BJJ is scarce, it is possible to conjecture that the decrease in pre catalase, which may come from accumulations of training during the week and other stressors, may have influenced the increase in LDH release after BJJ.

Carbonylated proteins are formed by the oxidation of protein side chains, mainly proline, arginine, lysine, and threonine. Derivatives of carbonyl groups can also be generated through oxidative cleavage of proteins by the α -amidation pathway or by oxidation of glutamyl side chains, making carbonylated protein a strong marker of oxidizing activity (Sanchez et al., 2021). This fact may explain the strong collection of DPPH markers and Protein carbonylated post BJJ. Although the sample was composed of elite-level athletes, the sample number could be greater. This limitation was due to the experimental procedure occurring between the European BJJ Championship (IBJJF) and the Pan American BJJ Championship (IBJJF), which implied the athletes' availability for such procedures.

None of them significantly affected the pre-and post-combat moments regarding oxidative stress markers. This result can demonstrate that a combat time of 10 minutes may not be enough for such an imbalance in the state of oxidative stress. This information tends to be useful since married fights have become commonplace within BJJ competitions. However, competitions with a high number of fights can increase these oxidative stress markers since the greater the volume of high-intensity physical activity, the greater the occurrence of oxidative lesions in biomolecules. This statement is supported by the study Gomes-Santos et al. (2019), where an increase in oxidative stress markers was found in MMA simulated combats.

Conclusions

We conclude that in a Brazilian jiu-jitsu match, the watery responses of cell damage biomarkers tend to increase right after the match, primarily when related to low pre-fight values of protective enzymes, such as catalase. Also, a strong relationship between DPPH and Protein carbonylated markers regarding oxidative stress markers can be observed in the post-combat. However, none of the oxidative stress markers are altered in the pre-and post-combat comparison. We believe that further studies should be carried out to ascertain these markers in a greater volume of stimulus and on consecutive days, given the existence of essential competitions with a high volume of fights, such as, for example, the ADCC and the Brazilian Jiu-Jitsu World Championship (IBJJF).

Conflicts of interest - None.

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