Acute effect of a fight of Mixed Martial Arts (MMA) on the serum concentrations of testosterone, cortisol, creatine kinase, lactate, and glucose

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ABSTRACT

The aim of this study was to analyse the serum concentrations of testosterone (T), cortisol (C), lactate (LAC), creatine kinase (CK) and glucose (GLU) on *mixed martial arts* (MMA) athletes, before and after a fight. We divided 20 MMA athletes into two groups of 10 fighters each, according to the result of a fight, and were then evaluated four times: 24 hours before (-24h), one hour before (-1h), immediately after (0h) and 24 hours after the fight (+24h). It was observed: a significant decrease in T and T/C between moment -24h and 0h and a subsequent increase between the moment 0h and +24h and a reverse behaviour in variables C, LAC and GLU (p<0.0001); a decrease in CK between moment -24h and -1h and an increase between moment -1h and +24h (p<0.0001); and differences between winners and losers T levels, in moments -24h, -1h, 0h and +24h (p = 0.009 e p < 0.001, p = 0.005 e p = 0,001, T and C, respectively), in T/C in the moments -24h and 0h (p=0.006 and p=0.001, respectively) and in GLU levels (p<0.0001) in the moment 0h. Therefore, it seems that an MMA fight leads to metabolic stress and muscle damage, regardless of the result of the fight. The coaches have now more biochemical and hormonal references and indicators in response to an MMA fight.

Keywords: hormones, blood biomarkers, MMA fighters

INTRODUCTION

Mixed Martial Arts (MMA) is widely popular among combat sports fans today. This modality combines various forms of martial arts disciplines, such as Judo, Karate, Jiu-Jitsu, Muay Thay, and Taekwondo. Strength and resistance capacities are inherent in its regular and systematic practice (Guerra Filho et al., 2014), a condition that translates into the high physical condition levels of MMA athletes (Dooley, 2013).

During the preparation period for the fight, some factors may reflect on the athlete's performance during the competition, as, for example, their physical condition, technique, and tactic, as well as psychological and physiological

factors. Stress which occurs before or during a competition has been associated with lack of focus and an increase in the athlete's muscle tension, which may lead to defeat and also expose the body to high levels of not only psychological but also physical stress (Santos, & Stefanello, 2010). This exposure to stress may also entail a set of chemical reactions, releasing a set of hormones into the blood flow, like testosterone (T), cortisol (C), adrenaline (ADR) noradrenaline (NA). Some products, subproducts, and metabolic enzymes are also released, like glucose (GLU), lactate (LAC), creatine kinase (CK) and lactate dehydrogenase (LDH). These may influence the athlete's

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performance and, at the same time, may serve as biochemical markers for the analysis of the impact of physical and psychological stress on the athlete (Brandao, Fernandes, Alves, Fonseca, & Reis, 2014; Filaire, Sagnol, Ferrand, Maso, & Lac, 2001) and distinguish between winners and losers.

Hormonal changes before and after a competition were initially observed by Mazur (1985), who hypothesizes the existence of an increase in the winner's T levels in a competitive situation and a decrease in C levels, having observed the opposite effect on losers. However, this effect was observed in competitions in which the physical component wasn't considered, but instead in computer game situations. Concerning competitions in which the physical component is involved, as is the case with fights, several authors have observed mixed changes in the hormonal response between winners and losers (Filaire, Sagnol, Ferrand, Maso, & Lac, 2001b; Papacosta, Nassis, & Gleeson, 2016; Suay et al. 1999).

Filaire et al. (2001b) observed in Judo athletes significantly higher T levels in the winners after a competition. This difference after a competition has been verified by Fry, Schiling, Fleck, and Kraemer (2011) and Kraemer et al. (2001). However, other authors (Alicia Salvador et al., 1987; Coswig et al., 2013; Papacosta et al., 2016; Suay et al., 1999) haven't observed this difference. Likewise, Filaire et al. (2001), haven't observed differences in the salivary T values between the participation in the regional championship and the inter-regional championship.

In literature, divergences arise in regard to the T levels response to a martial arts fight. Whereas some authors observed an increase in serum and salivary T values after a fight (Papacosta et al., 2016; Suay et al., 1999), others haven't observed significant changes (Filaire et al., 2001; Salvador, Suay, González-Bono, & Serrano, 2003).

In regards to the C hormone, its concentration in pre-competitive situations may be considered a stress indicator, due to a fear of failure or an uncertainty of one's ability to win, which may point to a "pre-competitive" alert state of the athlete (Girardello, 2004). Furthermore, there seem to be higher C levels in losers than in winners after a fight (Coswig, Neves, & Del Vecchio, 2013). However, Suay et al. (1999) observed higher values on winners. On the other hand, some haven't found any differences between winners and losers (Alicia et al. 1987, Filaire et al., 2001). Serum and salivary C values seem to increase between the pre and after fight moments (Filaire et al., 2001; Suay et al., 1999). However, Salvador et al. (2003) e Papacosta et al. (2016) haven't observed any significative changes in the pre and after fight moments.

Likewise, after a fight, the LAC concentrations in the skeletal muscle may increase due to the intensity of the efforts performed during the fight (Amtmann, Amtmann, & Spath, 2008; Viveiros, Costa, Moreira, Nakamura, & Aoki, 2011). Similarly, CK serum values may increase after a fight, reflecting the levels of muscle damage (Baird, Graham, Baker, & Bickerstaff, 2012; Bandeira, Moura, Souza, Nohama, & Neves, 2012; Coswig et al., 2013). This enzyme is found mostly in muscles and, on the occasion of fiber rupture due to muscle strain, is released into the blood flow. Therefore, it can be used as an indirect marker of muscular injuries and the intensity of the performed effort (Bandeira, Neves, Barroso, & Nohama, 2013; Bandeira, Neves, Moura, & Nohama, 2014; César, Bara Filho, Lima, Aidar, & Dantas, 2008).

Not a significant amount of research tried to observe the behaviour of biochemical and hormonal markers before and after competitions in combat sports, especially in MMA. Thus, the aim of this study was to analyse the T, C, LAC, CK and GLU concentrations in MMA highperformance athletes, before and after a fight.

METHOD

The athletes underwent anthropometric assessment (body mass, height, and estimated body fat percentage) 24 hours before the fight. It was calculated the estimated body fat percentage by measuring the skinfolds thickness using the method proposed by Jackson and Pollock (1978). To analyse the effect of a MMA fight on the hormonal levels (T and C) and biochemical markers (CK, LAC and GLU), the athletes were evaluated at four different times: 24 hours before (-24h), one hour before (-1h), immediately after (0h) and 24 hours after (+24h) after the fight.

Subjects

Twenty men, professional MMA athletes, competitors in an official event, were selected according to the following inclusion criteria: have practiced the sport for at least six years; have competed in at least three fights as a professional fighter; and not use anabolic steroids or other medicines which may influence the results. The subjects were divided into two groups of 10 fighters, according the outcome of the fight: winners (26.20 \pm 2.39 years old; 86.00 \pm 16.66 kg; 177.50 ± 8.11 cm; 15.62 ± 7.21 %G and 9.40 \pm 2.99 practice years) and losers (24.30 \pm 1.83 years; 87.20 ± 14.36 kg; 178.90 \pm 6,94 cm; $17.72 \pm 6,37$ %G e 7.70 \pm 2.16 practice years). This research was approved by the Research Ethics Committee of Centro de Ciências da Saúde (CCS) at Universidade Federal da Paraíba.

Experimental Procedures

All the athletes arrived at the event venue at least 48 hours before the fight and stayed at a hotel in the vicinity. The anthropometric assessment was carried out on this first day at the site. Afterward, 24 hours before the fight, a blood athlete's sample taken was at the accommodation. This sample, as well as the others, was taken by a qualified nurse. After this procedure, the athletes remained at their accommodation until two hours before the fight. At the venue and one hour before the fight, a second blood sample was taken, as well as a third sample immediately after. Each subject remained comfortably seated, with a tourniquet applied to the upper limb, as 10 ml of blood was collected from the athlete's median cubital vein using a venous blood collection system (BD Vacutainer®, New Jersey, EUA). The blood samples were transported to a certified laboratory immediately after being collected, where we centrifuged them at 1500 rpm for 10 minutes (Megafuge 1.0R, Heraeus, Germany). Afterward, the serum was removed and kept at -20 °C for subsequent laboratory analysis. The serum concentrations of testosterone and cortisol were then measured using radioimmunoassay kits (Cortisol Coat -

Count RIA, DPC Med Lab, Los Angeles, USA) (Rosa et al., 2014). The biochemical variables (CK, LAC, and GLU) were measured through the Integra 600® (Roche Diagnostics, Basileia, Suiça) equipment using the enzymatic colorimetric endpoint method (Coswig et al., 2013).

Statistical Analysis

The statistical analysis was carried out through the software Statistical Package for the Social Sciences (SPSS), version 21.0. The results are reported as means and standard deviations. The Shapiro-Wilk and Mauchly tests were used to verify the normality and sphericity of the data. The Student's t-test for independent samples was used to observe any significant differences between losers and winners at the initial moment in all variables. After this procedure, two-factor measures analysis of variance repeated (ANOVA), with post-hoc Bonferroni test, was performed with the model 4 moments (-24h, -1h, 0h, +24h) x 2 groups (winners, losers). The level of significance was set at 5%.

RESULTS

A significant moment effect was observed in all the hormonal and biochemical markers between the analysed moments ($F_{(3.54)}$ =333.985; p < 0.0001; $\eta_p^2 = 0.949$; $F_{(3,54)} = 58.129$; p < 0.0001; $\eta_p^2 = 0.774$; $F_{(3,54)} = 5.264$; p = 0.003; $\eta_p^2 = 0.236$; $F_{(3.54)} = 611.244;$ p<0.0001; $\eta_{\rm p}^2 = 0.971$; $F_{(3,54)} = 271.616$; $\eta_p^2 = 0.938;$ p<0.0001; $\eta_p^2 = 0.774$; $F_{(3,54)} = 53.448$; p<0.0001; $\eta_p^2 = 0.748$, T, C, T/C, LAC, GLU e CK respectively). Regarding the T and T/C variables, a significant decrease (p<0.0001) of their serum values was observed between the initial moment (-24h) and moment 0h (-27.53% and -29.72%, -39.39% and -54.44%, between -24h and -1h and between -1h and 0h, T and T/C, respectively), having later increased significantly (p<0.0001) between 0h and +24h (54.04% and 224.50%, T and T/C, respectively). Regarding the C, LAC and GLU variables, an opposite behaviour was observed, their values increasing significantly (p<0.0001) between moments -24h and 0h (22.74% and 47.49%, 53.62 and 519.34%, 16.18% and 107.90%, from -24h to -1h and -1h to 0h, C, LAC and GLU, respectively) and decreased significantly (p<0.0001) between moments 0h and +24h (-50.12%, -86.52% and -61.8%, C, LAC and GLU, respectively). Regarding the CK variable, a significant decrease (p<0.0001) of its serum values was observed

between moments -24h and -1h (-18.39%), as well as significant increase (p<0.0001) between moments -1h and 0h (13.35%) and moments 0h and +24h (187.23%) (see table 1 and 2).

Table 1. Mean \pm SD of Testosterone and Cortisol values in MMA fighters at selected moments

			- 1	
		Testosterone (nmol	.L ⁻¹)	
	-24h	-1h	0h	+24h
Winners	20.05 ± 2.13 *	15.90 ± 1.18 *	11.92 ± 1.79 *	17.75 ± 2.00 *
Losers	15.91±2.40*¶	10.16±3.52*¶	7.14±2.73*¶	11.61±1.79*¶
Total	17.98±3.06*	13.03 ± 3.90 *	9.53±3.33*	14.68 ± 4.02 *
		Cortisol (nmol.	L ⁻¹)	
Winners	580.63±83.18*	706.00±64.65*	949.98±59.20*	522.22±73.69*
Losers	$387.29 \pm 147.64 \Upsilon$ ¶	482.03 ± 180.55 *	$802.21 \pm 94.43*$	$351.69 \pm 109.86 \Upsilon$
Total	483.96±153.10*	594.02±144.99*	876.09 ± 107.84 *	436.96±126.26*
		Testosterone/Co	rtisol	
Winners	34.76±2.38Y	22.56±8.52*	12.49±1.10*	34.13±1.43Y
Losers	40.15±4.88*	22.85 ± 7.70 *	8.66 ± 2.42 *	10.57 ± 2.69 *
Total	37.45 ± 4.65 *	22.70±5.31*	10.57 ± 2.69 *	$34.30 \pm 4.73*$

^{*}p<0.05 compared with other moments; Υ p<0.05 compared with other moments with the exception of -24h and +24h; \P p<0.05 between Losers and Winners

A group effect was observed in variables T, T/C and GLU ($F_{(1,18)}$ =4.700; p=0.035; η_p^2 =0.217, $F_{(1,18)}$ =7.056; p=0.017; η_p^2 =0.293 and $F_{(1.18)}$ =17.929; p<0.0001; η_p^2 =0.499, T, T/C and GLU, respectively). Furthermore, a moment x group interaction was observed in variables T and GLU ($F_{(3,4)}$ =3.090; p=0.035; η_p^2 =0.154 and $F_{(3,54)}$ =15.454; p<0.0001; η_p^2 =0.462, T and GLU, respectively). Significant differences between winners and losers in their T levels were observed at moments -24h, -1h, 0h and +24h after the

fight (p=0.009, p<0.001, p=0.001 and p<0.0001, respectively); in T/C at moments -24h and 0h (p=0.006 e p=0.001, respectively); and in moment 0h in GLU values (p<0.001), having winners significantly higher T and GLU values at these moments and lower T/C values at -24h and higher at 0h. C values were significantly higher (p=0.023) on winners comparatively to the losers at -24h. No significant differences between winners and losers were observed at any other moment or with other variables.

Table 2. Mean \pm SD of Glucose, Lactate and Creatine Kinase Values in MMA fighters at selected moments

		Glucose (mg/d	1)	
	-24h	-1h	0h	+24h
Winners	4.48 ± 0.35 *	5.43 ± 0.83 *	11.73±2.30*	4.03 ± 0.21 *
Losers	$3.80 \pm 0.44 \Upsilon$	$4.20\pm0.49*$	8.27±1.33*¶	3.62 ± 0.18 *
Total	$4.14\pm0.52^*$	$4.81\pm0.92*$	10.00 ± 2.55 *	3.82 ± 0.28 *
		Lactate (nmol.L	-1)	
Winners	1.32±0.47*	2.02±0.61Y	2.37±3.18*	1.72±0.68Υ
Losers	$1.44 \pm 0-27$ *	2.22 ± 0.42 *	3.88 ± 1.30 *	1.82 ± 0.47 *
Total	1.38 ± 0.38 *	2.12±0.52*	13.13 ± 2.51 *	1.77 ± 0.52 *
		Creatine Kinase (J/L)	
Winners	510.17±288.12Φ	418.31±277.67 +	491.40±278.08+	1304.73±904.13*
Losers	553.13±128.31?	449.46±144.27 *	492.21±155.19 +	1520.48±609.66*
Total	531.15±218.19*	433.89±215.95*	491.81±219.17+	1412.69 ± 758.63 *
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^{*}p<0,05 compared with other moments; Y p<0,05 compared with other moments with the exception of -1h and +24h; Φ p<0,05 compared with other moments with the exception of -1h and 0h; + p<0,05 compared with other moments with the exception of -24h; ? p<0,05 compared with other moments with the exception of 0h; ¶ p<0,001 between Losers and Winners.

DISCUSSION

In this study, significant changes in C, T, GLU, LAC, CK values and T/C ratio were observed as a response to an MMA fight. This supports the thesis that this sport causes a high physical stress, which may equally reflect in psychological terms (Jorge et al., 2010). For this study, the sample consisted of a population of MMA athletes with a superior technical capability regarding the modality's national ranking, both in what concerns winners and losers.

Concerning the variation of the hormonal and biochemical markers studied, two distinct behaviours were observed: in the C, LAC and GLU variables, there was a gradual increase at the moments that precede the fight, peaking after the fight and decreasing at +24h; in the T variable, the opposite happened, with a gradual decrease before 0h and a return to its normal values at +24h. This behaviour corroborates with the other studies (Coswig et al., 2013; Moreira et al., 2012; Salvador et al., 2003; Suay et al., 1999). By contrast, CK serum values behaved differently from other variables. As CK is used as a muscle damage marker, it is anticipated an increase of its serum values after the fight, especially after 24 hours.

Regarding the C variable, the data obtained from this study suggests that its values were significantly higher before the fight (-24h and -1h) in winners, which corroborates what Passelergue and Lac (2012) observed when they analysed 15 wrestlers in the junior age category, internationally. and Papacosta et al. (2016) found significantly higher values in winners in comparison with the losers at Judo fights, the morning before the fight. Contrary to these studies, other authors didn't find any differences in the C values, both serum and salivary, between winners and losers, in different combat modalities, like Jiu Jitsu (Coswig et al., 2013) and Judo (Filaire et al., 2001). The various methodological approaches, like the number of subjects, age, combat sport, data collection time and C type analysed (serum or salivary) can explain the differences between the considered studies.

The reason behind the higher values on the winning athletes in comparison with the losers

found in this study may be related with a better pre-competitive preparation. Higher C levels before the competition can bring about a better perception about the situation at hand and its necessities, fostering faster information processing, which in turn enables a search for solutions, while selecting more adequate conducts and preparing the organism to act in a more effective and faster way (Margis, Picon, Cosner, & Silveira, 2003) as long as this increase is not too high, which otherwise can impair the athlete. Regardless of the result, when it comes to the behaviour of the C variable, a significant increase from -24h to 0h was found in the present study, such phenomenon may be related to a high physical strain from both athletes and a delayed physiological response to the fight (Salvador et al., 2003). These results are in accordance with studies developed by Suay et al. (1999) and Filaire et al. (2001), but they differ from results obtained by Papacosta et al. (2016). This difference may be related to the differences between the modalities chosen, MMA in this study and Judo in Papacosta et al. (2016). However, when it comes to the studies by Suay et al., (1999) and Filaire et al. (2001), the combat sport (Judo) did not differ from the one analysed by Papacosta et al. (2016). Thus, one of the possible explanations for the fact that Papacosta et al. (2016) did not find any increase in C between pre and post-fight moments may have been due to the technical level of the selected sample. In the Suay et al. (1999) study, the athletes all held between brown and black belts in the 3rd Dan while in the study by Papacosta et al. (2016), the athletes all held black belts, between 2nd and 3rd Dan. Therefore, the athletes from the study of Papacosta et al. (2016) probably had a higher technical level compared with those from the study by Suay et al. (1999), allowing them to fight in a more technical and tactical way at the expense of using their physical capacities. However, the technical level of the sample used in the Filaire et al. (2001) study was identical to the one in Papacosta et al. (2016). Like this, other methodological aspects could have influenced the different C hormonal responses regarding other studies, such different methods for extracting blood or saliva, collection times, the athlete's own training and fight experience, as well as the combat modality studied.

A significant decrease in C levels was observed 24 hours after the fight, reaching identical values to those observed 24 hours before the fight. When comparing the winning and losing athletes 24 hours after the fight, those who had won kept on showing significantly higher C levels than the losing athletes. This pattern was also observed in the moments before the fight. However, when normalized, the difference between winners and losers at -24h was not significant. Thus, we cannot state that winners were in a higher precompetitive alert state in comparison with the losers. It could be that the winners' basal values were already higher in comparison with the losers'. In following studies, moments before the pre-competitive 24h should be explored to clarify this aspect.

Regarding the T levels in the moments before the fight (-24h and -1h), there was a significant decrease, which is in accordance with the data gathered by Coswig et al. (2013), who collected similar samples, at breakfast and 90 minutes before the fight. This decrease in the T levels could be a reaction to pre-competitive stress. It could also have been influenced by a significant increase in C levels, which resulted in a decrease in T levels (Salvador et al., 1987). Nevertheless, as stated above, we cannot claim that the athletes were in pre-competitive stress. However, the same explanation can also apply for the significant decrease observed at 0h moment and supported by a return to the values of -24h, which also occurred with C levels. This decrease from the pre to the post-competitive moment does not corroborated with the literature where significant increases were observed (Papacosta et al., 2016; Suay et al., 1999) or where no changes were found (Filaire et al., 2001; Salvador et al., 2003). As it was mentioned before in regards with the C values, these differences could be related with methodology used in the different studies. In relation to the T values dependency of the final fight result, the winning athletes showed higher T values when compared with the losing athletes in all moments considered. This shows that the winners seem to use a different T regulation mechanism. These results corroborated with those observed by Filaire et al., (2001), following exercise, but go against Papacosta et al., (2016). The difference in the combat sports modality between this study and the referenced studies (MMA and Judo, respectively) may explain such results. MMA makes use of not only body projections and legs and arms keys, like in Judo, but also kicks and punches, which are more physical when comparing with the techniques mentioned before. The T response is more effective when the physical effort is more intense.

The T/C ratio behaves similarly to T, showing a decrease in the pre-fight moments (-24h and -1h) and immediately after fight (0h). This fact explains how competition is an indicator of physiological stress (Girardello, 2004). However, at +24h there was an increase that may be due to a tissue repair necessity, showing a potential for anabolism (Leite et al., 2011).

When comparing other biochemical markers, the LAC and GLU showed significant differences from the pre-fight moment (-21h and -1h) to immediately after moment (0h), which in turn demonstrates the predominant activation of lactic anaerobic metabolism in fighters (Coswig et al., 2013), considering that this increase in LAC levels post-fight may not only reveal muscle development, but also LAC conversion to GLU.

After the fight, different values between winners and losers in the GLU variable were observed with preponderantly higher levels registered on winners in comparison with the losing athletes. This may be due to a allegedly better technique showed by winning athletes, which in turn results in less effort and energy needed for them to win the fight. Reinforcing this idea, the same was observed by Brandão et al., (2014), after a Jiu Jitsu fight between elite and nonelite athletes.

Regarding CK values, there was a significant increase, as expected, at +24h. This reflects the occurrence of damage on the active muscles, which in turn affects the plasma membrane integrity and composition (Baird et al., 2012). CK levels observed immediately after fight in the present study corroborate with the results observed by Brandão et al., (2014), expressing that in order to detect changes in this variable and to try and understand the level of muscle injury

caused by the fight, the collection of a single sample after fight seems to be insufficient. This way, we suggest that a sample collection of this variable should be carried out for a longer period of time post-fight, as conducted in this study (+24h).

CONCLUSION

Based on the data presented in this study, it seems that an *MMA* fight causes post-competitive metabolic stress and muscle damage, regardless of the outcome of the fight. However, victory seems to be associated with higher testosterone serum levels. The behaviour of the biochemical and hormonal variables analysed in this study is different throughout the different considered moments. This way, MMA athletes' coaches now have access to benchmark biochemical and hormonal indicators for the response to an MMA fight.

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