Title: **Salivary hormones and anxiety in winners and losers of an international** judo competition.

Running title: Judo competition performance.

Keywords: winning performance, salivary testosterone, salivary cortisol, mucosal immunity, psychophysiological arousal

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Abstract

The purpose of this study was to investigate the responses of salivary hormones and salivary secretory immunoglobulin A (SIgA) and anxiety in winners and losers during an international judo competition. Twenty-three trained, male, national level judo athletes provided three saliva samples during a competition day: morning, in anticipation of competition after an overnight fast, mid-competition, and post-competition within 15 min post-fight for determination of salivary cortisol, salivary testosterone, salivary testosterone/cortisol ratio, SIgA absolute concentrations, SIgA secretion rate and saliva flow rate. The competitive state anxiety inventory questionnaire was completed by the athletes (n=12) after the first saliva collection for determination of somatic anxiety, cognitive anxiety and self-confidence. Winners were considered 1-3 ranking place (n=12) and losers (n=11) below 3rd place in each weight category. Winners presented higher anticipatory salivary cortisol concentrations ($p=0.03$) and a lower mid-competition salivary testosterone/cortisol ratio ($p=0.003$) compared with losers with no differences for salivary testosterone. Winners tended to have higher SIgA secretion rates ($p=0.07$) and higher saliva flow rates ($p=0.009$) at mid-competition. Higher levels of cognitive anxiety ($p=0.02$) were observed in the winners, without differences according to the outcome in somatic anxiety and self-confidence. The results suggest that winners experienced higher levels of physiological arousal and better psychological preparedness in the morning, and as the competition progressed, the winners were able to control their stress response better.

Keywords: winning performance, salivary testosterone, salivary cortisol, mucosal immunity, arousal

Introduction

Increased plasma cortisol levels are associated with anxiety and physical exertion (Viru & Viru, 2004), whereas acute elevations of this glucocorticoid can be euphorogenic and neurostimulatory (Duclos, 2010). The biosocial model of status (Mazur, 1985) suggests that elevated testosterone levels during competitive situations are associated with dominance, fearlessness of the opponent, confidence and situation-specific aggression. Salivary testosterone concentrations in males were reported to be associated with situation-specific aggression and willingness to engage in competitive task (Carre and McCormick, 2008), traits that could positively influence judo competition-performance.

Suay et al. (1999) observed that winners of a judo competition had higher serum cortisol levels throughout the competition, despite no differences in testosterone and prolactin and similar physical effort of the athletes. Similarly, Balthazar, Garcia, and Spadari-Bratfisch (2012) observed that higher early-morning anticipatory salivary cortisol levels were associated with winning performance during a triathlon competition. However, no differences in anticipatory salivary cortisol levels between winners and losers were observed in relation to judo fights (Salvador, Suay, Gonzalez-Bono, & Serrano, 2003). Serum testosterone was reported to rise in anticipation of a competitive match, with larger increases during the pre-fight anticipatory values in those who win a wrestling (Fry, Schilling, Fleck, & Kraemer, 2011) and a weight-lifting competition (Passelergue, Robert, & Lac, 1995). Winning can also lead to subsequent elevations in circulating testosterone, which stimulate competitiveness described as the “winners’ effect” (Booth, Shelley, Mazur, Tharp, & Kittok, 1989). Higher levels of post-competition testosterone were observed in the

winners of a badminton competition (Jimenez, Aguilar, & Alvero-Cruz, 2012), a wrestling competition (Fry et al., 2011) and a judo competition (Filaire, Maso, Sagnol, Ferrand, & Lac, 2001a). The evidence suggests that responses of cortisol and testosterone during competition could be related to the outcome; however, findings reported for elite athletes are limited.

Saliva secretory immunoglobulin A (SIgA) responses to acute exercise are not consistent, with some studies reporting decreases (Mackinnon, Ginn, & Seymour, 1993; Nehlsen-Cannarella, et al., 2000; Nieman, et al., 2002), some studies reporting increases (Blannin, et al., 1998; Sari-Sarraf, Reilly, Doran, & Atkinson, 2007) and some studies reporting no change of SIgA after acute exercise bouts (Walsh, Blannin, et al., 1999; Sari-Sarraf, Reilly, & Doran, 2006). The overall intensity of the exercise bout appears to influence the post-exercise SIgA response, with short duration, high-intensity exercise reported to induce increases in SIgA secretion rate (Allgrove, Farrell, Gleeson, Williamson, & Cooper, 2008). In general, increases are seen in response to short bouts (<30 min) of high intensity exercise (>80% VO_2max), whereas no change or falls are seen with very prolonged exercise (>2 h) (Bishop & Gleeson, 2009). However, studies examining SIgA responses to competitive situations are limited, showing no change in SIgA levels after a Brazilian jiu-jitsu (Moreira, Franchini, et al., 2012) and a basketball competition (Moreira, Bucurau, et al., 2013). However, no studies on SIgA responses during judo competitions exist.

The association between arousal and performance has been demonstrated in an inverted U-shaped relationship, illustrating that optimal performance is accomplished at a moderate level of arousal; thus poor performance is related to very low levels of arousal, progressively enhances at moderate levels of arousal until it

deteriorates at very high arousal levels (Hardy & Parfitt, 1991). Arousal results from both physiological and psychological response to a stressor, whereas practically it could be interpreted as the physiological response of the sympathetic nervous system and the cognitive anxiety of the competing athlete. Filaire, Sagnol, Ferrand, Maso, & Lac, (2001b) observed that interregional judo competitions elicited high levels of somatic and cognitive anxiety and lower self-confidence along with increases in salivary cortisol levels, suggesting that neuroendocrine response and anxiety are positively related in judo athletes. Arousal is closely interrelated to anxiety, whereas in athletic population it could be interpreted as the perception of the athletes' physiological/somatic response and/or psychological/cognitive response to a stressor, which is usually the subsequent competition. It has been previously suggested that mood disturbance as measured by the Profile of Mood States did not predict actual or predicted cycling performance (Murgia, et al., 2015). However, the revised competitive anxiety inventory 2 questionnaire is a commonly used, validated multidimensional construct for assessing scales of somatic anxiety, cognitive anxiety and self-confidence and one of the most used measures in sport psychology (Cox, Martens, & Russell, 2003). Judo is a combat sport with high body contact where the athlete should "read" the moves of the opponent; thus the mental/psychological capacity and arousal of the athletes is especially important to the combat outcome. However, evidence are lacking to whether anxiety measures and self-confidence could influence the outcome of a judo competition.

Therefore, the aims of this study were to investigate the responses of salivary cortisol, salivary testosterone and SIgA during an international judo competition and to identify whether these salivary immunoendocrine responses and anxiety measures could differentiate winners and losers of the competition.

Methods

Participants

Twenty-three trained, male, national level competitive judo athletes volunteered to participate in the current investigation (age 22 ± 4 years; height 178 ± 7 cm; body mass 78.6 ± 13.2 kg; body fat 11.3 ± 5.6%; VO_2peak 52.8 ± 5.4 $ml \cdot min^{-1} \cdot kg^{-1}$; training experience 8 ± 4 years). Athletes were aged 19 - 35 years and all athletes had competed in judo for at least five years and trained at least 3 times per week. They competed in weight categories within 60 - 100 kg and were officially registered under the National Judo Federation. All participants were from Cyprus, from 3 different cities. Athletes trained with different coaches at 3 different clubs (one in each city). Most of the athletes (60%) came from city 1, and less athletes came from cities 2 and 3 (20% each city). All were experienced athletes and received relatively the same training (and pre-competition recovery) by their coaches. Athletes were familiar with each other, from previous training camps and competitions. They were non-smokers, not taking any form of medication, refrained from alcohol consumption and were free from illness during the study. The athletes did not exercise or train on the previous day. Prior to the study, all participants completed an informed consent and a health screening questionnaire. Ethical approval for this study was obtained by the national ethics committee.

Judo competition

The study took place during an international judo competition in November 2012. Competition began at 9:30 and ended at 15:00. The competition day began with registrations and weigh-ins of the athletes in the morning after an overnight fast

(08:00-08:30), around 1.0 - 1.5 h before their first scheduled fight. Saliva samples were collected three times in total, before, during and after the competition. Immediately after the first saliva collection and ~1 h before the competition began, half of the athletes (n=12) in a randomised order completed the revised competitive anxiety inventory-2 questionnaire, as suggested by Cox et al. (2003). Using this questionnaire, athletes rated their anxiety symptoms on a scale of 1 (not at all) to 4 (very much so) and subscales of somatic anxiety, cognitive anxiety and self-confidence were then calculated for each athlete on a scale of 10 to 40. Reproducibility of the anxiety questionnaire for this sample of athletes was ICC=0.78. Athletes were familiar with saliva collection procedures and the anxiety questionnaire. Then the athletes were divided into their weight categories and draws determined the opposing couples within each category. When the athlete lost the fight, he was disqualified from the tournament except when he competed in a repechage round to determine the third place. During this judo competition, athletes had no limitations or control in regards of fluid or food consumption, and they were asked to keep their regular habits; however, no food or drink was consumed before weigh-ins and the first sample collection. At end of judo competition according to the final rankings, athletes were divided into winners (first, second and third place) and losers (fourth place and below), at each weight category for the subsequent statistical analysis. Personal interviews revealed that in the week preceding the competition 80% of the athletes underwent a weight reduction of 2-5% of body weight, without differences between the groups of winners and losers.

Saliva collection and analysis

Saliva samples were collected in the morning after an overnight fast and before warm-up (08:00 – 08:30), mid-competition, after 2 fights and 10 min before the third fight (10:30 – 11:30), and post-fight within 15 min after their final fight (14:00 – 14:30). Subjects were instructed to swallow to empty their mouth before an unstimulated saliva sample was collected. Prior drinks or food consumption was not permitted for at least 10 min prior to the saliva collection. Saliva collections were made with the participant seated, head leaning slightly forward with eyes open, and making minimal orofacial movement while passively dribbling into a sterile vial (Sterilin, Caerphily, UK). The collection time was 2 min at least or until an adequate volume of saliva (~1.5 ml) had been collected. Saliva was then stored in the same vials at −30ºC and were transported frozen to the Loughborough University laboratories for analysis. Concentrations of salivary cortisol, salivary testosterone and SIgA were determined in duplicate using commercially available ELISA kits (Salimetrics, PA, USA). Mean intra-assay coefficients of variation were 3.6 %, 2.5 % and 2.6 % for salivary cortisol, salivary testosterone and SIgA, respectively. Saliva volume was estimated by weighing the vial before and immediately after collection and assuming that saliva density was 1.00 $g \cdot ml^{-1}$ (Cole & Eastoe, 1988). Saliva flow rate was then calculated by dividing the total saliva volume collected in each sample (in ml) by the time taken to produce the sample (in min). The SIgA secretion rate ($\mu g \cdot min^{-1}$) was calculated by multiplying absolute SIgA concentration ($\mu g \cdot ml^{-1}$) by saliva flow rate ($ml \cdot min^{-1}$).

Statistical analysis

Data was checked for normality, homogeneity of variance and sphericity before statistical analysis. If Mauchly's test indicated that assumption of sphericity was

violated the degrees of freedom were corrected using Greenhouse-Geisser estimates. According to the outcome the athletes were divided into groups of winners (n=12) and losers (n=11). The values of salivary cortisol, salivary testosterone, salivary testosterone/cortisol ratio and SIgA concentrations and secretion rates between winners and losers were analysed across time using a two-way analysis of variance (ANOVA) for repeated measures (time x group) with Bonferroni adjustments. Anxiety responses between winners and losers were analysed using a one-way between measures ANOVA. From the subscale of cognitive anxiety, two outliers (>2 SD from the mean) were removed from the data set. Statistical significance was set at $p \leq 0.05$. The 95% confidence intervals (CI) for relative differences and size effects (ES) from simple planned contrasts were calculated to confirm meaningful significant differences. All data are presented as mean ± SD. Data was analysed using SPSS (SPSS v. 22.0; SPSS Inc, Chicago, IL, USA).

Results

Salivary hormones

Individual athletes' data for salivary cortisol, salivary testosterone and salivary testosterone/cortisol ratio is presented in figures 1(a), 1(b) and 1(c), respectively, and mean data for salivary hormones in presented in table 1. Winners presented higher concentrations of salivary cortisol compared with losers in the morning ($p=0.03$, ES=0.58, CI 36 to 165%). No significant effects of time and interaction ($p>0.05$) showed that mean salivary cortisol responses were similar across the competition. Mean salivary testosterone concentrations were higher in the morning compared with post-fight values ($p=0.01$, ES=0.52, CI 10 to 190%); however, no significant effects

of group and interaction showed no differences in salivary testosterone between winners and losers of the competition ($p>0.05$). Significant effects of time ($p=0.02$; ES=0.60) and group ($p=0.03$, ES=0.53) but not interaction ($p>0.05$) showed that mean salivary testosterone/cortisol ratio fell mid-competition compared with morning values (CI -27 to -173%) and winners presented lower salivary testosterone/cortisol ratio in the morning (CI -43 to -156%) and mid-competition (CI -28 to -171%) compared with losers.

Salivary SIgA

No significant effects of time, group and interaction were found for SIgA absolute concentration and secretion rate ($p>0.05$); however, winners tended to have higher SIgA secretion rates at mid-competition [$p=0.07$, ES=0.35, CI -12 to 212%, figures 2(a), 2(b); table 1].

Saliva flow rate

A significant effect of group ($p=0.009$, ES=0.51) showed higher saliva flow rates in the winners at the mid-competition time-point (CI 23 to 173%). Significant effects of time ($p=0.02$, ES=0.46) and interaction ($p=0.007$, ES=0.53) showed that saliva flow rate was lower in the morning compared with mid-competition (CI -5 to -204%) and post-fight [CI -3 to -368%, figure 2(c); table 1].

Somatic anxiety, cognitive anxiety and self-confidence

Levels of cognitive anxiety were higher for the winners compared with losers (p=0.02, ES=0.72, CI 23 to 177%). No significant differences between winners and losers were found on somatic anxiety and self-confidence (p>0.05, figure 3).

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Discussion

This study showed that winners had higher salivary cortisol concentrations in the morning of the competition, higher saliva flow rate and a tendency for higher rates of SIgA secretion mid-competition compared with losers. In addition, winners had higher levels of cognitive anxiety compared with losers; no differences were found in levels of somatic anxiety and self-confidence according to the outcome. Therefore, this study suggests that higher levels of psychophysiological arousal in the morning of a judo competition may be related with enhanced performance.

This study presented higher morning salivary cortisol concentrations in the winners of the judo competition; thus, morning salivary cortisol concentrations ranged 5-17 $nmol \cdot l^{-1}$ in the winners and 4-10 $nmol \cdot l^{-1}$ in the losers. Similar findings in judo athletes were observed by Suay et al. (1999), presenting higher anticipatory, pre-competition serum cortisol but not testosterone concentrations in the winners of judo competition. Comparable findings were presented in triathletes, with higher morning salivary cortisol concentrations in those who performed better, thus presenting a positive relationship between early-morning cortisol levels and ranking place during

a triathlon competition (Balthazar et al., 2012). From a physiological perspective there is evidence to suggest that acute rises in cortisol can have ergogenic effects via its neurostimulatory, anti-inflammatory/analgesic and metabolic functions (Duclos, 2010); whereas modearate elevations in cortisol are considered to be advantageous for increasing arousal. Salivary cortisol and salivary alpha-amylase are both considered markers of stress, as the hypothalamic pituitary-adrenocortical system (cortisol) was reported to mirror the responses of the sympathetic-adrenomedullary system (salivary alpha-amylase) at 30 min post-exercise in children in response of a taekwondo competition (Capranica, et al., 2012). Possibly in our study, the higher morning salivary cortisol levels in the winners could reflect the activation of the sympathetic nervous system which was associated with the "fight or flight" stress response; consequently this finding could be related to the higher levels of physiological (and mental) alertness in the winning athletes, which in turn could have prepared the body (and mind) for action at the onset of the competition.

Winners also presented higher levels of cognitive anxiety, without any significant differences in ratings of somatic anxiety and self-confidence between winners and losers. Our findings disagree with the findings of Filaire, Maso, et al. (2011a) that winners of a judo competition present lower levels of cognitive anxiety. However, our findings are in line with the catastrophe theory, whereas an intermediate level of arousal could mediate enhanced performance (Hardy & Parfitt, 1991). Another study in judo athletes (Filaire, Sagnol, et al., 2001b) showed that cortisol and cognitive anxiety were related pre- and post-competition, thus these authors suggested that elite athletes may actually utilise the high levels of cognitive anxiety to enhance performance. Hence, these authors suggested that winning judo performance is actually dependant on the ability of each athlete to control the physiological arousal

that accompanies the increased cognitive anxiety (Filaire, Sagnol, et al., 2001b). The importance of cortisol in sustaining and facilitating cognitive functions has been demonstrated in a study in female elite water polo athletes, where a lower than normal cortisol secretion was reported to be related to dysfunctional mood state during two months of training and competitions (Di Corrado, Agostini, Bonifazi, Perciavalle, 2013). Judo is a sport where high mental alertness is required in order to face the opponent during combat, whereas the participating judokas in our study were national, experienced, elite level athletes, with possibly good control over competition stress situations. Therefore, the higher levels of cognitive anxiety along with higher cortisol concentrations in the winners of our study could indicate better psychophysiological arousal which has possibly been a factor for promoting winning performance.

Concentrations of salivary testosterone presented no differences between winners and losers; our findings contradict the biosocial model of status (Mazur, 1985) and disagree with the findings of studies reporting higher pre-competition testosterone concentrations in the winners of a weight-lifting competition (Passelergue et al., 1995) and higher post-competition testosterone in the winners of a badminton (Jimenez et al., 2012), a wrestling (Fry et al., 2011) and a judo competition (Filaire, Maso, et al., 2001a). A lower salivary testosterone/cortisol was observed in the winners in anticipation and at mid-competition; however it is probably of low physiological value as it has reflected salivary cortisol concentrations.

The discrepancy in our findings regarding anticipatory endocrine responses could be explained by the dual-hormone hypothesis, as proposed by Mehta & Josephs (2010). These authors suggested that cortisol and testosterone concentrations during acute

stress situations jointly interact and compensate for each other to modify dominance; thus only when cortisol is low should higher testosterone promote higher status and reversely when cortisol is high, higher testosterone may actually decrease dominance and sequentially motivate lower status. This theory could actually explain the discrepancy in our findings showing no differences in testosterone levels between winners and losers, which is in contrast to other studies (Fry, et al., 2011; Passelergue, et al., 1995). Thus, no differences in salivary testosterone concentrations between winners and losers could be related and actually explain the higher salivary cortisol levels in those who won the judo competition. However, an additional saliva collection on a resting day could provide further evidence for this suggestion. SIgA secretion rate tended to be higher in the winners mid-competition, whereas this was accompanied by significantly higher rates of saliva flow. Salivary responses can illustrate the activity of autonomic nervous system, since saliva is regulated by both sympathetic and parasympathetic nervous system activity; saliva elicited by sympathetic stimulation reduces saliva flow rate due to vasoconstriction of the blood vessels supplying the salivary glands, whereas parasympathetic nerve activation nerve stimulation results in a higher volume of watery saliva (Chicharro, Lucía, Pérez, Vaquero, Ureña, 1998). However, it is well known that sympathetic and parasympathetic nervous systems, work in cooperation rather than in opposition. The function of the parasympathetic nervous system is to actually work along with the sympathetic nervous system for calming the body after the arousal. Therefore, the higher saliva flow rate mid-competition in the winners could suggest increased participation (or less inhibition) of the parasympathetic nervous system, which in that case, aided to control the sympathetic nervous system activation; thus practically, the winning athletes were the ones that were able to control their stress

response better during competition. Mean SIgA concentrations and secretion rate did not change from pre to post-competition, agreeing with the findings of Moreira, Arsati, et al. (2010) and Moreira, Franchini, et al. (2012) that competition may have a minimal effect on this marker of mucosal immunity.

Limitations of this study were the measurement of hormonal responses and anxiety during only one competition day; therefore, it is possible that many other factors have also influenced performance in these judo athletes. In addition, the fact that testosterone concentrations were not associated with the competition outcome, as was expected due to its physiological role in performance, should be better explored. One could argue that the higher cortisol concentrations in the winners could be attributed to exogenous use of stimulants. However, we can say with confidence that cortisol concentrations at this time point were not affected by previous food or drink (i.e. coffee or ergogenic substances) intake or previous exercise, since saliva was collected just before weigh-ins as the competition took place before the change in weigh-ins procedures (International Judo Federation, 2012) and warm-up, when athletes did not consume anything (not even water) in order to maintain their body mass. It is important to note that doping control was present at the day of the competition and all participants were tested. Furthermore, values of salivary cortisol in the winners were within the normal range (although higher) and not unusually higher than those of the losers. Therefore, it seems unlikely that there was any previous ergogenic substance use by these athletes.

In conclusion, this study suggests that winning competition performance in judo may be influenced by the levels of psychophysiological arousal. Winners presented higher levels of pre-competition psychophysiological arousal, as evidenced by the

higher salivary cortisol concentrations and higher self-ratings of cognitive anxiety in the morning of the competition; subsequently, as the competition progressed, the winners were the ones that managed to control their stress response better, as evidenced by higher saliva flow rate at mid-competition. Practical application of this study could suggest increasing the levels of arousal in the athletes, before competition. A study in rugby union players suggested pre-game presentation of motivational strategies to athletes involving specific video footage and coach feedback can provide effective mental arousal strategies for enhancing match performance (Cook & Crewther, 2012). Further studies could focus on strategies for increasing arousal levels before competition in judo.

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525 Table 1. Mean ± SD responses of salivary hormones, SIgA and saliva flow rate.

		morning	mid-competition	post-competition
salivary cortisol	Winners	11.57 ± 4.44 †	11.73 ± 4.34	9.30 ± 3.57
($nmol \cdot l^{-1}$)	Losers	6.76 ± 2.26	10.08 ± 4.05	7.37 ± 4.88
salivary testosterone	Winners	330.04 ± 92.38	231.42 ± 118.78	235.30 ± 81.84 *
($pmol \cdot l^{-1}$)	Losers	338.70 ± 102.34	276.59 ± 107.30	248.44 ± 102.00 *
salivary testosterone/cortisol	Winners	31.33 ± 9.27 †	23.08 ± 8.46 †*	32.13 ± 21.92
ratio	Losers	54.93 ± 19.89	36.97 ± 15.62 *	45.70 ± 24.97
SIgA absolute	Winners	119.67 ± 76.46	137.69 ± 92.66	95.60 ± 53.12
concentrations ($mg \cdot l^{-1}$)	Losers	126.98 ± 90.90	132.97 ± 79.20	89.34 ± 58.45
SIgA secretion rate	Winners	78.46 ± 56.27	138.64 ± 104.51	86.89 ± 71.88
($\mu g \cdot min^{-1}$)	Losers	85.02 ± 77.46	80.85 ± 49.27	77.59 ± 56.26
saliva flow rate	Winners	0.70 ± 0.21	1.03 ± 0.42 † *	0.94 ± 0.45 *
($ml \cdot min^{-1}$)	Losers	0.66 ± 0.26	0.58 ± 0.32	0.93 ± 0.65 *

526 † indicates significantly different from losers ($p<0.05$); * indicates significantly
527 different from morning ($p<0.05$).

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Figure 1. Individual responses of (a) salivary cortisol, (b) salivary testosterone and (c) salivary testosterone/cortisol ratio across time in winners and losers. Filled dots indicate the winners, empty dots indicate the losers and horizontal lines indicate the mean for each group. † indicates significantly different ($p<0.05$) from losers.

Figure 2. Individual responses of (a) SIgA absolute concentrations, (b) SIgA secretion rate and (c) saliva flow rate across time in winners and losers. Filled dots indicate the winners, empty dots indicate the losers and horizontal lines indicate the mean for each group. † indicates significantly different ($p<0.05$) from losers.

Figure 3. Individual responses of somatic anxiety, cognitive anxiety and self-confidence in winners and losers. Filled dots indicate the winners, empty dots indicate the losers and horizontal lines indicate the mean for each group. † indicates significantly different ($p<0.05$) from losers.

Figure 1 (a), (b), (c).

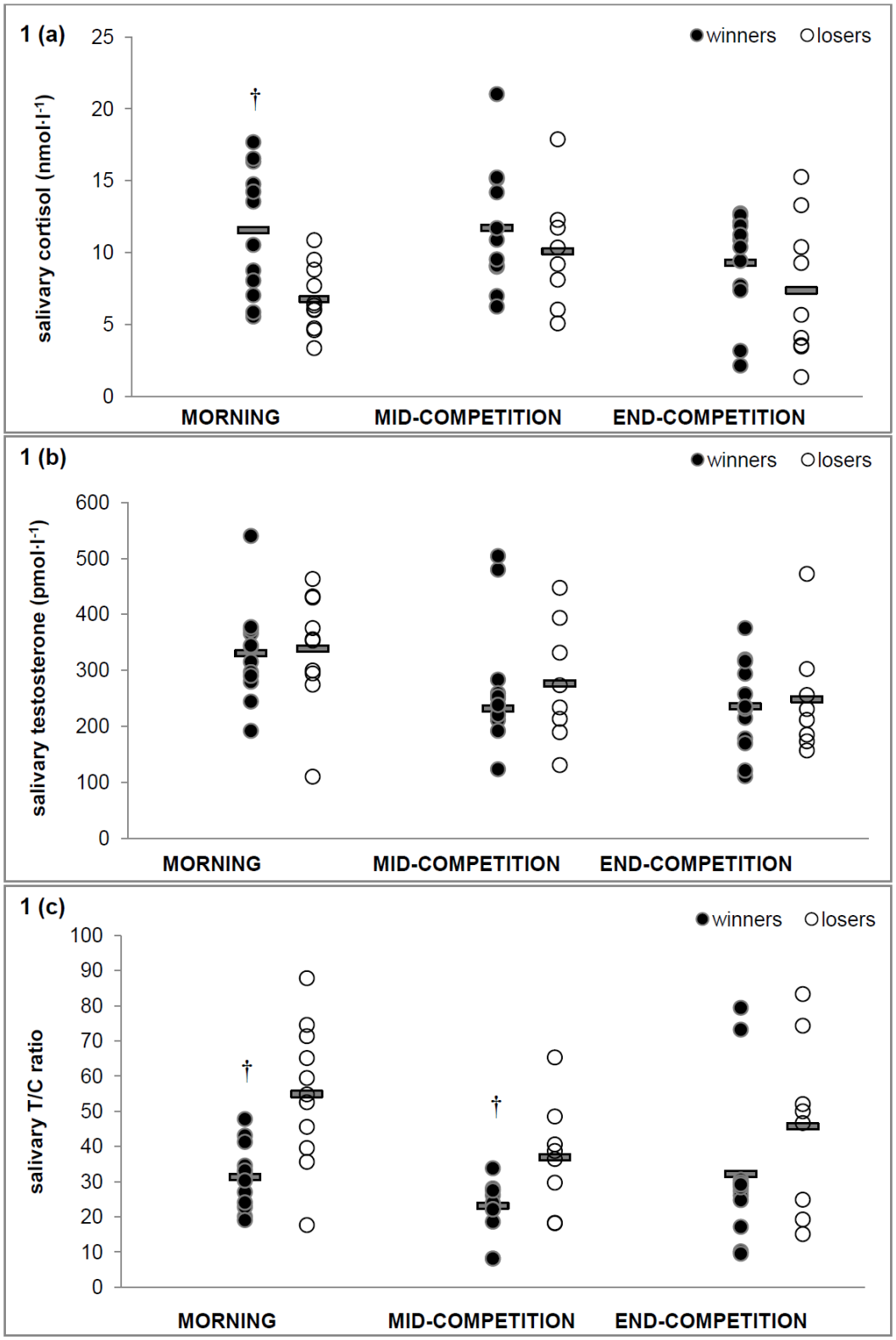

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Figure 2 (a), (b), (c).

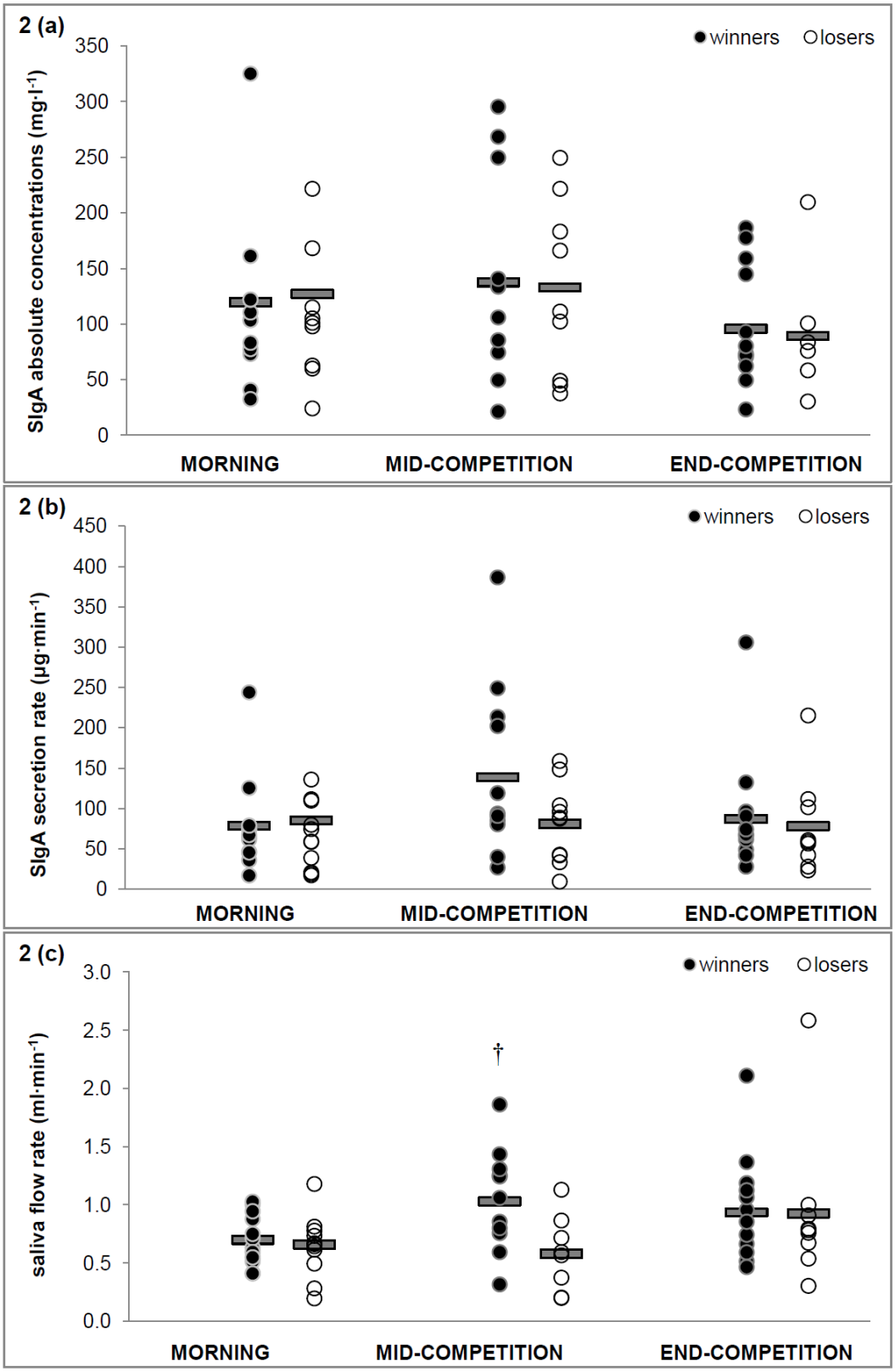

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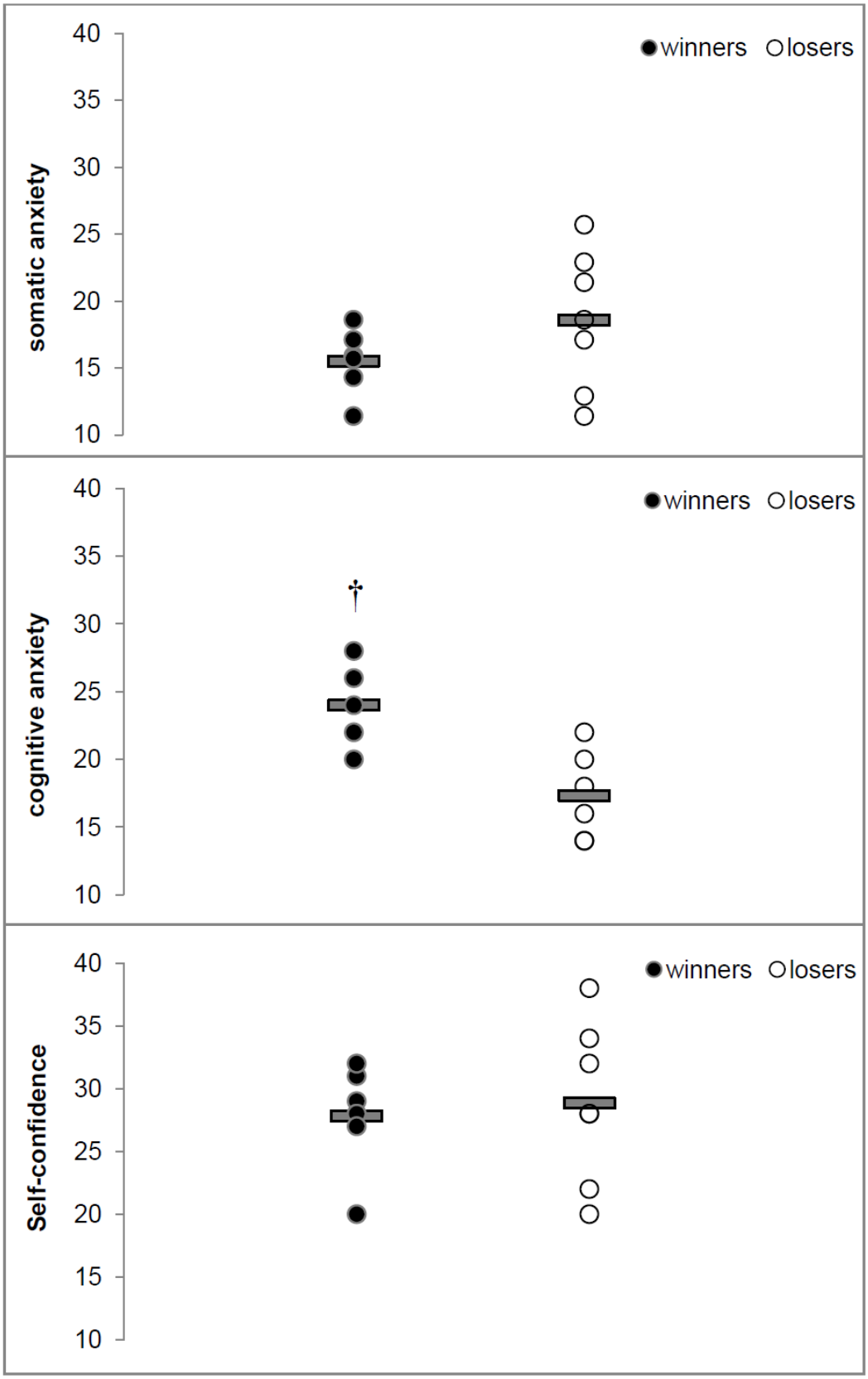
Figure 3.



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