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Testosterone:cortisol ratio as a predictor of podium in adolescent rowing athletes

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ABSTRACT

Purpose: There is a lack of data regarding the stress and motivation response in adolescent athletes during competitions. The athletic performance can be highly influenced by stress rather than appropriate training, at this age. The aim of this investigation is to evaluate the level of stress markers in adolescent rowers in different competition settings that might alter their stress status and performance.

Methods: Adolescent rowing athletes (12–18 yrs) have been tested for determining saliva content of stress biomarkers, cortisol and testosterone, before and after competitions that have been performed indoor and outdoor. Specifically, samples have been taken in the morning, before and after the race in 2 different settings: 1) an indoor rowing competition with an ergometer, 2) an outdoor rowing competition on boats.

Results: A reduction in cortisol levels has been observed in athletes right before the outdoor race, while testosterone levels increased at the same time point before either the ergometer or boat competition and kept rising at the end of the race. Significant differences have been found comparing the testosterone/cortisol ratio between indoor and outdoor data, being higher in the indoor race at all considered time-point. Furthermore, the linear regression demonstrated that the increased ratio correlated with a better podium position in the indoor race.

Conclusion: Despite the age differences among athletes might have an influence on their hormone levels, these data suggest that rowing athletes subjected to different kind of competitions show a different stress and motivation response profile that might influence their performance.

1. Introduction

The athletic performance in adolescence can be highly influenced by stress rather than appropriate training, as stress hormones have substantial variability during puberty. There is a lack of data regarding the stress and motivation response in adolescent athletes during competitions. Generally, the hypothalamic-pituitary adrenal-axis (HPA) is activated due to psycho-socially related stressors [1, 2], leading to high cortisol secretion from the adrenal gland, which plays an essential role both in the regulation and utilization of energy derived from carbohydrates and proteins as well as the production of reserve materials for metabolic adaptations to physical

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effort [3]. The adrenal gland, also releasing adrenalin, induces an increase in testosterone levels from testes, a masculinizing hormone whose classic functions depend on the age of the subject [4]. In relation to both hormones, training and competition have been shown to induce distinct responses; in particular, competition is characterized by different variables which are not always present in training [5].

Rowing can be considered either a team and an individual sport since the same athlete, in non-elite races, may compete in single or multiple scull boats, thus the technical difficulties of the required skills, the nature of the event, and the competition level (regional or national) may influence the physical and psychological response of the athletes [6,7]. The subjective response to stress is dynamic and complex includes multiple variables, especially during different official level events [8].

Some Authors have demonstrated a higher stress-associated response in competition than training in sports such as running, soccer, golf and Jiu-Jitsu [5]. The anticipatory stress, also known as CAR (Cortisol Awakening Response) combines characteristics of a reactivity index (the starting of a new day) with the circadian cycle (it repeats every day at the same time), providing information regarding the HPA reactivity of subjects to psychosocial, cognitive and health-related stressors [9]. In rowers has been noted a differential response to mental and physical stressors, probably related to the type of performance which is highly demanding either in terms of technical skills and muscular efforts, involving the explosive force together with the resistance [10].

The daily fluctuations of testosterone during adolescence range in the morning (9:00 to 13:00 a.m.) from 35.4 to 20.4 pg/ml with a net 40 % decrease due to the circadian rhythm [11], thus changes in this normal variations may account for different behavioral responses.

It is known that the importance of the race can also change the type of hormonal response and the level of the two hormones can be modulated as an anticipatory response to an upcoming competition to prepare the organism to the physiological and behavioural demands. Monitoring hormonal responses of cortisol and testosterone during training and competition seems to provide precious stress level information, in order to properly plan training and to avoid overtraining. Very few studies have investigated the variation of hormonal markers associated with training or competition in adolescents practicing rowing. As a matter of fact, the testosterone/cortisol ratio is useful to emphasize the way in which the variations in these two hormones take place during prolonged exercise and training periods, in other sports it has been observed that changes in testosterone and cortisol levels either affects performance and contribution to team outcome, but are in turn affected by the venue and the importance of the competition itself [12–15].

The evaluation of testosterone/cortisol ratio has been studied in several settings and a recent meta-analysis [16] showed that most of the changes in this ratio are achieved during aerobic and power exercise rather than endurance training. If this is true for most sports, which are predominantly aerobic or anaerobic, there could be differences in the specific case of rowing since it is an aerobic and anaerobic sport with a lactacid and a-lactacid component during the race [17]. In addition, in young amateur rowing athletes very little is known regarding testosterone/cortisol ratio and its possible role as a predictor of performance beyond the classical use to determine adequate recover after training in elite sportsman [18].

The study arises from the need of comparing the differences in bio-humoral response during indoor and outdoor races. As a matter of fact, these two types of races have peculiar differences especially in amateur athlete, in fact during indoor races there is a greater physical stress with a lower influence of coordination technique as compared to the outdoor race were the coordinative and technical skills prevail. In addition, different other factors could mentally influence the athlete's performance in outdoor races, such as weather conditions and the assigned lane. These peculiarities could account for an increase in testosterone during the indoor race and a more stressed attitude during the boat race. Therefore, the aim of this investigation is to evaluate the level and variations of the two hormones, cortisol and testosterone, important for the response to the race, in adolescent rowers in different competition settings that might alter their stress status and performance.

2. MATERIALS and method

2.1. Subjects and training protocol

The study was carried out in accordance with Helsinki declaration and approved by the rowing team coach of Circolo Canottieri Peloro (based in Messina, Italy), the medical staff, and by the Ethics Committee of Messina University Hospital (approval protocol number 4157/2021). Written informed consent was released by the parents of all participants to the corresponding author. Seventeen Sicilian teenage male rowers (age 13.5 ± 2.4 years; height 164 ± 12 cm; weight 60.41 ± 14.56 body mass index (BMI) 21.89 ± 2.97 kg/m^2) of the rowing team Circolo Canottieri Peloro participated in this study (Table 1).

Of the 32 adolescent male athletes of our team 15 were not included for the following reasons.

- 2 athletes were elite rowers of the national team;

Table 1
Characteristics of the amateur young athletes enrolled.

Characteristics	Mean ± SD
Age (years)	13.5 ± 2.4
Height (cm)	164 ± 12
Weight (kg)	60.41 ± 14.56
BMI (Kg/m^2)	21.89 ± 2.97

- 5 athletes had a BMI above 25.0 kg/m^2;
- 8 athletes were not authorized by parents to undergo the saliva test.

The rowers were all second/third year competitors and both indoor and outdoor races were regionals. None of the enrolled subject could be considered as an elite athlete, but mostly amateurs. All subjects followed a training routine of 6 days per week, based on a mesocycle preparation program, training sessions for races were specifically assessed 6 weeks before competitions (Table 2).

In detail, before the indoor race athletes did 5 sessions of training per week, 2 on the ergometer, 1 wt and running and 2 boat training sessions with intensive endurance and load volume.

On the other hand, the 6 weeks before the outdoor regatta the number of boat sessions become 3 with high intensity training at 70–80 % of the best training load of the athletes.

In both cases, the week before the race the focus of training is on physiological recovery implying less volume at 50 % of their best training load. The last days before the competition, training sessions are relatively short though intensive, tactical situations like starts and sprints are studied with the coach, and 3 days before the race, a simulated session is dedicated to test the race distance on the ergometer.

2.2. Collection of saliva samples

Saliva collection was performed in 3 different moments: early morning (T0, between 6:00 and 7:30 a.m.), 5 min before the race (T1), and 15 min after the race (T2) both on a day of indoor and on a day of outdoor race. Saliva samples were self-collected by the athletes using Salivette devices Sarstedt (Medical Systems, Genova, Italy) according to manufacturer's instructions. All participants and their parents have been instructed for sample collection as follows: the T0 sample was taken immediately after awakening before getting out from the bed, drinking anything, eating, and washing their mouth; as a matter of fact, the salivette device was left on the nightstand the night before and to be sure that the instructions were followed properly a tutorial video was sent to all participants through a chat group. The T1 sample was taken 5 min before the race, between 10:00 and 13:00 a.m. for the indoor and between 9:30 and 13:30 a.m. for the outdoor. For the boat race the athlete was instructed to carry the salivette on the boat and do the saliva collection when the equipages of the preceding race were called for alignment on the starting line, this would account for 5 min before the actual start of the race for the rower. While for the T2, the time for sampling was set at 15 min after the race in either competition settings.

2.3. Biochemical tests

Total salivary proteins were quantified using the standard Bradford assay and salivary cortisol and testosterone were measured by indirect competitive ELISA kits (Abcam, Prodotti Gianni, Milan, Italy) as previously reported [15]. Salivary proteins were used to normalize hormone concentrations. Cortisol levels were expressed as ng/ml in all results and were transformed to nmol/L using an online tool (https://unitslab.com/node/110) to calculate the ratio with testosterone (results shown in Tables 5 and 6, and Fig. 4). Testosterone levels were always expressed as pg/ml.

2.4. Statistical analyses

The numerical data were expressed as means and standard error (SEM). The distribution of examined variables was not found normal (for cortisol and testosterone values at baseline), as verified by Kolmogorov–Smirnov test. Then, the nonparametric Kruskal Wallis test and Mann–Whitney *U* test were used for comparison of the 3 data sets (T0-T1-T2) and the two-by two comparisons between conditions (indoor and outdoor competitions). The Spearman correlation test was applied to determine the existence of any significant interdependence between the examined variables, as previously reported [15]. Linear regression model was adopted to evaluate the association of testosterone/cortisol ratio with the podium position in both competitions. A post-hoc power calculation (T test Wilcoxon signed-rank test) was performed on cortisol saliva levels during the indoor race, considering the median values of the 10 enrolled subjects at pre-race and post-race with a probability of error $\alpha = 0.05$. The post-hoc power calculation, including 10 enrolled subjects as sample size with median cortisol saliva levels of 4,64 ng/mL at pre-race and 12,35 ng/mL ($\pm$ SD = 7,68) at post-race and with an α error probability = 0.05, showed an effects size d = 1.142857 and a power (1-β error probability) = 87.6 %. The CAR was calculated by subtracting cortisol levels at awakening (T0) from levels before the race (T1). A P value < 0.05 was considered statistically significant.

Table 2
Training protocol for indoor and outdoor race.

	6 weeks before indoor race	6 weeks before outdoor race
Ergometer	1 time/week 12 km; 1 time/week 40 min interval training	1 time/week 4 repetitions 2250 m (rest 5 min)
Weights and running	1 time/week 60 min weights and 40 min running	1 time/week 60 min weights and 40 min running
Rowing	1 time/week endurance 12 km; 1 time/week 8 reps of 750 m high intensity	1 time/week 3 reps of 3 km high intensity; 1 time/week 1 × 8 km (top performance); 1 time/week 4 repetitions of 1 km (top performance)

Table 3
Cortisol and Testosterone levels on the day of the indoor race. Values are mean ± SD of the 17 athletes.

Timing	Cortisol ng/ml	Testosterone pg/ml	P value
Morning	4.96 ± 2.01	113.23 ± 63.30	
Pre-competition	6.53 ± 6.15	104.68 ± 48.63	Cortisol T1 vs T0 <0.05
Post-competition	10.27 ± 6.86	173.62 ± 69.12	Cortisol T2 vs T0 and T1 <0.01 Testosterone T2 vs T0 and T1 <0.05

Table 4
Cortisol and Testosterone levels of the 10 athletes evaluated at the indoor and outdoor event. Values are mean ± SD of the 10 athletes. *outdoor vs indoor.

Timing	Indoor	Outdoor	P value
Cortisol ng/ml			
Morning	4.64 ± 1.88	12.73 ± 7.62	<0.01*
Pre-competition	8.75 ± 6.76	11.10 ± 7.26	0.40
Post-competition	12.35 ± 7.68	14.20 ± 9.36	0.53
Testosterone pg/ml			
Morning	111.40 ± 44.25	41.34 ± 21.61	0.09
Pre-competition	118.84 ± 43.97	46.53 ± 15.12	0.02*
Post-competition	183.43 ± 63.14	61.61 ± 23.13	0.05

Table 5
Testosterone (pg/ml)/Cortisol (mmol/l) ratio at the three time points evaluated, indoor vs outdoor race. *outdoor vs indoor.

Testosterone (pg/ml)/Cortisol (nmol/l) ratio	Indoor race	Outdoor race	P value
Morning	10.2 (±5.1)	1.6 (±1.1)	<0.01*
Pre-competition	8.2 (±6.3)	2.3 (±1.6)	<0.01*
Post-competition	9.8 (±11.6)	2.1 (±1.8)	0.02*

Table 6
Testosterone/cortisol ratio according to expected or un-expected results at the indoor or outdoor race. The final outcome of the race was used to divide the athletes in the two groups according to their ability to perform better or as expected. *expected vs un-expected.

Testosterone(pg/ml)/cortisol (nmol/l)	Expected (mean ± SD)	Un-expected (mean ± SD)	P value
Indoor			
T0	10.33 ± 4.92	9.90 ± 6.09	0.351
T1	5.68 ± 5.02	11.95 ± 6.68	0.528
T2	5.13 ± 2.69	16.85 ± 16.34	0.015*
Outdoor			
T0	1.80 ± 1.28	1.03 ± 0.66	0.099
T1	2.77 ± 1.71	1.10 ± 0.26	0.048*
T2	2.53 ± 2.06	1.20 ± 0.61	0.195

Graphics were performed using Graph Pad Prism software and the post-hoc power calculation was calculate using G Power 3.1 [19].

3. Results

3.1. Effects of training on performance

The enrolled subjects (n = 17) were all adolescent male athletes doing rowing from at least 2 years, and both indoor and outdoor races were regionals.

All athletes were subjected to the same training protocol reported in Table 2. The protocol was specifically studied to obtain the maximum performance for the two types of competitions and lasted 3 months before the indoor and 3 months before the outdoor race. The week before race all athletes performed a test on the ergometer to evaluate the improvements, and to test race distance.

3.2. Cortisol and testosterone changes during the indoor competition

The first set of results, obtained during the indoor competition on 17 athletes, showed that salivary cortisol had a different trend from subject to subject over time. However, 13 out of 17 athletes had similar values ranging between 0 and 10 ng/ml, as shown in the graph below.

As depicted in Fig. 1 A and Table 3, cortisol mean values were 4,96 ± 2,01 ng/ml in the morning, 6,53 ± 6,15 ng/ml pre-race and 10,27 ± 6,86 ng/ml post-race. In general, there was an increase in cortisol levels before the competition, compared to the morning values ($p < 0.05$). However, these levels further increase 15 min after the race compared to the morning values ($p < 0.01$). The same level of significance ($p < 0.01$) was observed when comparing the levels of this stress hormone in the post-competition vs pre-competition. A reduction in cortisol levels at T1 and T2 compared to T0 was found only in 2 athletes, probably due to their young age (11 years). When recalculating means excluding these 2 outliers cortisol mean values were 4,74 ± 1,87 ng/ml in the morning, 7,15 ± 6,32 ng/ml pre-race and 11,46 ± 6,51 ng/ml post-race with no substantial changes into significance.

Salivary testosterone levels (Fig. 1B and Table 3) from the indoor competition, showed a similar trend to cortisol, even if no significant differences were found between the morning values and those obtained before the race.

However, there was a statistically significant increase after the competition ($p < 0.05$), probably due to the agonism of the race; the

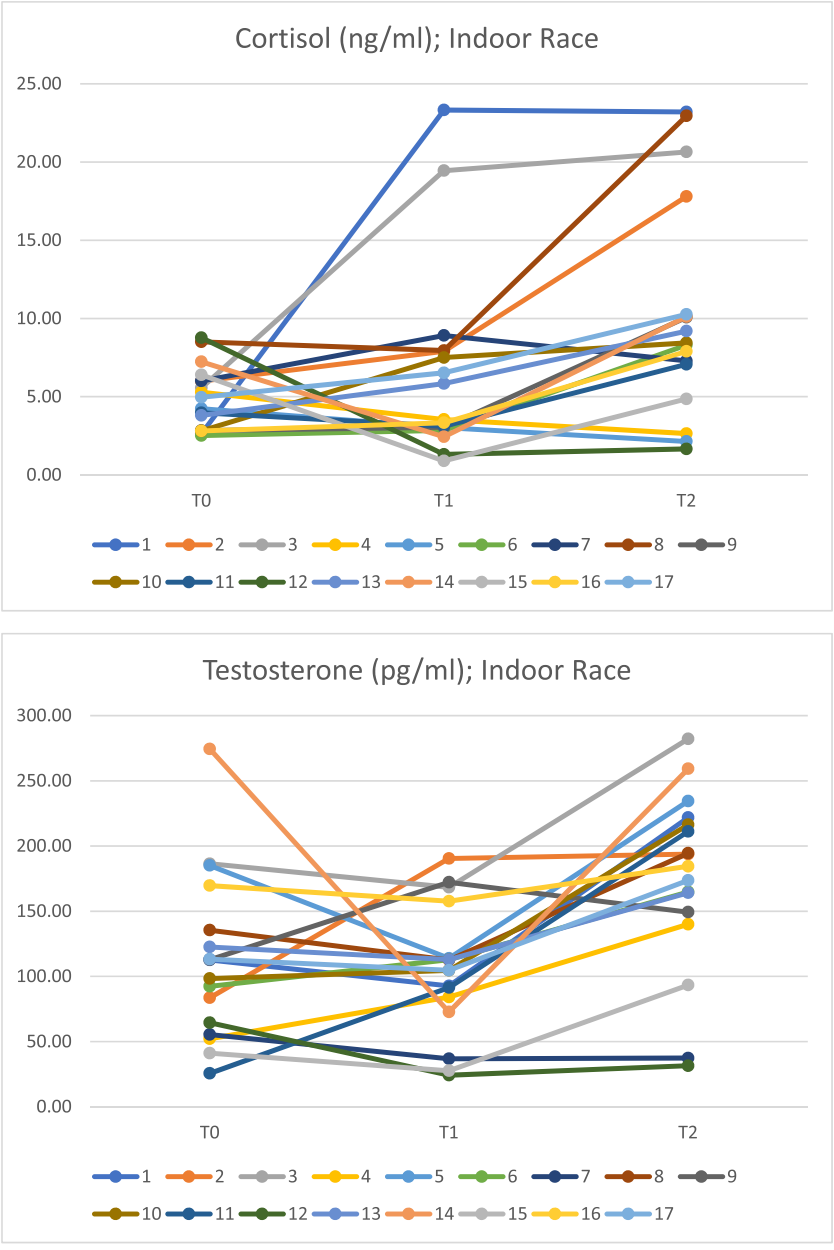

Fig. 1. ALevels of cortisol (ng/ml) at awakening (T0), before (T1) and after (T2) the indoor race. Each color identifies a single athlete. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.) BLevels of testosterone (pg/ml) at awakening (T0), before (T1) and after (T2) the indoor race. Each color identifies a single athlete as in Fig. 1A. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

post-competition increase in testosterone was significantly higher versus the other values. A further similarity between the results of the two hormones is the reduction in testosterone values in the same two athletes in the post-competition evaluation. When recalculating means excluding these 2 young rowers testosterone mean values were 120.84 ± 64.13 pg/ml in the morning, 115.28 ± 42.40 pg/ml pre-race and 193.51 ± 47.90 pg/ml post-race with no substantial changes into significance.

3.3. Cortisol and testosterone changes during the outdoor competition

For the outdoor competition the 2 youngest athletes were not included in the evaluation and 5 other athletes were not able to participate because were in quarantine for testing positive to COVID-19, this resulted in a homogeneous sample of athletes aged 14–18 yrs. Fig. 2A and B depict the individual values of the 10 athletes (using the same identification color used in Fig. 1A and B) regarding either cortisol and testosterone. Table 4 includes the values of cortisol and testosterone of the 10 athletes that performed both races on

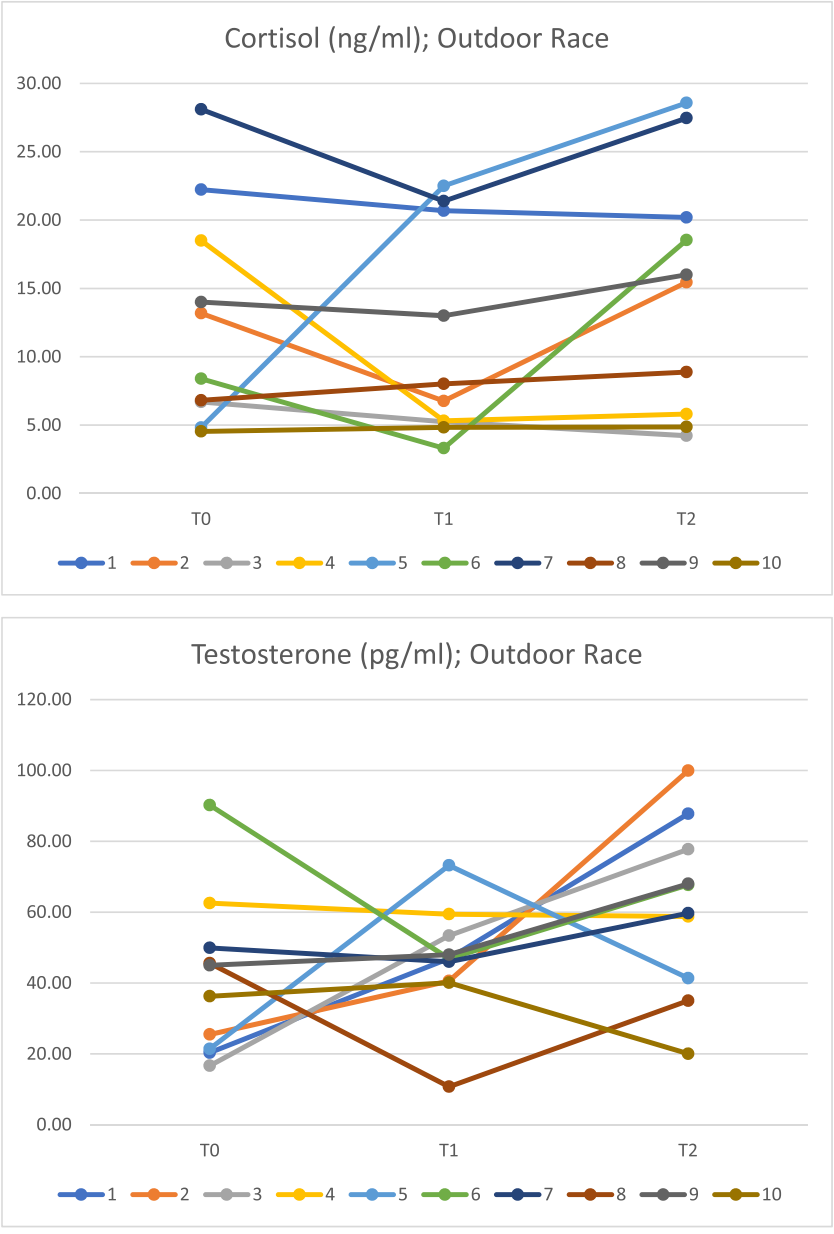

Fig. 2. ALevels of cortisol (ng/ml) at awakening (T0), before (T1) and after (T2) the outdoor race. Each color identifies a single athlete. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.) BLevels of testosterone (pg/ml) at awakening (T0), before (T1) and after (T2) the outdoor race. Each color identifies a single athlete. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the day of the indoor competition.

The day of the outdoor competition, the athletes showed a very similar pattern of distribution of cortisol values, in particular the morning expression was significantly higher (12.73 ± 7.62 vs 4.64 ± 1.88 ng/ml; $p < 0.01$) as compared to the indoor race (Table 4). The pre- and post-race values were almost similar (pre-race 11.10 ± 7.26 and post-race 14.20 ± 9.36 ng/ml) although slightly reduced right before the competition (Table 4). No difference was found comparing the levels of the stress hormone in the two competition settings pre- and post-competition (Table 4).

Testosterone levels were lower in the morning of the outdoor race (41.34 ± 21.61 pg/ml) compared to the ergometer session (111.40 ± 44.25 pg/ml), even if there was no significant difference (Table 4). The aggressiveness hormone increased following the race as observed also in the indoor competition, although a difference was found only when comparing the two competition settings pre-race values (Table 4; $p = 0.02$).

The CAR plays an important role in determining the ability of an athlete to cope with stressors as it easily happens during competitions, rather than training sessions, in our sample we observed a good cortisol response in all subjects involved in the indoor race; while 4 out of 10 athletes of the outdoor competition demonstrated higher levels of anticipatory stress, having a difference of more than 2.5 ng/ml in their cortisol levels compared to baseline.

Considering the stress generated by the competition itself, at the indoor race 4 out of 10 had increased cortisol levels at the end (T2) of the competition, while 5 athletes (4 of which did not cope well with stress either at the indoor race) were more stressed after the outdoor competition.

Considering the CAR as the difference between the levels of cortisol at T0 and T1, 4 subjects at the indoor and only 1 at the outdoor race, demonstrated a greater anxiety on the day of the competition. This has been done taking into account that all the T0 and T1 evaluations have been done in the same time range, representing single assessments for each athlete.

We further considered for each of the 10 athletes that completed the indoor and outdoor races the percentage of increase in either

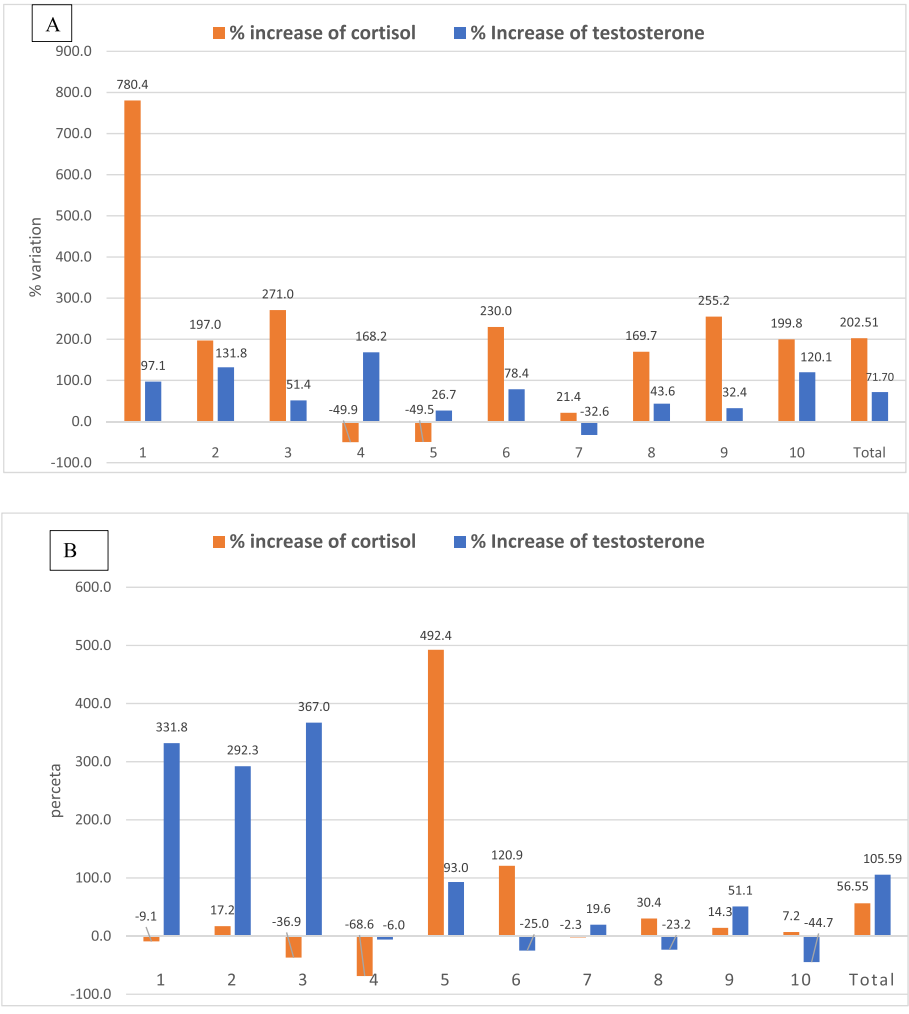

Fig. 3. Percentage change of the values of cortisol and testosterone stratified for each athlete, for both indoor (A) and outdoor race (B). Each number on the X-axis corresponds to a specific athlete.

cortisol and testosterone, as shown in Fig. 3 (A and B). The graph shows that 8 out of 10 athletes were more subjected to an increase in cortisol levels and 9 out of 10 had an increase in testosterone during the indoor race (Fig. 3A). As demonstrated by data, rowers #4 and #5 had an increase in testosterone levels only, while rower #7 showed augmented levels of cortisol only (Fig. 3A). As far as the outdoor race is concerned, we observed a percentage increase in both hormones in 6 out of 10 athletes (Fig. 3B). In this setting rowers #1, #3, and #7, had increased levels of testosterone only, while rowers #6, #8, and #10 increased only their cortisol levels; while rower #4 had a peculiar condition of reduced release of both hormones when the whole time span was considered (Fig. 3B).

Significantly higher values of the Testosterone/Cortisol ratio were observed during the indoor race than to outdoor competition, at all time points (Table 5). In addition, intra- and inter-individual differences were reported for the three time points for each competition in Fig. 4A and B.

To better understand the influence of the hormonal response on the performance of the 10 athletes that competed either in the indoor and the outdoor races, we interviewed the coach (Mr. Dario Femminò) to identify those who had an expected or an un-expected race result. According to his evaluation we identified 3 out of 10 that performed un-expectedly at the indoor and 4 out of 10 at the outdoor. The un-expected performance correlated with a higher testosterone/cortisol ratio at the end of the indoor race (T2), while athletes that performed as expected by the coach had a more aggressive behavior right before the race (T1) in the outdoor competition as demonstrated in Table 6.

Since the testosterone/cortisol ratio can be used as a predictor of performance, the podium results were correlated to this ratio revealing that at least for the indoor race the higher levels of this ratio correlated with the highest podium positions (Fig. 5A; $B = -0.266$; $p = 0.032$). The same relationship was not found on the day of the outdoor competition, although a certain pattern or correlation was evidenced (Fig. 5B; $B = -0.144$; $p = 0.795$).

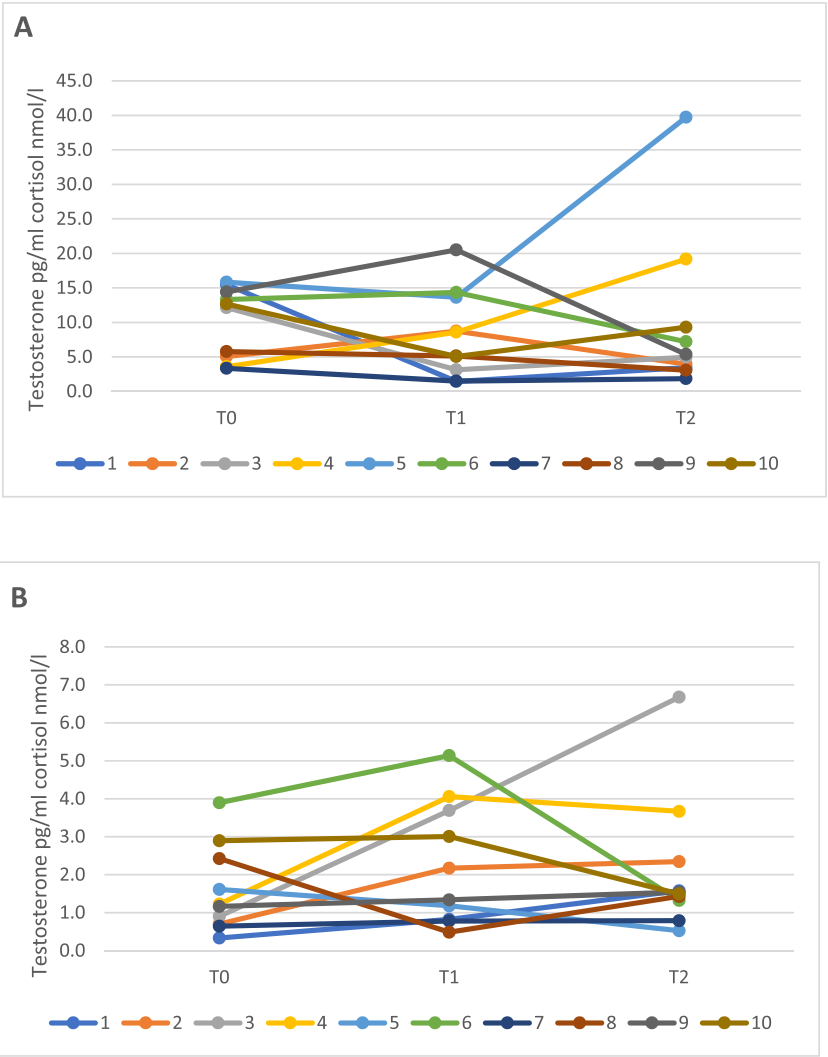

Fig. 4. Testosterone (pg/ml)/Cortisol (nmol/l) ratio stratified for each athlete in indoor (A) and outdoor (B) race.

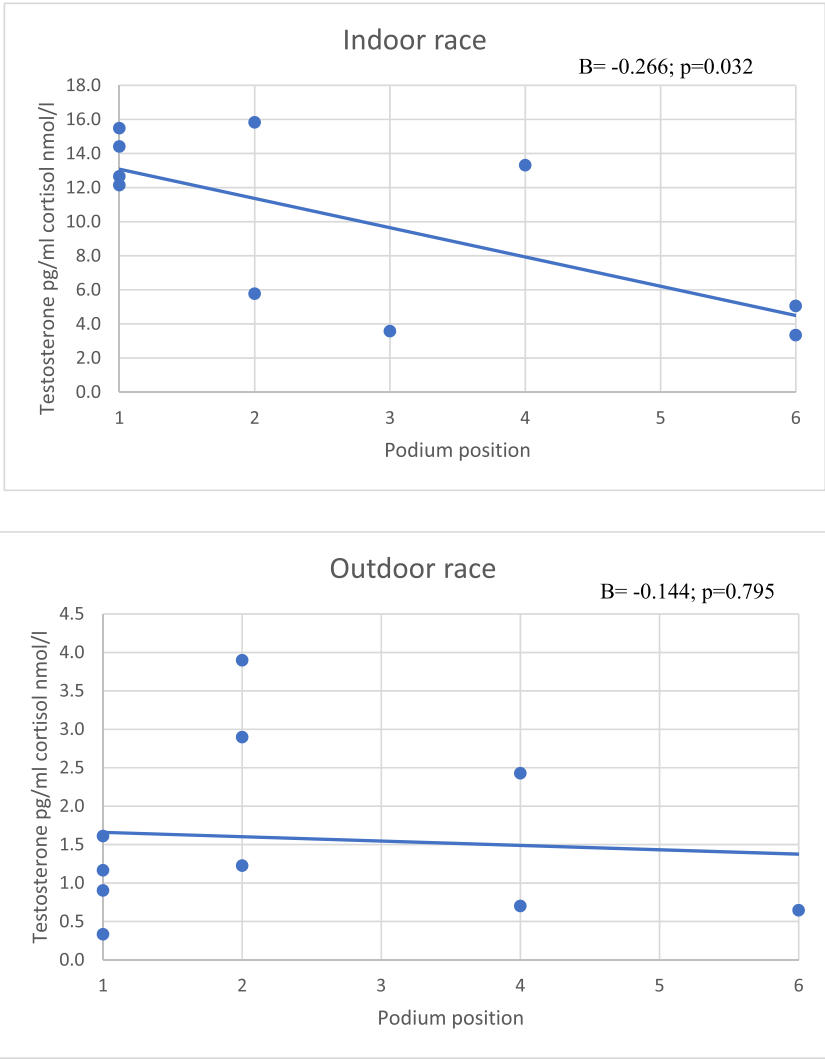

Fig. 5. The testosterone/cortisol ratio significantly influenced the position podium (B = −0.266; p = 0.032) in indoor race. In particular, higher testosterone/cortisol ratio values were associated with the better podium positions (A). Instead, no significantly associated was observed in outdoor competition (B = −0.144; p = 0.795), (B).

4. Discussion

The changes in cortisol and testosterone levels in players might be due to a winning/losing situation

or to the social support of team mates, families and coaches. Even in the context of a dominance contest, aggressive behavior (demonstrated by higher testosterone levels) depends less on motivational and hormonal factors, directly linked to dominance, and more on stress and its endocrine basis [20]. It is known that at least for men, latent power motivation is positively correlated with

baseline levels of testosterone [20].

According to the dual-hormone hypothesis, both testosterone and cortisol regulate dominance motivation in men and women. However, high cortisol levels reduce or even halt testosterone response [21].

This same pattern of hormonal response was also observed in our sample where the variability in testosterone and cortisol levels was mostly related to the general setting of the race. The dual-hormone response has been observed in our athletes during the indoor race, their raised cortisol levels were paralleled by a reduced increase in testosterone levels. As a matter of fact, during indoor races all the attending people is close to the athletes, the coach and the public are right behind the rower and this provides strong feelings of support and stress, which can be similar to the stress of competing home or away in other sport settings [22]. In fact, cortisol levels on the day of the race were lower when measured at the time of awakening for the indoor as compared with the outdoor competition, this can be considered as a normal response, known as CAR (cortisol awakening rise) [23]. This might also be due to mental factors, as the

awareness that the real race for a rower is on the boat rather than on the ergometer. Despite a blunted CARs before a competition may reflect burnout and exhaustion, however an exaggerated cortisol increase reduces testosterone leading to reduced aggressivity, which may be responsible for a poor performance. As known CAR is considered an adaptive response to prepare for forthcoming demands; but no signs of overtraining were observed in our rowers. In addition, the nature of the two competitions (regional races) might also have played a role in the reduced stress responsiveness. Similar results have previously described in elite rowers either as a positive function of repeated exercise or habituation to competition [24].

Indeed, testosterone fluctuations demonstrated a linear increase from morning values (with no difference between awakening and pre-race) to post-competition in the indoor race probably due to the close presence of team mates and coaches causing an excessive aggressive and motivational response. Also, the type of physical effort at the ergometer implies a maximal muscular response and less technical skills that may justify the rise in testosterone levels after the race, independently of the winning/losing outcome.

On the other hand, pre-competition levels of cortisol were similar in the two settings, showing a little decrease that was independent of the time of the day, considering that in both cases the races have been performed in the morning, so the time of the day has not an influence on these values. These same hormonal values, measured in saliva samples, were already observed in a previous work that compared the levels of cortisol in light and heavy-weight rowers in a day of competition and during the week of the race [10].

The day of the outdoor race the levels of testosterone followed the trend of cortisol, showing an increase at the last evaluation, this parallel activation seems to be specific of adolescence [25] to influence the psychology of dominance and competition. It has to be considered also the metabolic role of testosterone that plays a pivotal role in gluconeogenesis via the proteolytic pathway, in the storage of glycogen and in protein synthesis at muscular level [26]. The higher increase noted in testosterone following the indoor race, was probably induced by adrenaline stimulation, and by the stimulatory effect of lactate which is mostly produced during ergometer sessions rather than boat races [27]. It is also important to note that in adolescent non-elite athletes the improvement of the technical approach is important to determine a better physical response, also in terms of stress-related biomarkers and to maximize the effort of the performance.

As far as the cortisol response is concerned during the indoor competition the race was between 10 and 13 a.m., in this time frame normal cortisol levels for age-matched adolescents decrease from 3.95 to 1.81 ng/ml [28], while in our athletes was 4.64 ng/ml at T0 (between 7 and 7:30 a.m.), 8.75 ng/ml at T1 and 12.35 ng/ml at T2, clearly indicating a higher level of stress. During the outdoor competition races were between 9:30 and 13:30 a.m. and the mean cortisol levels at T0 (at 6:00 a.m.) were 12.73 ng/ml, 11.10 ng/ml at T1, and 14.20 ng/ml at T2; these values strongly suggest a higher stress level also compared to the indoor race. In the context of the outdoor race, the athlete is stressed for several factors which may include the early awakening (usually at 6:00 a.m.), the trip to the race venue (usually lakes or rivers far from cities), the boat and oars setting, and the race itself. In the specific condition evaluated in this study, the coach Mr Dario Femminò ruled out the stress deriving from boat setting because his team of coaches and helpers, usually travels one day before the competitions to the venue to set up all the equipment for the athletes, thus the stress derives only from the early wake up and the bus trip (usually 2–3 h), plus the race itself. These stressors are not the same as during the indoor race, in fact the venue is a gym in the city which minimize travel time and does not force the athletes to an early wake up, additionally the only equipment needed is the ergometer that is already set up when the competition starts. Considering also the above mentioned variables is not difficult to understand the predictability of the different stress levels observed in our athletes in the two race settings.

In the case of testosterone, the mean salivary levels reported in age-matched adolescents [11] in the morning (9:00 to 13:00 a.m.) range from 35.4 to 20.4 pg/ml with a net 40 % decrease, while in our sample values ranged from 111.4 to 183.4 pg/ml in the indoor race, while the values obtained for the outdoor race ranged from 41.3 to 61.6 pg/ml. In both competitions our athletes demonstrated an increased aggressivity related to the race that highly affected the circadian rhythm of testosterone, as could be expected during such a demanding condition.

As the HPA is activated due to several types of stressors from psychological to socially related, CAR is considered an adaptive response to prepare for forthcoming demands; in this context, the

competition-related increases in salivary cortisol clearly supports the activation of the hypothalamo-pituitary adrenal axis. During adolescence some instability of the HPA could be responsible for exaggerated responses both in the anticipatory and in the stress-related response as demonstrated in different cohorts of subjects [29,30].

Considering the overall results, the positive relationship between cortisol and testosterone levels may be related to stimulation of the adrenal gland in response to physical stress, since both hormones are synthesized by the same stimulatory axis, even though testosterone is secreted mainly by testes. Indeed, in most of our athletes a very similar pattern of response for both hormones was noted.

The testosterone:cortisol ratio is commonly used to determine the level of overtraining and sometimes of stress and aggressiveness for athletes of different sports [16] however in young athletes this is not always true due to the hormonal changes typical of adolescence. A recent metanalysis [16] showed that following resistance exercise there are little changes, while most of the alterations in this ratio have been observed during aerobic and power exercise. In this context, considering that rowing is an aerobic and anaerobic sport with a lactacid and a-lactacid component during the race [17], the variation in the testosterone:cortisol ratio might have a peculiar pattern. In addition, it has been little investigated as relative to a single event or in young amateur athletes since this ratio is usually considered a parameter to determine adequate recover after training in elite sportsman [18].

In response to physical exercise, or at least until overreaching when a negative correlation between cortisol and testosterone levels is reported, testosterone increases linearly with peak concentrations at the end of training sessions [31]. The testosterone:cortisol ratio has been also shown to be reduced by at least 30 % when the athletes suffer from overtraining [32]. It has been proposed that during training conditions the increase of free testosterone is at least partially due to the contribution of the adrenal cortex which counteracts the depressor effect of cortisol and the normal circadian fluctuation. Additional evidence obtained in runners sustain the hypothesis

that this type of acute biphasic response may correlate to the intensity of the stimulus, as a matter of fact, endurance runners have a reduced increase in testosterone as compared to middle-distance runners following an acute effort [33,34]. On the other hand, amateur runners demonstrated increased cortisol levels two days before the marathon, while professionals had increased testosterone levels, probably due to a reduced HPA activation in response to the training volume [35,36]. In the present investigation, the testosterone/cortisol ratio was intended to be a marker of the ability of the athlete to cope with stressors in the two different race settings, despite the young age of the subjects the obtained results indicate that the outdoor competition greatly stimulates the competitiveness and combative attitude since the testosterone dynamic was significantly modified. This result was to be expected since the percentage increase in both cortisol and testosterone, evaluated to understand if the two different competitions could influence the hormonal levels, demonstrated the prevalence of the aggressivity hormone in the outdoor race. These results suggest that boat races, usually with multiple rowers racing together determine a lower rate of pre-competition stress and aggressive-agonist behavior as compared to the ergometer where the athlete is alone. As a matter of fact, our hypothesis was that the more stressful event was meant to be the boat race, as it could be considered "the real race" for a rower, surprisingly this was instead the competition that had a greater stimulus on the aggressive and competitive attitude of the athletes. Clearly this result could be due mainly to the fact that our athletes were amateur and thus approached the race with a lower stress level. The overall data comparing the 2 competition settings (Fig. 3A and B) show a more stressful condition during the indoor rather than outdoor race, this could be indeed an expected outcome given the several stressors that are present during an indoor race. On the other hand, testosterone levels increased more, as a percentage, during the outdoor competition (Fig. 3A and B) as compared to the indoor when considering the overall data.

As a comprehensive review article suggests [37], people naturally exhibit variations in several aspects, such as their level of concern regarding victory and defeat, the enjoyment derived from competitive activities, the attribution of performance to personal effort or external factors, and their perception of their own competence and potential for success in a given competition. These differences may influence the way hormonal responses are experienced during competitive situations. Evidence suggests that alterations in testosterone levels, particularly those that occur after the outcome of a competition is determined, can be associated with the psychological experience of winning and losing, although not consistently. Simultaneous changes in testosterone levels during competition may impact an individual's decisions regarding whether to engage in future competitions, depending on the person and the specific circumstances. Individual characteristics, in conjunction with the context, undoubtedly play a significant role in this regard. One key personal characteristic could be their level of competitiveness. People vary in their willingness to embrace competition, yet the hormonal connections to competitiveness remain relatively unexplored. Another personal factor, cortisol, has the potential to modulate the psychological effects of testosterone in a wide range of competitive situations.

As previously shown blood and saliva levels often correlates and in young athletes is far easier to use saliva as a non-invasive, nor stressing method for determining levels of this useful biomarkers [15,26]. Additionally, as suggested by other studies cortisol changes are not strictly linked to an agonistic activity but also by the cognitive and emotional perception of the event [38,39]. The rise in cortisol and testosterone allows the organism to adapt to environmental demands following the activation of HPA, wich influences behavioral responses to physical and/or situational changes [40].

Some limitations in our study should be taken into account, first of all the relatively small sample that does not allow to generalize the obtained data; second, we do not have hormone data from training sessions; third we did not assay the emotional status of the athletes with an appropriate questionnaire. A further limitation is that we did not find any correlation between the percentage variation of the evaluated hormones and the athlete performance during the race, a bigger sample would have probably provided such information. Nonetheless considering that participation was on a voluntary base it was not possible to expand the sample, but our data provide similar results to those previously described even if are the first ones to report the differences between two race settings in non-elite adolescent rowers. Another important result emerging from this study is the possible role of the testosterone/cortisol ratio as a predictor of performance during a race since it was associated with podium positioning, at least during the indoor race. In the future it will be important to prolong the study observations and to include a female group to determine if the salivary levels of these hormones and their ratio, could be predictive of performance outcomes in young rowers. In addition, due to the hormonal changes observed in the two 11-year-old subjects in our study, other future studies could evaluate the hormonal difference according to the age groups of the adolescents that could have a different type of response to the stress of competition. According to literature data a future further analysis could be performed on the concentration of some myokines as irisin [41], or cytokines, as IL-1β and IL-6 that were positively associated to anger and anxiety during as exams [42] which, in some extent, might cause a similar socio-cognitive stress to a competition, at least for adolescents.

Declarations

4.1. Ethics statement

The protocol has been reviewed and approved by the Ethics Committee of Messina University Hospital (approval protocol number 4157/2021).

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Author's contribution: conceived and designed the experiments (GF, AB, DMD); performed the experiments (GF, DC, AB); analyzed and interpreted the data (GF, AB, MR, CD, DMD, FT); contributed reagents, materials, analysis tools or data (AB, DMD, FT); wrote the

paper (GF, AB, MR).

Data availability

The datasets used and analyzed during the current study is available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Giovanni Ficarra: Conceptualization, Investigation, Writing – original draft. **Daniela Caccamo:** Data curation, Investigation, Methodology. **Michelangelo Rottura:** Data curation, Formal analysis, Writing – original draft. **Alessandra Bitto:** Conceptualization, Funding acquisition, Methodology, Project administration, Writing – original draft, Writing – review & editing. **Fabio Trimarchi:** Methodology, Supervision, Writing – review & editing. **Debora Di Mauro:** Conceptualization, Methodology, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

No competing interest is declared by any of the authors. Alessandra Bitto is co-founder of the Academic Spin-off of the University of Messina.

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