European Journal of Sport Science

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tejs20

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To cite this article: Diana Vaamonde, Juan Manuel García-Manso, Carolina Algar-Santacruz, Asghar Abbasi, Samuel Sarmiento & Teresa Valverde-Esteve (2021): Behaviour of salivary testosterone and cortisol in men during an Ironman Triathlon, European Journal of Sport Science, DOI: 10.1080/17461391.2021.1955011

To link to this article: https://doi.org/10.1080/17461391.2021.1955011

Published online: 30 Aug 2021.

EUROPEAN JOURNAL OF SPORT SCIENCE
https://doi.org/10.1080/17461391.2021.1955011

Behaviour of salivary testosterone and cortisol in men during an Ironman Triathlon*

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ABSTRACT
Endurance exercise induces notable acute hormonal responses on the gonadal and adrenal hormones. The purpose of this study was to assess the changes in salivary testosterone (*Ts*), salivary cortisol (*Cs*) and *T/C* ratio during long-distance triathlon. Ten well-trained male triathletes participated in the study and were assessed for hormonal changes at four time-points (pre-competition, post-swimming, post-cycling, and post-running phases). *Ts* decreased from pre-competition to post-swimming (from 93.37 pg/mL to 57.63 pg/mL; $p < .01$) and increased during two other parts of the competition to almost pre-competition values (cycling: 79.20 pg/mL, $p = .02$; running: 89,66 pg/mL, $p = .04$, respectively). *Cs* showed a similar behaviour; decreasing in the post-swimming phase (1.74 pg/mL) and increasing in the other transitions (post-cycling: 7.30 pg/mL; post-running: 13.31 pg/mL), with significant differences between pre-competition and post- competition values ($p = .01$). Conversely, *T/C* increased significantly from pre-competition to post-swimming phase ($p = .04$) to later decrease until the end of the competition. Overall, *T/C* significantly decreased ($p < .05$). In conclusion, during an Ironman triathlon, hormone values fluctuate in response to the demands of the competition. *Ts* and *Cs* decrease after-swimming, increase after-cycling and reach the maximum values after-running. *T/C* reflects overall catabolic status.

KEYWORDS
Hormones; sex-steroids; stress-response; ultraendurance; competition

Introduction

Triathlon is a sport consisting of sequential swimming, cycling and running events. Today, triathlon is one of the fastest growing endurance sports events. Although there are many variants of triathlon (super-sprint, sprint, olympic, Ironman triathlon and Ironman™), one of the most popular forms is the Ironman or Full Triathlon (swimming: 3.86 km; cycling: 180 km; running: 42.2 km). This is an exhaustive exercise requiring high training volumes (Elite runners: 30-35 h; Contenders: 25-30 h; Finishers: 15-25 h; amateur runners: 7-14 h). All participants must do it in less than 17 h. Due to the nature of this sports event and especially the fact that several sports must be completed in succession, its effect on the organism may differ slightly from other endurance sports. Athletes in this discipline are a small portion of the athletics population. The practice of long-distance endurance modalities entails acute hormonal responses to long-duration exercise (Ginsburg, O'Toole, Rimm, Douglas, & Rifai, 2001). Testosterone and cortisol, indicators of anabolic and catabolic status in endurance sports, are usually measured to assess the stress imposed by these types of sports (Anderson, Lane, & Hackney, 2016; Vaamonde, Da Silva-Grigoletto, Fernandez, Algar-Santacruz, & García-Manso, 2014).

It is well known that prolonged exhaustive exercise induces remarkable acute hormonal responses on the

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pituitary-testicular and pituitary-adrenal axis (Sgrò et al., 2014). Acute endocrine responses to ultraendurance events have also been reported (Tremblay, Copeland, & Van Helder, 2005), particularly during sports events that are similar to the one analysed in this study (Sylta et al., 2016). Having a reliable biological marker to monitor the stress response of the endocrine system to an ultra-endurance event is necessary. Previous studies have reported a wide range of biochemical, hematological, and physiological markers to be stress indicators in the athletes as exercise intensity is progressively increased (Fry et al., 1993). Among them, cortisol (C) and testosterone (T) have been recommended as good markers of physical stress and performance (Sgrò et al., 2014). The effect of physical exercise on cortisol and testosterone concentration have been widely reported with particular emphasis to the fact that cortisol and testosterone change in the opposite direction (catabolic and anabolic, respectively), thus showing that physical exercise produces an imbalance between the anabolic hormone of testicular origin and the catabolic hormone of adrenal origin (Elloumi, Maso, Michaux, Robert, & Lac, 2003). Consequently, many studies (Jones, Howatson, Russell, & French, 2017; Lac & Berthon, 2000; Seidman et al., 1990; Vaamonde, Algar-Santacruz, Abbasi, & García-Manso, 2018) have evaluated the *T/C* ratio to clearly emphasize the way in which the variations in these two hormones take place during prolonged exercise and training periods.

Although the most commonly used tissue to determine hormonal levels in response to exercise is blood, it would be advantageous to be able to measure the hormonal levels with a noninvasive method such as saliva sampling. Analysing hormonal levels in saliva has the advantage of permitting assessments to be made without altering the normal course of the different phases of the competition or increasing the stress to which the athletes are subjected during the competition itself (Moreira, Arsati, de Oliveira Lima-Arsati, Franchini, & De Araújo, 2010; Papacosta & Nassis, 2011). The procedure to collect saliva is easy, completely non-invasive, and very useful in field studies regarding exercise and sports. Thus, it presents many advantages in comparison to using other biological fluids such as serum/plasma. On the other hand, accepting some limitations (Fiers et al., 2014), previous studies have demonstrated the positive correlation between blood and salivary cortisol and testosterone (Crewther, Lowe, Ingram, & Weatherby, 2010), indicating that saliva is a viable source to evaluate hormonal changes in response to exercise.

Studying salivary hormone behaviour during ultra-endurance exercise is a valuable tool to non-invasively detect hormonal changes during endurance competitions, revealing the catabolic or anabolic response of the organism to competition. Moreover, this might also be used in regular training to assess important changes such as those taking place in overreaching/overtraining syndromes. Some studies in triathlon have aimed at describing salivary T and C in type 1 diabetes mellitus athletes (Boehncke et al., 2009), the effect of a 10-day training on T and C response in elite triathletes (Hough, Robertson, & Gleeson, 2015), or to determine the T and C levels at different moments during the day of a triathlon and after 7 days (Balthazar, Garcia, & Spadari-Bratfisch, 2012). However, no study has determined the T and C levels after each transition during a triathlon. Therefore, the purpose of the present study was to assess the changes in salivary testosterone (*Ts*), cortisol (*Cs*) and *T/C* ratio at pre-competition (t0), and three post-exercise time points including post-swimming (t1), post-cycling (t2), and post-running (t3) during an official ultra-endurance event (Ironman Lanzarote).

Materials and methods

Subjects

Ten experienced and highly trained male triathletes (finishers and amateurs) voluntarily participated in the study (age: 34 ± 5.6 years; height: 177.5 ± 4.57 cm; weight: 73.6 ± 6.53 kg). All of the subjects were experienced athletes and had undergone triathlon training in a systematic manner for at least 12 ± 7.62 years, and they had all previously participated in events of this nature. None of the athletes suffered from acute or chronic diseases or reported intake of medication, including antioxidants and nicotine abuse. Informed written consent was obtained from each subject, and the study was approved by the University Ethics Committee. This study meets the ethical standards for research in sports and exercise as described by Harriss and Atkinson (2011).

Exercise programme

All the athletes performed an official ultra-endurance event (Ironman Lanzarote) under competition conditions. The event started at 08:00 AM on an almost warm spring day (24°C) (Ironman Lanzarote-Spain).

Saliva collection

The subjects abstained from food and caffeine products for at least 2 h prior to saliva collection. Salivary samples were collected at four different time-points: 60 min before starting the competition (baseline), transitions zones (post-swimming phase, post-cycling phase), and post-running phase (immediately at the end of the competition). Saliva was collected during transition times.

The athletes were asked to have *ad libitum* water intake during the whole competition in order to minimize dehydration as much as possible (Sousa et al., 2019). Moreover, the solid intake periods were recorded throughout the competition previously adjusted to all the participants with the aim of not effecting the final result of the measurements. The protocol for the recollection of the salive measurements, uses a collection pad that is inserted into the athlete's mouth, either under the tongue or in the cheek. The absorbent pad was kept in the mouth to absorb saliva during 2 min, and removed afterwards and putted into a storage container. Immediately after collection, the saliva samples were frozen and stored at −80°C until assayed for hormonal concentration.

Measurement of salivary hormone levels

Saliva was analysed for testosterone and cortisol at baseline (t0), post-swimming (t1), post-cycling (t2) and post-running (t3) using a nonradioactive ELISA kits (Salivary Testosterone EIA Kit and HS Salivary Cortisol EIA Kit of Salimetrics LLC, Suffolk UK). Sensitivity was < .003 µg/dL for *Cs*, and 1 pg/mL for *Ts*.

Statistical analysis

Values were expressed as mean ± standard deviation. Normal distribution of the data was verified through the Shapiro–Wilk test for the variables of *Ts*, *Cs*, and *T/C* ratio at four different moments (t1, t2, t3, t4). Repeated-measures analysis of covariance with adjustments for multiple comparisons was used to examine within-group (phases) differences with Bonferoni post hoc adjustments for multiple comparisons. A criterion alpha level of $p \leq .05$ was used to determine statistical significance. Cohen's effect size (ES) was also calculated by $ES=(M_1-M_2)/SD_{pooled}$, where $SD_{pooled}=\sqrt{((SD_1^2+SD_2^2)/2)}$ ($ES \leq .2$, small; $.2 < ES \leq .6$, moderate; $ES > .6$, large) (Cohen, 1988). We controlled age in analyses of *Ts, Cs* and *T/C*, and performed all statistical analyses using SPSS version 17.0 for Windows (IBM, Chicago, IL).

Results

All athletes completed the ultra-endurance event. Table 1 summarizes the general results of this event.

Figure 1 shows the behaviour of the analysed hormones (*Ts* and *Cs*), as well as their ratio (*T/C*) during the different phases of the competition.

Table 1. Results obtained in the Ironman Lanzarote (Total Time) and its different phases (Swimming, Cycling, Running and Transitions Phases).

Value	Final result (h, min, s)	Swimming (h, min, s)	Cycling (h, min, s)	Running (h, min, s)	Transitions (min, s)
Mean	12:20:11	1:00:30	6:36:56	4:31:54	14:35
Range	9:34:00 14:59.00	57:30 1:11:46	5:20:00 7:00:00	3:00:00 6:00:00	

Note: The ranges correspond to the approximate scores achieved by the best and worst subjects to better characterize the sample. Exact scores are not shown to guarantee the confidentiality of the participants.

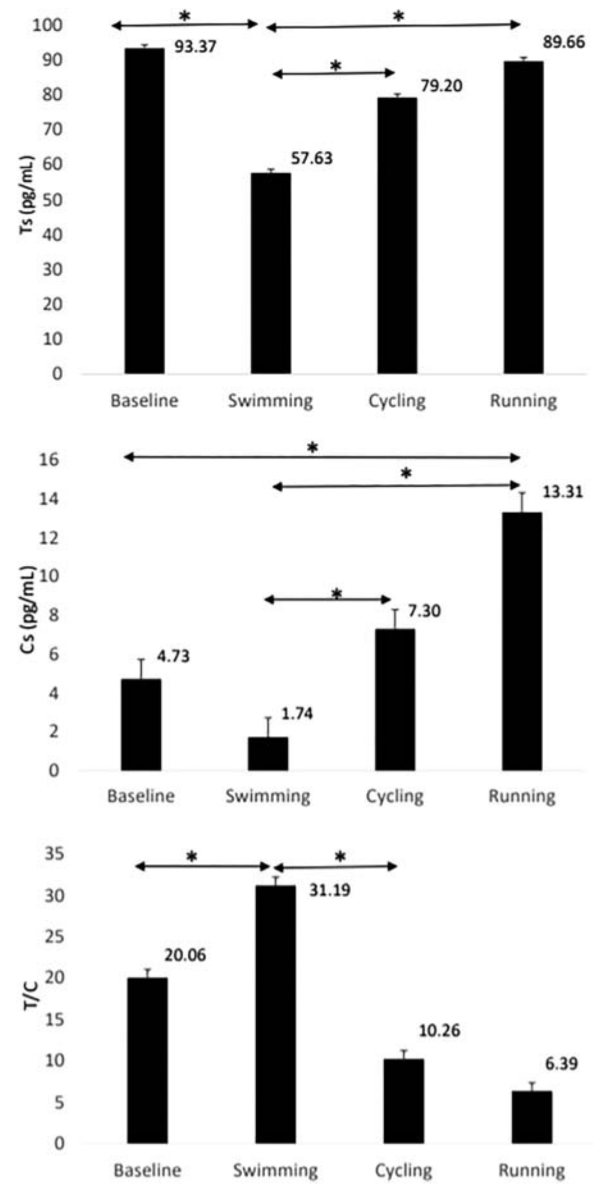

Figure 1. shows the behaviour of the analysed hormones (Ts and Cs), as well as their ratio (T/C) during the different phases of the competition. Values are mean ± standard deviation. Samples were taken once at each time point. *denotes significant difference ($p < .05$) between phases.

Statistical differences were found for the analysed hormones and ratio *T/C* during the different phases of the Ironman competition. For *Ts*, there was an initial significant decrease between baseline and post-swimming values (Change: 36.5%, $p < .01$, Cohen's *d*: 1.20). Significant increases in *Ts* values were observed after the cycling and running phases with regard to the values after the swimming phase (Change: 26.2%, $p = .001$, Cohen's *d*: .77; 27.8%, $p = .033$, Cohen's *d*: 1.17, respectively). Although slightly higher, no significant differences were found between baseline pre-competition values and the values obtained after the running phase at the end of the competition.

With regard to *Cs* values, a similar behaviour to that of *Ts* was observed with an initial significant decrease between baseline and post-swimming phase values (Change: 63.2%; $p < .001$; Cohen's *d*: 1.88). *Cs* then significantly increased after the cycling and running phases with regard to the values observed after the swimming phase (Change: 54.3%, $p < .001$, Cohen's *d*: 3.88; 62.4%, $p < .001$, Cohen's *d*: 3.44, respectively). Unlike the *Ts* values, significant differences were found between baseline and post-running *Cs* values (Change: 181.4%, $p < .001$).

The behaviour of *T/C* showed a significant increase between baseline and post-swimming phase values (Change: 34.8%, $p = .003$, Cohen's *d*: .67) to later significantly decrease after the cycling and running phases until the end of competition (Change: 68.8%; $p < .001$, Cohen's *d*: 1.64; Change: 82.3%, $p < .001$, Cohen's *d*: 2.02), with regard to swimming values. A significant decrease in *T/C* was observed overall from the baseline values to the values obtained at the end of the competition (68.1%, $p = .05$).

Discussion

The main objective of the present study was to evaluate the behavioural changes of *Ts* and *Cs* during the different phases of one of the most demanding and famous triathlons in the world (Lanzarote's Ironman Triathlon). At the end of the race, the *Ts* values decreased slightly in all subjects (4.0%), while the *Cs* values were significantly higher (181.4%) than those obtained prior to the competition. Moreover, the *T/C* ratio decreased by 68.1% at the end of the competition. These hormonal trends are consistent with those reported in other studies in which the accute or chronic response is assessed during the endurance work (Tauler, Martinez, Moreno, Martínez, & Aguilo, 2013) and, especifically, after the Ironman performance. In that last study, the authors determine the urinary steroid profile in triathletes after performing an Ironman race and detect a similar behaviour as in our study (Testosterone: ↓27.2%; Cortisol: ↑28.3%; *T/C*: ↓11.8%). Therefore, Ginsburg et al. (2001) report a decrease of 58% in the levels of Testosterone in 38 athletes (males) who participated in the Hawaii Ironman. However, our data are different from the changes detected by Chang, Tseng, Tan, Hsuuw, and Lce-Hsieh (2005) in the values of *Ts* after the performance of lower duration Triathlon (Olimpic Triathlon: 1.5 km swimming, 40 km bike course, 10 km running). In such work, the authors observed non significant increases in two groups of triathletes of different ages (Young: 19.9%; Middle Aged: 39.2%).

An in-depth analysis of the concentration of these hormones in each phase of the competition provides relevant information regarding the behaviour of the pituitary-testicular and pituitary-adrenal axes in competitions of such long duration and with highly variable neuromuscular and bioenergetics characteristics.

Although not assessed here, it should be noted that the pre-competition values of these two hormones are usually slightly higher than those found during the resting periods (Kivlighan, Granger, & Booth, 2005). This hormonal response appears to be related to pre-competition emotional stress and is often important for the physical, mental and psychological preparation required to optimally meet initial demands for energy and achieve adequate performance.

Once the event gets underway, *Cs* and *Ts* do not behave in the same manner in all the phases of the competition. The duration of the competition, changing environmental conditions, the extraordinary stress to which the athlete's organism is subjected, and subsequent functional changes are among the main causes of changes that may occur in the endocrine system.

Testosterone behaviour in the different phases of the ironman

The reproductive endocrine system seems to be particularly sensitive to exercise-related stress. Testosterone is the main male hormone and is a steroid hormone primarily secreted in the testes of males and the ovaries of females, although small amounts are also secreted by the adrenal glands. This hormone, with a host of receptors on different tissues and the resulting signalling processes, are an important stimulator of muscle growth (Urban, 2011) and performance (Vingren et al., 2010). In the present study, testosterone showed a statistically significant decrease at the post-swimming phase compared to pre-competition values. However, after swimming phase, *Ts* levels were increased during cycling and running until end of the competition (55.6%).

A decrease in testosterone values during a swimming competition has been previously described (Cumming, Wall, Quinney, & Belcastro, 1987), postulating that such a response is associated with changes in hepatic blood flow resulting from the horizontal posture and/or immersion-related hemodilution. During the last two phases (cycling and running) of the Ironman competition, an increase in *Ts* values was observed. However, it is interesting to note that the increases observed during the second transition (swimming-cycling), and until the end of the competition were not of the same magnitude.

These increases in *Ts* are not sufficient to reach the values observed in the subjects prior to the competition. Several mechanisms could potentially be responsible for decreased circulating testosterone levels during and following prolonged exercise, including suppressed gonadotropin release or elevated prolactin, cortisol, or catecholamine levels directly on the testis or indirectly via suppression of gonadotropins (Cumming, 2000).

The progressive increase in testosterone levels during the final phases of a prolonged, low-intensity competition have also been described by Vuorimaa, Ahotupa, Häkkinen, and Vasankari (2008) who were evaluating two types of athletes (middle-distance and marathon runners) during an exercise of 40 min in duration (Vuorimaa et al., 2008). This seems to suggest that the increase in testosterone, when athletes have completed a significant part of the competition, may be linked to a protective metabolic response that is triggered in situations in which glycogen stores are severely compromised. It should be noted that the presence of testosterone improves the efficiency of oxidative metabolism and the efficiency of ATP in mitochondria (Er, Michels, Gassanov, Rivero, & Hoppe, 2004). Consequently, the increase in *Ts* could be associated to a greater metabolic efficiency in these phases of the competition (Zahavi & Perel, 2011). Zahavi and Perel (2011) reported improved efficiency of the proton pump in the mitochondria when ATP is produced, although they suggest that this metabolic advantage could be associated with increased oxidative damage. It should also be noted that indirect evidence suggests that fat utilization is actually increased by means of greater lipoprotein lipase activity with testosterone supplementation in humans (Schroeder et al., 2004). In rodents, testosterone treatment has been reported to increase lipid use and reduce exercise glycogen use (Van Breda, Keizer, Kuipers, & Granenburg, 2003), leading to an efficient metabolic strategy to meet the energy demands of ultraendurance competitions.

During the last part of the cycling phase and during the running phase, the athletes already suffer a high degree of specific fatigue and a functional abnormality that have been documented by means of triathlon race or simulations (Millet, Bentley, & Vleck, 2007; Nosaka et al., 2010). The multiple changes that are observed include depletion of glycogen stores (Hausswirth, Bigard, Berthelot, Thomaidis, & Guezennec, 1996), respiratory muscle fatigue (Boussana et al., 2003) and moderate dehydration with an increase in core temperature (Hausswirth, Bigard, & Guezennec, 1997). Such alterations lead to a decreased running economy (Hausswirth et al., 1996; Miura, Kitagawa, & Ishiko, 1999) and less efficient metabolism. This phenomenon is also illustrated by the fact that, despite the steady increase during the last two phases of the Ironman (cycling and running), the final *Ts* values do not reach the baseline values. This behaviour has also been observed in long-distance races such as marathons (Karkoulias et al., 2008), ultra-marathons (Fournier, Stalder, Mermillod, & Chantraine, 1997), triathlons and the Ironman competition (Ginsburg et al., 2001). The decrease in testosterone during extreme exercises may be related to circulating catecholamine levels, a decrease in testicular blood flow by increased vasoconstriction via elevated sympathetic drive (Jeukendrup, Hesselink, Snyder, Kuipers, & Keizer, 1992) or to the greater use of androgens directed towards tissue repair (Cumming, 2000).

The reason for decreased testosterone level following prolonged endurance exercise is still unclear. In fact, some authors even report increased testosterone levels in response to endurance exercise (Tremblay et al., 2005) or no changes (Duclos, Corcuff, Rashedi, Fougere, & Manier, 1996) after low intensity exercise of mid to long duration. Tremblay et al. (2005) report that for changes in testosterone levels to be significant, the intensity of the exercise must be high or prolonged sufficiently over time in the case of moderate or low intensity exercise.

Cortisol behaviour in the different phases of the Ironman competition

Similar to the *Ts*, exercise involves a physiological challenge for the hypothalamo-pituitary-adrenal axis. The identification of mechanisms that condition the regulation and, specially, the misregulation of the cortisol response towards the most trained athletes is conditioned by a high quantity of variables that should be taken into consideration. Among the most important, we highlight the type of activity and the magnitude of the task (Luger et al., 1987). In our study, the *Cs* showed a similar behaviour to *Ts*, significantly decreasing from baseline values to those obtained in the post-swimming phase (63.2%). This drop in values could be chiefly due to the intensity and environmental characteristics of this phase of the competition.

Most triathletes take advantage of the swimming phase to get out of the water in a good position without an excessive energy demand that could adversely affect the rest of the competition. This means that the swimming phase of an Ironman is always completed at a lower speed than the maximum potential of each subject in order to avoid excessive fatigue that could interfere with the final performance (Peeling & Landers, 2009). This circumstance, and the fact that the athletes must concentrate on the technical and tactical aspects of the competition may lower the pre-competition stress athletes often experience prior to the event.

Moreover, it should be noted that *Cs* values were linearly related to exercise intensity and duration (Jacks, Sowash, Anning, Mcgloughlin, & Andres, 2002). Previous works have shown that when the exercise is of low intensity, increases in cortisol levels are not observed if the volume is not sufficiently high (Tremblay et al., 2005). The cited authors found that increases in plasma cortisol are only detected after 120 min of exercise (55% of the $VO_{2max.}$). They highlighted that volume is the determining factor in increased cortisol in this type of exercise.

Water temperature is another factor to consider in this phase of the Ironman. Galbo et al. (1979) found that when athletes swim in moderately cold water (21° C), their body temperature drops and cortisol decreases. This situation could also apply to the Ironman competition where triathletes swim in open water at a temperature of 19°C. Our results showed a progressive increase in *Cs* levels following the swimming phase, which are maintained until the end of the competition. Other works on endurance events also report increases in cortisol at the end of prolonged competitions (Karkoulias et al., 2008; Vuorimaa et al., 2008). During the running phase, the degree of elevation is likely to depend on the duration of the race (Tremblay et al., 2005). In our study, we observed a 319.5% increase in average values during the second transition time (cycling-running), and a smaller increase thereafter until the end of the race. The level of *Cs* at the end of the race was also much higher than the level observed in the subjects at the beginning of the race.

Behaviour of the testosterone/cortisol ratio in the different phases of the Ironman

The *T/C* ratio shows an opposite behaviour to that of the hormones described. Initially, a significant increase is observed, which then decreases in a statistically significant manner until the end of the competition, with the greatest decline found during the second transition. The changes observed between the beginning and the end of the event show a 68.1% decrease. These values indicate the intense catabolic status of the organism during ultraendurance competitions.

A limitation of the present study is the fact that, although the athletes were instructed to drink ad libitum, one cannot be sure that they did not experience a certain degree of dehydration by the end of the competition. Indeed, differences in dietary intake cannot be confirmed, probably impacting hormonal findings. At any rate, *Ts* and *Cs* seem to be independent of secretion rate and salivary volume. Yet even in intensive exercises of a long duration, non-specific mechanisms like hemoconcentration and decreased hepatic blood flow cannot alone explain the increase in serum hormone levels (Vuorimaa et al., 2008).

It is well known that competitions of a very long duration lead to intense multisystemic changes in the organism. These changes largely manifest themselves at the endocrine level and can be detected by analysing salivary hormones such as testosterone and cortisol. During an Ironman competition, both hormones behave in a similar manner, but differ in terms of the intensity of the changes. Hormone levels decrease during the swimming phase, but later increase during the cycling and running phases. The final *Cs* levels are significantly higher than at the beginning of the competition, while *Ts* does not return to the initial levels. Further studies need to be conducted in order to gain deeper insight into these changes as the tactical behaviours observed in the different parts (beginning, middle and end) of each phase of the event (swimming, cycling and running) suggest the existence of subtle hormonal changes linked to the course of the competition.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

Organism/Institution: Junta de Andalucía- Consejería de Turismo, Comercio y Deporte [Grant number: IMD2010SC0001].

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