







## RESPONSE OF INDICATORS MUSCLE AND KIDNEY DAMAGE TO A HALF-DISTANCE TRIATHLON IN NON-PROFESSIONAL TRIATHLETES

*Daño muscular y hepático tras un triatlón de media distancia en triatletas no profesionales*

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DOI: <https://doi.org/10.17398/1885-7019.19.35>

Recibido: 29/09/2021; Aceptado: 05/15/22; Publicado: 20/02/2023

### OPEN ACCESS

Sección / Section:

Fisiología del Deporte y el Ejercicio  
Physiology of sport and exercise

Editor de Sección / Edited by:

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Citación / Citation:

Camacho-Cardenosa, M., Crespo, C., Camacho-Cardenosa, A., González-Custodio, A., Timón, R., & Olcina, G. (2023). Response of indicators muscle and kidney damage to a half-distance triathlon in non-professional triathletes. E-balonmano Com, 19(1), 35-42.

Funding:

This research was supported by the GAEDAF Research Group under Grant GR15020; and the Ministry of Education, Culture and Sports, under Grant FPU15/00450 and FPU15/00452.

Conflicts of Interest:

All authors declare no conflict of interest

### Abstract

Triathlon is a sport modality that has been on the rise in recent years, and represents a great physical load for the body. Therefore, the first goal of the present study was to investigate physiological response in non-professional half-distance triathlon participants regarding to muscle and kidney damage. Secondly, we evaluated the short-term recovery of this competition. Blood parameters of muscle and kidney damage of seventeen trained and experienced male triathletes were assessed before (pre) and after (post) the competition and 24h (24h REC) and 48 h (48h REC) after the individual finish. After race there were significant increases in the concentrations of creatine kinase and Total Protein ( $\leq 0.005$ ) compared with Pre- race. A significant decrease in the total bilirubin ( $\Delta = -24.5\%$ ;  $p < 0.05$ ) and Total Protein ( $\Delta = -11.4\%$ ;  $p < 0.05$ ) values compared with post-race were shown 24h after race finish. At 48h of recovery, Total Protein decrease significantly compared with post- ( $\Delta = -10.8\%$ ;  $p < 0.05$ ) and shown lower values compared with Pre- race ( $\Delta = -5.7\%$ ;  $p < 0.05$ ). Significant muscle damage is caused in non-professional triathletes as result of half-distance race competition and 48h of recovery could be not sufficient to the decrease of serum creatine kinase.

**Keywords:** muscle damage; endurance; triathlon; performance; creatine kinase; recovery

### Resumen

El triatlón, modalidad deportiva que ha tenido un gran auge en los últimos años, representa una gran carga física para sus participantes. Así, el objetivo de este artículo es conocer la respuesta fisiológica de triatletas no profesionales ante una prueba de media distancia, en relación al daño muscular y hepático, y estudiar la recuperación a corto plazo.

Parámetros sanguíneos relacionados con el daño muscular y hepático de 17 triatletas experimentados fueron evaluados antes (pre), después (post) y a las 24h (24h REC) y 48h (48h REC) después de la prueba. Después de la competición, se observó un incremento significativo en la concentración de creatina quinasa y proteínas totales ( $p \leq 0.005$ ) comparado con el Pre-. Además, se observó un descenso significativo en la bilirrubina total ( $\Delta = -24.5\%$ ;  $p < 0.05$ ) y las proteínas totales ( $\Delta = -11.4\%$ ;  $p < 0.05$ ) a las 24h REC- comparado con el final de prueba. A las 48h REC, las proteínas totales mostraron un descenso significativo en comparación con el Post- ( $\Delta = -10.8\%$ ;  $p < 0.05$ ) y Pre- ( $\Delta = -5.7\%$ ;  $p < 0.05$ ). Como resultado de la participación en un triatlón de media distancia, triatletas no profesionales sufren un daño muscular, el cual necesita al menos de 48h de recuperación para conseguir descensos en los niveles de creatina quinasa.

**Palabras clave:** daño muscular; resistencia; triatlón; rendimiento; creatina quinasa; recuperación.

## Introduction

Triathlon competitions have been gaining great popularity in recent decades (Jeukendrup, 2011), especially in the amateur sport scenario. Distance competitions vary from short to ultralong distance. Therefore, it consists of 300-m swim, 8-10 km bike and 2,5 km run (Supersprint), 750-m swim, 20-km bike, and 5-km run (Sprint Triathlon), 1500-m swim, 40-km bike, and 10-km run (Olympic Triathlon), 1900-m swim, 90-km bike, and 21,1-km run (Half distance Triathlon), 3800-m swim, 180-km bike, and 42,2-km run (Full distance Triathlon), or 7600-m swim, 360-km bike, and 84.4-km run (Double Ultra Triathlon) (Knechtle et al., 2010).

As in other endurance competitions, these modalities may involve great physical load, a large energy demands (Kimber et al., 2002) and the development of different fatigue mechanisms (Sharwood et al., 2004). Fatigue during a triathlon competition is a complex factor related to different physiological markers (Areces et al., 2015). Strenuous physical exertion could lead to muscle disintegration that is known as exertional rhabdomyolysis (ER; (Tietze & Borchers, 2014)). Markers as the release of myoglobin (MB) or the content in blood of creatine kinase (CK) or lactate dehydrogenase (LDH) are wide used to identify the ER (Brancaccio et al., 2010). Previous studies have demonstrated significant increases in traditional indicators of muscle damage after half-distance triathlons (Areces et al., 2015; Brancaccio et al., 2010; Del Coso et al., 2014; Tietze & Borchers, 2014). Also, this muscle damage has been indicated as one of the determining factors in athletes' performance during a triathlon race (Coso et al., 2012).

But not only muscle damage could affect to these athletes. Combination of factors such as workload, dehydration and heat strain can trigger acute kidney injury (AKI) (Beker et al., 2018), which is defined as a deterioration of renal functionality over a relative short period of time (hours to days) (Han & Bonventre, 2004). These conditions can be found in sports that are carried out for prolonged time and are exposed to adverse environmental conditions, as triathlon. Markers such as blood ureic nitrogen (BUN) or serum albumin (ALB) are usually evaluated for the diagnostic of AKI (Laws et al., 2016). Glutamate-oxalacetate transaminase (GOT), also known as aspartate aminotransferase (AST), and glutamate-pyruvate transaminase (GPT), known as alanine aminotransferase (ALT), are enzymes that mainly reflect liver activity and are also used as kidney damage markers. Current studies have observed changes in these biochemical markers after long-distance exercises, which would indicate a temporary AKI (Hoppel et al., 2019; Rojas-Valverde et al., 2019).

But between other factors, the distance covered and running intensity may differently affect physiological responses in adult non-elite runners (Jastrzębski et al., 2015; Shin et al., 2016). In scientific literature, there are few scientific findings that can explain the extent to which this physical load affects half-distance triathletes (Coso et al., 2012; Del Coso et al., 2014). Due to the lack of information in this sense, the first goal of the present study was to investigate physiological response in non-professional half-distance triathlon participants regarding to muscle and kidney damage. Secondly, we evaluated the short-term recovery after the completion of the half-distance triathlon based on these physiological parameters.

## Materials and Methods

### **Participants**

Seventeen trained and experienced male triathletes agreed to voluntarily participate in the present study. The competition organizers provided a list of participants and we contacted them by phone or email two weeks before the competition where the measurements were development. None of the participants suffered from any serious illness and they were not taking any medication. After agreeing to participate in the study, all the participants signed an informed consent form approved by the Bioethics and Biosafety Committee of the [remote to review], according to the latest version of the Declaration of Helsinki.

## **Design and Procedures**

All the selected participants arrived at the starting area of the competition in advance and we performed the measurements. They had eaten breakfast at least one hour before arriving at the area of measurement. Upon arrival and before the start of the competition, we performed the first assessment (pre). Then, we performed a second assessment (post) after completing the competition. Finally, the participants were evaluated 24h (24h REC) and 48 h (48h REC) after the individual finish. General data of each participant, such as weekly training hours or years of experience, were recorded at the beginning of the first assessment. Participants began the competition without any instruction in terms of rhythm, strategy of hydration, nutrition, or any other issue that could affect their normal performance. They could eat and drink ad libitum during the race; there were no special provisions in this regard. The half-distance competition consisted of 2.1-km swim, 83-km cycling, and 18.7-km run, and drafting was not allowed. The triathlon was held in May in a city located at 269 meters above sea level. Weather conditions during the competition were: average temperature of 16 °C (10 °C - 21 °C); 68% humidity (44-97%); wind velocity of 12 km.h-1; and no rainfall. Temperature in the different parts of the event was measured by the judges themselves. The swimming competition was held in a swamp with a water temperature of 16.5 °C. Therefore, the use of the neoprene wetsuit was allowed. The average temperature during the cycling competition and the running was 17 °C. The results of the competition in terms of overall time and speed, and by sections are shown in Table 2. When the competition was completed, the participants went to the area of assessment accompanied by a member of research team to undergo the same assessment protocol collected before the competition using the same material. Collecting of blood samples was development 3 min after that the triathletes passed from the finish line. The participants were asked to avoid any food or liquid intake from the finish line to the assessment area.

**Body composition:** body weight was measured according to standard protocols in kilograms, with a precision of 0.1 kg, using a scale (SECA 769, GmbH & Co. KG, Hamburg, Germany). Height was measured using a stadiometer (SECA 769, GmbH & Co. kg, Hamburg, Germany) and expressed in centimeters, with an accuracy of 1 millimeter. Body mass index (BMI) was calculated using weight and height, according to the accepted mathematical equation ( $BMI = \text{weight}/\text{height}^2$ , expressed in  $\text{kg}\cdot\text{m}^{-2}$ ). The same researcher measured the six skinfolds (abdominal, triceps, subscapular, suprailliac, medial leg and thigh) using a skinfold calliper (SATA, AMEFDA, Spain) on the left side of the body, and following the recommendations of the International Biological Program (American College of Sports Medicine, 2014). Obtained values for each fold was the average value of the three measurements (Tremblay et al., 1994). Percentage of fat mass and fat-free mass were estimated based on Spanish Group of Kinanthropometry (Alvero Cruz et al., 2010):

$$\text{Fat (\%)} = \text{Body weight} \times (3.64 + (\sum \text{skinfolds} \times 0.097)) / 100$$

$$\text{Muscle Mass (\%)} = (\text{Muscular weight} / \text{Body weight}) \times 100$$

**Blood simples:** blood was drawn from the antecubital vein. A part of the blood sample was used to assess lactate in whole blood by means of a portable analyser (Lactate Scout, SensLab GmbH, Germany). Another part was previously centrifuged for measuring haematocrit, Total Proteins, total bilirubin (TBIL), BUN, GOT, GPT, LDH and CK values, using a dry clinical chemistry analyser (Spotchem EZ SP-4430; Arkray, Inc. Kyoto, Japan) by means of the corresponding test strips.

## **Statistical analysis**

For the statistical analysis, we used the SPSS statistical package, version 20 for MAC (IBM, New York, USA) and calculated descriptive statistics for the variables in the study. In addition, prior to the analysis, we confirmed the normality of each variable using Shapiro-Wilk test and Levene's test was used to determine the homogeneity of variance. Absolute change percentage (%) was reported between time measurement in each variable as follow:  $\Delta (\%) = (\text{Pre-Post}) / \text{Pre} \times 100$ . To compare responses in each variable across the two time points it was used ANOVA for repeated-measures.

When a global difference over time was determined, Bonferroni post hoc analysis was used to identify where changes occurred. The criterion  $p < 0.05$  was used to determine statistical significance. The effect size (Cohen's  $d$ ; (Cohen, 1992)) was calculated for all variables between pre-and post-competition states. Magnitude of change considered was: small (0.2), moderate (0.5) and large (0.8).

## Results

### Participants

The Table 1 shows the training status and morphological characteristics of the study participants.

**Table 1.** Morphological characteristics and training status of study participants

	mean $\pm$ SD (n=17)
Age (yr)	37.7 $\pm$ 4.6
Experience as triathletes (years)	8.4 $\pm$ 7.4
Training/week (h)	15.5 $\pm$ 3.8
Muscle mass percentage (%)	51.2 $\pm$ 17.5
Fat percentage (%)	9.2 $\pm$ 1.7
Body mass (kg)	74.8 $\pm$ 4.8
BMI (kg/m <sup>2</sup> )	23.9 $\pm$ 1.4

BMI: Body mass index

### Race Performance

Related to race performance, the comparisons between our study sample and the all-race participants indicate that the study participants represent the whole range of all participants (Table 2).

**Table 2.** Race performance of study participants compared to all finished

	Study Participants (n=17)	Race Participants (n=64)
Swimming Time (min)	38.45 $\pm$ 4.28	42.06 $\pm$ 6.38
Cycling Time (min)	152.32 $\pm$ 10.36	161.26 $\pm$ 14.15
Running Time (min)	87.30 $\pm$ 9.88	92.50 $\pm$ 12.18
Total race time (min)	278.15 $\pm$ 20.52	296.16 $\pm$ 29.00
Percentage respect winner time (%)	14.52 $\pm$ 8.40	
Swimming Velocity (m/s)	0.92 $\pm$ 0.09	
Cycling Velocity (km/h)	32.84 $\pm$ 2.31	
Running Velocity (km/h)	12.99 $\pm$ 1.42	
Lactate (mmol/L)	2.14 $\pm$ 0.27	

Results are expressed as means  $\pm$ SD. 11 of 75 race participants (14.67%) did not finish the race; drop-outs not included.

**Response of Tissue Damage Markers at half-distance triathlon**

Values of markers for tissue damage before and after race are shown in Table 3. After race there were significant increases in the concentrations of CK and Total Protein ( $\leq 0.05$ ) compared with Pre- race. Both parameters showed large effect sizes (CK  $d=1.49$ ; Total Protein  $d=1.00$ ).

**Table 3.** Response of tissue damage markers to half-distance triathlon

	Pre- (A)	Post- (B)	$\Delta$ A-B (%)	ES A-B (d)
CK (Ui/L)	258.3 $\pm$ 205.8	590.3 $\pm$ 239.1	128.5*	1.49
LDH (Ui/L)	306.33 $\pm$ 92.8	459.3 $\pm$ 112.2	49.9	1.49
TBIL (mg/dL)	0.5 $\pm$ 0.2	0.7 $\pm$ 0.0	32.1	2.00
GOT (Ui/L)	31.0 $\pm$ 19.7	40.0 $\pm$ 17.3	29.0	0.49
GPT (Ui/L)	21.7 $\pm$ 9.3	29.7 $\pm$ 5.0	36.9	1.12
BUN (mg/dL)	12.7 $\pm$ 2.5	14.67 $\pm$ 2.3	15.8	0.82
Total Protein (mg/dL)	7.5 $\pm$ 0.2	7.9 $\pm$ 0.6	5.7*	1.00

Results are expressed as means  $\pm$ SD.

Pre-: before race assessment; post-: after race assessment;  $\Delta$ : absolute change percentage; ES: effect size (d Cohen); CK: creatinephosphokinase, LDH: Lacticacid dehydrogenase, TBIL: Bilirubin, GOT: Glutamic-oxaloacetictransaminase, GPT: Glutamatepyruvate transaminase, BUN: blood urea nitrogen. \*  $p < 0.05$  for difference between Pre- and Post-

**Response of Tissue Damage Markers at short-term recovery**

Regarding to obtained values during a short-term recovery of 24h and 48h (Table 4), a significant decrease with large effect sizes in the TBIL ( $\Delta = -24.5\%$ ;  $p < 0.05$ ;  $d = 6.00$ ) and Total Protein ( $\Delta = -11.4\%$ ;  $p < 0.05$ ;  $d = 2.00$ ) values compared with Post- race were shown 24h after race finish. At 48h of recovery, Total Protein decrease significantly with large effect size compared with Post- ( $\Delta = -10.8\%$ ;  $p < 0.05$ ;  $d = 1.78$ ). In this time assessment, Total Protein shown lower values compared with Pre- race with a large effect size ( $\Delta = -5.7\%$ ;  $p < 0.05$ ;  $d = 1.60$ ).

**Table 4.** Response of tissue damage markers to 24 (24h REC) and 48 hours (48h REC) of recovery of finish race

	CK (Ui/L)	LDH (Ui/L)	TBIL (mg/dL)	GOT (Ui/L)	GPT (Ui/L)	BUN (mg/dL)	Total-Protein (mg/dL)
24h-REC (C)	1095.3 $\pm$ 810.4	360.3 $\pm$ 113.0	0.4 $\pm$ 0.1	58.3 $\pm$ 43.6	29.3 $\pm$ 19.0	15.7 $\pm$ 0.6	7.0 $\pm$ 0.3
48h-REC (D)	856.3 $\pm$ 887.3	306.0 $\pm$ 98.3	0.7 $\pm$ 0.5	62.3 $\pm$ 37.7	25.0 $\pm$ 15.0	11.3 $\pm$ 1.5	7.1 $\pm$ 0.3
$\Delta$ A-C (%)	324.0	17.6	-24.5	88.2	35.4	23.7	-6.3
ES A-C (d)	1.65	0.52	0.67	0.86	0.54	1.94	2.00
$\Delta$ B-C (%)	85.6	-21.6	-42.9*	45.8	-1.2	6.8	-11.4*
ES B-C (d)	0.96	0.88	6.00	0.60	0.03	0.71	2.00
$\Delta$ A-D (%)	231.5	-0.1	26.4	101.1	15.4	-10.6	-5.7 $\beta$
ES A-D (d)	1.09	0.00	0.57	1.09	0.27	0.70	1.60
$\Delta$ B-D (%)	45.1	-33.4	-4.3	55.8	-15.7	-22.8	-10.8 $\$$
ES B-D (d)	0.47	1.46	0.00	0.81	0.47	1.77	1.78

Results are expressed as means  $\pm$ SD. CK: creatine kinasa, LDH: lacticacid dehydrogenase, TBIL: Bilirubin, GOT: Glutamic-oxaloacetictransaminase, GPT: Glutamatepyruvate transaminase, BUN: blood urea nitrogen; 24h- REC: 24h recovery post-race; 48h REC: 48h- recovery post-race;  $\Delta$ : absolute change percentage; ES: effect size (d Cohen).

\*  $p < 0.05$  for difference between Post- and 24h REC

$\beta$   $p < 0.05$  for difference between Pre- and 48h REC

$\$$   $p < 0.05$  for difference between Post- and 48h REC

## Discussion

First goal of the present study was to investigate physiological response in non-professional triathletes regarding to muscle and kidney damage. Secondly, we evaluated the short-term recovery after the completion of the race based on these physiological parameters. After race, our results shown significant increases of the CK and Total Protein blood values. At 24h of recovery, TBIL and Total Protein values decreased significantly compared with post-race. At 48h race finish, Total Protein values continued decreasing, shown lower values than times previous at competition.

Serum CK is shown as the main in the identification of muscle damage, which is considered when the CK values is set at  $>1000\text{Ui/L}$  (Stahl et al., 2020). In the present study, CK values were measured before and just after the competition, showing an increase of 207%. These changes have already been reported previously in the scientific literature in half-distance triathlon (Arecas et al., 2015; Del Coso et al., 2014) or Olympic distance triathlon (Olcina et al., 2018). Some authors have showed a positive correlation between CK and race time (Del Coso et al., 2014). Exercise-induced muscle damage caused loss of skeletal muscle performance and soreness (Owens et al., 2019), which could have consequences on race time during triathlon (Del Coso et al., 2014). Muscle damage in triathletes can result from mechanical causes—such as the impact on the steps during the race—or metabolic causes due to the lack of carbohydrate reserves in the lower body muscle groups (Del Coso et al., 2014). The mechanical impact on the steps during the race, which is the most critical phase for the final performance (Figueiredo et al., 2016), has an effect on red blood cells, causing intravascular haemolysis. When CK values are analysed in athletes, it is important take into account that a large variability of CK concentrations post-exercise has been reported (Kim & Lee, 2015). Dependence of serum CK is showed on body composition, metabolic dysfunctions, and inflammatory effects due to immune responses to exercise between other factors (Hoppel et al., 2019). In this sense, CK leakage to the blood could be a physiological response to prolonged exercise (Baird et al., 2012; Mougios, 2007) and increases in serum CK alone may be a consequence of normal muscular activity is not an accurate reflection of structural muscle damage (Hoppel et al., 2019).

Although significant differences were not found, ER was shown at 24h finish race ( $1095.3 \pm 810.4\text{ Ui/L}$  serum CK) with a large effect size ( $d$  Pre-24h REC=1.65;  $d$  Post-24h REC=0.96). At 48h of recovery of finish race CK values returned to a healthy range at 48h of recovery ( $856.3 \pm 887.3\text{ Ui/L}$ ) with large effect size compared with Pre- race ( $d$  Pre-48h REC=1.09). These tendencies during the recovery have already been reported by previous authors: serum CK may have a maximum peak at 24-36 hours (Brown, 2004) after exercise and recovers the basal states at 48-72h later (Vogt et al., 2012). However, these results should be interpreted with caution. A recovery of 48h could not be sufficient to return to baseline values of serum CK. Further investigations could study the response of longer-term recovery after a half-distance triathlon race. Therefore, additional measure at 72h could be interesting to know the complete kinetics of this molecule. However, it possible that athletes don't ensure full recovery the weeks preceding competing, and they came to the competition already exhibiting muscle fatigue. In this case, the observed values at 24h of finish race could be the results of accumulative fatigue of competition and training. In this sense, it would be advisable to study and monitor the work carried out during training in the weeks preceding the competition to ensure full recovery of the athletes.

Other markers related to dysfunction renal showed significant change after the race. Total Proteins increased significantly after the competition. Puggina et al., (2014) described also changes in the urinary excretion of protein, and these were associated to the exercise-induced modifications in the glomerular membrane permeability and to the endocrine variables. After 24h of recovery, TBIL and Total Protein shown a significant decrease compared with Pre race. After 48h of finish race, Total Protein parameter decreases significantly compared with Pre and Post assessment. These results could be in agreement with other previous, where renal dysfunction returned to baseline within 24-48 h (Hodgson et al., 2017). However, the values of these parameters did not increase above the reference values and the magnitude of the changes is not sufficient to indicate liver overload (Olcina et al., 2018). Therefore, important kidney damage could be not occurring after a half-distance triathlon. Although previous authors reported increases of GOT and GPT after 5h de recovery of an Ironman, these were associated to increments to the muscle damage and not to kidney damage (Mujika

et al., 2017). For all this, muscle damage could be the most important response after this type of competition. When the distances of the event increments – i.e. endurance trail running-, the kidney damage markers could be incremented (Rojas-Valverde et al., 2019). In adult non-professional runners, the distance covered or running intensity of competition may differently affect to physiological responses (Hoppel et al., 2019). Concretely the skeletal muscle damage, liver and kidney function could be directly related to the distance covered (Shin et al., 2016).

A small sample size is the main limitations in this investigation. However, its ecological validity, due to their similitude with real world and getting data from real competition instead of laboratory test could be considered as strength. Furthermore, amateur athletes must regularly combine their sporting activities with their work, which means that recovery processes, rest, hydration and nutrition may be less controlled (Aldasoro et al., 2019).

## Conclusions

In conclusion, muscular damage is caused in non-professional triathletes as result of half-distance race competition and 48h of recovery could be not sufficient to the decrease of serum CK. This way, recovery strategies that can reduce those issues could be necessary due to muscle fatigue is associated with declines in the performance of these athletes. The study and monitor the work carried out during training (with indicators as the POMS scale o cortisol basal) in the weeks preceding the competition to ensure full recovery of the athletes would be advisable too (Santos, 2018).

**Author Contributions:** MCC: methodology, analyses and writing; CC: design and methodology; ACC: methodology and writing; AGC: methodology; RT: design and review; GO: design, analysis and review.

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