



Article **Proteinuria and Significant Dehydration in a Short-Steep Triathlon: Preliminary Observational Report**

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Abstract: Background/Objectives: Endurance triathlons impose substantial physiological stress, yet the effects of short-course formats remain relatively unexplored. This preliminary study presents novel findings on proteinuria and hydration levels in well-trained triathletes. Methods: 27 participants (41.9 ± 7.4 years) who completed a sprint triathlon consisting of a 1500 m swim, 26 km cycle, and 8 km run. Urine samples were collected before and after the race. Results: Our results revealed a significant increase in post-race proteinuria cases from four to nine (p = 0.03) and the first reported case of post-race urobilinuria and ketoacidosis in this context. Additionally, pre-race glucosuria, present in nine cases, decreased to three post-race. Hematuria cases decreased from six to two (p = 0.13) and pre-race leukocyturia resolved post-race. There was a significant increase in urine specific gravity (from 1.018 to 1.023, p = 0.03), indicating dehydration. Conclusions: Short-course triathlons significantly induced post-race proteinuria, urobilinuria, and dehydration, highlighting the substantial physiological stress on kidney function and hydration status despite the shorter distances. These findings underscore the importance of monitoring urinary biomarkers and hydration levels in athletes before and after competition.

Keywords: physical endurance; renal function; exercise-induced dehydration; athlete health; urinary biomarkers; physiological stress

1. Introduction

Endurance sports, such as triathlons, impose considerable physiological stress on athletes, demanding exceptional cardiovascular fitness, muscle strength, flexibility, and mental stamina to complete [1]. The triathlon, consisting of sequential swimming, cycling, and running legs, is renowned as one of the most physically demanding single-day endurance



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Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). events [2]. While the physiological demands of long-course triathlons like the Ironman (140.6 miles in 8–17 h) have been extensively investigated [3], the effects of shorter and high-intensity formats such as sprint (750 m swim, 20 km bike, 5 km run) and Olympic distance (1.5 km swim, 40 km bike, 10 km run) triathlons on markers of kidney function and hydration status remain relatively unexplored. These shorter races enable athletes to maintain higher percentages of their maximal oxygen uptake (VO₂max) and heart rate across all legs, resulting in greater physiological strain despite the reduced total distance [4].

Numerous studies have examined the physiological responses, including dehydration and proteinuria, in various running events and distances equivalent to short triathlons [5]. Instead, the key difference between triathlons and running races is the sequential nature of the three disciplines (swim, bike, run) in a triathlon [4]. This multi-modal aspect introduces additional challenges compared to a single running event, which can affect hydration status and kidney function differently than a sustained running effort [6,7].

Exercise-induced proteinuria, characterized by the transient elevation of abnormal protein levels in the urine following physical exertion, is a well-documented phenomenon in endurance athletes [8]. This phenomenon has been widely reported in the context of long-distance triathlons and ultramarathons, where high rates of post-competition proteinuria have been observed [9,10]. The underlying mechanisms are thought to involve increased kidney blood flow and altered glomerular permeability during intense exercise, leading to the leakage of proteins into the urine [11,12]. Although exercise-induced proteinuria is typically transient and reversible, recurring instances may indicate underlying kidney damage or dysfunction [13,14].

Dehydration is another major concern in endurance sports like triathlons, which can exacerbate exercise-induced proteinuria by increasing blood viscosity, promoting renal vasoconstriction, and altering glomerular permeability [15–18]. The impacts of dehydration on kidney function have been well-documented in the context of longer triathlon and ultra-endurance events. However, the relationship between hydration status and proteinuria in the specific setting of short, high-intensity triathlon formats still needs to be better understood.

Furthermore, it is important to provide some background on the urinary biomarkers assessed in this study. Glucosuria, or the presence of glucose in the urine, can indicate impaired glucose regulation, which the metabolic demands of endurance exercise in trained athletes may exacerbate [19]. Proteinuria, the abnormal presence of proteins in the urine, is a well-documented phenomenon in endurance athletes and can provide insights into exercise-induced alterations in kidney function and permeability [8]. Leukocyturia, or the presence of white blood cells in the urine, may reflect inflammatory processes within the urinary tract that can be affected by the physiological stress of triathlon competition [20]. Examining these urinary biomarkers in the context of short-course triathlons can elucidate the unique physiological challenges this athlete population faces.

While various physiological responses to triathlon events have been studied, proteinuria and hydration status were chosen as the primary focus of this investigation for several reasons. Proteinuria is a well-established marker of exercise-induced stress on renal function [8], and its occurrence in short-distance triathlons has not been extensively studied. Moreover, the relationship between exercise intensity and proteinuria [11] makes it particularly relevant to shorter, more intense triathlon formats. Hydration status, as indicated by urine specific gravity, is crucial for maintaining performance and preventing heat-related illnesses in endurance events [15,18]. The unique combination of three disciplines in triathlons, each with different thermoregulatory demands, makes understanding hydration dynamics in these events particularly important [4,6,7]. Additionally, dehydration can exacerbate exercise-induced proteinuria [17], creating a potential interplay between these two factors that warrants investigation in the context of short-distance triathlons.

Therefore, this preliminary study aimed to provide novel insights into the physiological responses, including proteinuria and hydration status, to shorter and more intense triathlon events. Examining these markers in the context of short-course triathlons, where the potential kidney stress and dehydration-induced impacts may differ from longer endurance competitions, could inform medical guidelines and athlete preparation strategies for this increasingly popular endurance sport discipline. Our primary objective was to assess the occurrence and extent of proteinuria and dehydration in well-trained athletes participating in a short-distance triathlon and to explore potential relationships between these physiological responses and race performance.

2. Methods

2.1. Design

This was a repeated-measures observational study conducted during a competitive short-distance triathlon event. Data were collected both before and after a competitive hilly triathlon that started with a 1500 m swim in a temperate 25 m indoor pool, followed by a 26 km cycling leg with a total ascent of 1100 m, and concluded with an 8 km run featuring a total ascent of 180 m. This classification is common practice when any segment of the triathlon is significantly shorter or is realized in a different environment that reduces the physical demands of the competition. In this study, the organizers selected shorter cycling and running distances considering participants' abilities, safety, and logistical constraints. The swimming segment was conducted in a pool instead of open water to ensure safety and control over environmental variables (e.g., waves, currents, water temperature). These protocol variations, influenced by the event organizers, provide meaningful data on the physiological impacts of short-distance triathlons and can inform future research and practice.

Athletes were not monitored or recorded for their calorie and hydration intake during training and racing to maintain ecological validity. This approach ensured that participants maintained their usual training load, lifestyle habits, and nutritional practices throughout the assessment. By not influencing their behavior, we aimed to observe the physiological impacts under typical conditions, thus reflecting a more realistic and natural setting.

The event occurred under environmental conditions with temperatures ranging from 23 °C to 25 °C and relative humidity between 60% and 75%. Throughout the triathlon, participants' performance and distances were continuously monitored using smartwatches provided to each athlete. Additionally, urine samples were collected before and after the race to assess the physiological impact on kidney function and hydration status, analyzing markers such as proteinuria, urobilinuria, glucosuria, hematuria, and leukocyturia.

2.2. Participants

Twenty-seven well-trained triathletes participated in the study, comprising twenty men and seven women with a mean age of 41.9 ± 7.4 years, body mass of 70.1 ± 13.7 kg, stature of 1.78 ± 0.38 m, and a VO₂max of 57.6 ± 6.4 mL/kg/min. These participants were recruited from a pool of highly experienced ultra-endurance triathletes boasting an average of 12.4 ± 5.2 years of experience in the field. Inclusion criteria required participants to undergo rigorous training, involving 5 to 6 weekly sessions lasting between 300 to 400 min/week, and not participating in a competition between the VO₂max assessment and the competition (minimum 15 days between events). Additionally, male participants were expected to achieve a season best of under 20 min in a 5 km run, while female participants were expected to achieve a season best of under 25 min in the same running distance.

Before participating in the study, athletes received comprehensive information detailing the potential risks and discomforts associated with the testing procedures. They provided their informed consent, and the research adhered to the guidelines outlined in the Declaration of Helsinki (2013). Furthermore, the study underwent a thorough review and received approval from an Institutional Review Board (Reg. Code 139/2020).

2.3. Measurements

2.3.1. Anthropometry

Body mass measurements were obtained using a digital scale (Elite Tanita-Ironman[®] Series BC554, Arlington Heights, IL, USA) with a sensitivity of 0.1 kg. Stature measurements were taken using a stadiometer (SECA 213, Hamburg, Germany) to the nearest 0.1 cm.

2.3.2. Physical Performance

Two weeks before the competition, each participant's maximum oxygen consumption (VO_{2max}) was assessed in a laboratory setting using a gas analyzer device (VO 2000, MedGraphics[®], St. Paul, MN, USA) and the BreezeSuite[®] software, version 8.5. The VO_{2max} testing was conducted on a treadmill (T5, Lifefitness, Rosemont, IL, USA) following an incremental intensity protocol developed by Martin & Coe [21]. The protocol consisted of a 3 min warm-up, followed by a gradual increase in speed (1 km/h) every 3 minutes until the participants reached volitional fatigue. The gas analyzer had an accuracy of within $\pm 3\%$ for absolute volume measurements [22].

During the triathlon competition, athletes' speed, distance, and time for each discipline were monitored using a smartwatch (Forunner 745, Garmin, Olathe, KS, USA). Participants independently completed the laps between disciplines and transitions, with distances verified by event judges at the conclusion.

2.3.3. Urine Collection and Analysis

Before and following the triathlon, urine samples were collected on-site using sterile 30 mL polypropylene containers (Nipro Medical Corp., Osaka, Japan). Subsequently, these samples underwent analysis employing highly sensitive and accurate dipsticks specifically designed for urine screening (Combur10Test M, Roche, Mannheim, Germany) [8,23]. Two independent microbiologists conducted the analysis immediately after collection and compared the results against the color scale provided by the manufacturer. In case of discrepancies between the two observers, a third researcher's opinion was sought to reach a consensus. None of the participants reported any urination difficulties or discomfort.

The urine samples were screened for several parameters, including leucocytes, erythrocytes, bilirubin, ketones, nitrites, protein, glucose, and urobilinogen. Trace amounts were considered negative, while scores greater than one were recorded. The interpretation of the urine test results was as follows: a score above one indicated leucocytes counts greater than 10 cells/ μ L, erythrocytes count greater than 5 cells/ μ L, bilirubin levels higher than one, ketones higher than one, positive nitrites, protein levels exceeding 30 mg/dL, glucose levels higher than 50 mg/dL, and urobilinogen levels higher than 1 mg/dL.

2.3.4. Hydration Status

To assess the hydration status of the participants, urine specific gravity (USG) was determined from urine samples collected immediately before and after the triathlon competition. This measurement involved an evaluation of urine solids, with USG values confirmed utilizing a handheld digital refractometer (Palm AbbeTM, Misco, Solon, OH, USA) [24]. Before use, the refractometer underwent appropriate cleaning and calibration. The classification of hydration status based on USG values was categorized as follows: well-hydrated (USG < 1.01), minimal dehydration (USG = 1.01–1.02), significant dehydration (USG = 1.02–1.03), and severe dehydration (USG > 1.03) [15]. Urine samples were collected and analyzed close to the start and finish of the triathlon to provide insights into the participants' hydration levels during the competition.

Regarding the timing of urine sample collection, it is important to note that fluctuations in urine concentration can occur naturally throughout the day, with the highest concentrations typically observed in the morning and more stable levels in the afternoon and evening hours [25]. In this study, urine samples were collected immediately before the start and after the completion of the triathlon event, ensuring that the pre- and post-race measurements were taken within a relatively narrow timeframe and under similar conditions. While the NATA-recommended cut-off values were used to classify hydration status, the timing of sample collection helps mitigate potential confounding effects of diurnal variation in urine concentration.

2.4. Data Analysis

The data are presented as mean and standard deviation (SD) or frequencies for relevant distributions. A significance level 0.05 was predetermined, and the critical value was calculated based on the sample size and alpha level. Statistical analysis of the data collected was conducted using IBM SPSS® (IBM Corp., Armonk, NY, USA, https://www.ibm.com/spss, accessed on 1 March 2024). The association between pre- and post-event urine component measurements was assessed using a Chi-squared test for within-subjects (McNemar's Test). Additionally, the Wilcoxon (Z) non-parametric test was employed to explore the differences in the number of cases of specific urinary conditions (pre vs. post). Furthermore, to analyze the changes in urine specific gravity (USG) from pre- to post-competition, a paired *t*-test was used. The Shapiro–Wilk Test confirmed the normal distribution of the USG data, allowing for the appropriate application of the paired *t*-test to compare the mean USG values before and after the triathlon event. A Spearman's Rank Correlation (ρ) test assessed the strength and direction of associations between ordinal and continuous variables. Spearman's ρ is a non-parametric test that evaluates the monotonic relationship between two variables, making it suitable for data that are either ordinal or exhibit nonlinear relationships. This method does not assume normality, which is appropriate for the current ordinal and categorical variables dataset. Correlations with $\rho > 0.5$ were considered significant, indicating moderate to strong relationships.

3. Results

The triathletes completed the swimming section at an average pace of $2:00 \pm 0:12 \text{ min}/100 \text{ m}$, the cycling section at an average pace of $15.43 \pm 2.23 \text{ km/h}$, and the subsequent running section at a pace of $5:38 \pm 0:31 \text{ min/km}$. These paces resulted in a total time of $30.00 \pm 3.00 \text{ min}$ in the swimming section, $101.10 \pm 14.63 \text{ min}$ in the cycling section, and $45.06 \pm 4.13 \text{ min}$ in the running section. The triathlon total time was $176.16 \pm 21.76 \text{ min}$.

Table 1 presents the number of cases per urine component based on the results and assessment time-point. The findings indicate the presence of glucosuria (n = 9) and proteinuria before the start of the event (n = 4). Moreover, there was a 133% increase (five new cases) in proteinuria cases following the event. Additionally, one case of urobilinuria and ketoacidosis was reported.

Table 2 presents the number of positive cases of urobilinuria, ketoacidosis, glucosuria, proteinuria, hematuria, leukocyturia, and urine nitrates. Pre–post associations were observed in cases of urobilinuria, ketoacidosis, and urine nitrates, with each health condition having only one case. Additionally, there was a statistically significant change in the number proteinuria cases (Z = 29.60, p = 0.03). The paired *t*-test results indicated a significant increase in USG when comparing pre vs. post values (1.018 ± 0.009 vs. 1.023 ± 0.005 ; t = 2.26, p = 0.03).

Finally, a positive correlation was found between ascorbic acid and pH, with $\rho = 0.61$. A strong positive correlation ($\rho = 0.72$) was found between erythrocytes and ketones. A moderate positive correlation ($\rho = 0.54$) was found between protein levels and erythrocytes in the samples. A moderate negative correlation ($\rho = -0.50$) was found between pH and protein levels in the samples. Finally, there was no correlation ($\rho < 0.5$) between USG and protein levels or erythrocytes.

17	0.1	Pre-	Race	Post-Race		
variable	Outcome –	п	%	п	%	
Bilirubin	Negative	27	100	27	100	
Urobilinogen	Negative	27	100	26	96.30	
	70 mg/dL	0	0.00	1	3.70	
Ketones	Negative	27	100	26	96.30	
	1+	0	0	1	3.70	
Glucose	Normal	18	66.66	24	88.90	
	50 mg/dL	9	33.33	3	11.10	
Protein	Negative	23	85.20	18	66.70	
	30 mg/dL	2	7.40	3	11.10	
	100 mg/dL	2	7.40	5	18.50	
	500 mg/dL	0	0.00	1	3.70	
Erythrocytes	Negative	21	77.80	25	92.60	
	5–10 cells/µL	6	22.20	1	3.70	
	250 cells/μL	0	0.00	1	3.70	
Nitrites	Negative	26	96.30	26	96.30	
	Positive	1	3.70	1	3.70	
Leucocytes	Negative	25	92.60	27	100	
	10–25 cells/μL	2	7.40	0	0.00	

Table 1. Distribution of urine components by outcome and measurement time-point.

Table 2. Association between cases above normal/negative values by evaluation time-point.

Variable	Pre- n	-Race %	Pos n	t-Race %	Z (Wilcoxon)	<i>p</i> -Value	X ²	<i>p</i> -Value	McNemar's Test Value
Bilirubin (≥1+)	0	0.00	0	0.00	0.00	1	-	-	-
Urobilinogen ($\geq 8 \text{ mg/dL}$)	0	0.00	1	3.70	0.00	1	22.00	< 0.01	1
Ketones (>1+)	0	0.00	1	3.70	1	0.32	22.00	< 0.01	1
Glucose ($\geq 50 \text{ mg/dL}$)	9	51.90	3	11.10	2.12	0.03	0.95	0.33	0.07
Protein (>30 mg/dL)	4	14.80	8	29.60	1.16	2.49	1.20	0.28	0.39
Erythrocytes ($\geq 5 \text{ cells}/\mu L$)	6	22.20	2	7.40	1.41	0.16	0.83	0.83	0.29
Nitrites (positive)	1	3.70	1	3.70	0.00	1	22	< 0.01	1
Leucocytes ($\geq 10 \text{ cells}/\mu L$)	2	7.40	0	0.00	1.41	1.16	-	-	-

4. Discussion

This preliminary study aimed to assess proteinuria and hydration status in athletes participating in a short-distance triathlon. Several noteworthy findings emerged from the study, including proteinuria, urobilinuria, and significant dehydration induced by participation in the short-distance triathlon.

The observed increases in post-race proteinuria, urobilinuria, and dehydration were consistent with previous research on the physiological responses to endurance exercise [9,10,26]. While the frequency of cases observed in our study is lower than that reported in previous studies of ultramarathon athletes [10], the mechanisms underlying exercise-induced proteinuria likely involve heightened renal blood flow and altered glomerular permeability resulting from extreme physical exertion [12], as well as the diversion of blood flow away from the kidneys and toward the muscles and skin during exercise [11,14]. One notable case of post-race urobilinuria was reported, indicating potential acute kidney injury that warrants further medical investigation [17]. While this finding is interesting, it is important to note that our study design did not include monitoring of participants' dietary intake or nutritional status before or during the race. Therefore, we cannot conclude the specific factors that may have contributed to this observation. Future studies investigating urinary biomarkers in short-distance triathlons should consider in-

corporating dietary assessments to provide a more comprehensive understanding of the physiological responses to this type of endurance event.

The observed decrease in pre-race hematuria and leukocyturia after the competition is an intriguing finding that warrants further investigation. While this result appears counterintuitive given the high-intensity nature of the event, it is important to note that the complex physiological responses to endurance exercise can vary among individuals and may depend on factors such as training status, hydration, and pre-existing conditions. This unexpected outcome highlights the need for more comprehensive studies to elucidate the underlying mechanisms of urinary biomarker changes in response to short-distance triathlons.

On the other hand, some cases of glucosuria, proteinuria, hematuria, leukocyturia, and dehydration were observed before the competition. Interestingly, pre-race glucosuria did not worsen with triathlon exertion; instead, it decreased post-race. This contrasts with previous findings indicating that endurance exercise can exacerbate glucosuria in athletes with diabetes [19]. The progressive nature of the cycling and running course in the short-distance triathlon may have enhanced glucose uptake by active muscles, potentially through the activation of cellular signaling pathways responsible for glucose transport [27]. These factors may have contributed to the observed decrease in post-race glucosuria in the current study, although this aspect requires further investigation in the context of triathlon events. High-intensity training and carbohydrate-rich diets may contribute to pre-race glucosuria, hematuria, and proteinuria, potentially reflecting renal strain from muscle breakdown [8,28,29]. Overtraining or pre-race stress might also lead to leukocyturia due to immune system compromise or inflammation [30]. These findings suggest further study on how training loads and environmental exposure affect athletes' pre-race physiological states.

Finally, participation in the triathlon significantly increased USG, indicating dehydration. This aligns with studies demonstrating a worsening of hydration status in longer triathlons [26]. Dehydration can exacerbate exercise-induced proteinuria by increasing blood viscosity, renal vasoconstriction, and glomerular permeability [17,26]. It is essential to note that dehydration often leads to exercise-associated hyponatremia, where sodium concentration in blood, plasma, or serum decreases to abnormally low levels (<135 mmol/L). This condition can manifest symptoms ranging from mild, such as nausea, muscle cramps, and weakness, to severe, including confusion, seizures, and even coma (exercise-associated hyponatremic encephalopathy) [31]. However, this aspect has received limited attention in the context of short-distance triathlons [32].

The observed correlations highlight key physiological responses to the intense physical stress experienced by athletes during the triathlon. The positive relationship between ascorbic acid and pH suggests a potential role for ascorbic acid in maintaining acid-base balance, possibly influenced by dietary intake or metabolic adjustments under exertion. The strong association between erythrocytes and ketones may reflect dehydration and increased fat metabolism, common responses to energy depletion in endurance sports [33].

Furthermore, the relationship between proteinuria and hematuria points to possible renal stress, likely due to the combined effects of dehydration and high physical exertion. The negative correlation between pH and protein levels suggests that a more acidic urinary environment could exacerbate kidney stress or injury during high-intensity activities like triathlons [29,34,35].

However, it is important to note that using urine strips for these measurements limits the certainty of the results. While these strips provide valuable preliminary insights [23,36], their semi-quantitative nature means that the findings should be interpreted with caution. More robust methods, such as laboratory-based biochemical analysis, would be required to confirm these physiological patterns more accurately.

4.1. Limitations

This study provides valuable insights into the physiological responses to short-distance triathlons, particularly regarding proteinuria and hydration status. Our rapid dipstick urinalysis allowed for immediate assessment of multiple urinary biomarkers in a field setting, providing a comprehensive snapshot of athlete health immediately post-race. Including well-trained triathletes ensured our findings were relevant to the population most likely to participate in these events. Furthermore, our study is among the first to examine these specific physiological responses in the context of short-distance triathlons, filling an important gap in the literature.

However, some limitations should be considered when interpreting our results. The relatively small sample size (n = 27) may have led to statistically significant results for small changes, highlighting the preliminary nature of our findings. The absence of a non-exercising control group limits our ability to distinguish between exercise-induced changes and potential diurnal variations in urinary biomarkers. Our reliance on a single post-race urine sample restricts our ability to track the duration of observed changes or identify peak levels of proteinuria. While dipstick urinalysis enabled rapid screening results, the utilization of more advanced urine and blood markers such as urine proteincreatinine ratios, cystatin-C, urinary neutrophil gelatinase-associated lipocalin (NGAL), and urinary kidney injury molecule-1 (KIM-1) could enhance the precision of the analysis, particularly regarding conditions such as acute kidney injury. Additionally, the unexpected improvement in some biomarkers post-exercise, such as the decrease in pre-race haematuria and leukocyturia, warrants further investigation to understand the underlying mechanisms in the context of high-intensity, short-distance triathlons. Also, it is interesting for future studies to realize correlations, regressions, and factor analysis results to check which factors are related to specific urine components pre- and post-race, or if individual variables such as VO₂max, total finishing time, age, sex, or dietary intake affected the proteinuria and dehydration variables before and after the race. Despite these limitations, our study provides a solid foundation for future research. It offers valuable preliminary data that can inform athlete preparation strategies and medical guidelines for short-distance triathlons.

4.2. Practical Recommendations

From a practical perspective, these findings suggest that athletes should engage in postcompetition monitoring of urine biomarkers to understand the physiological challenges endurance athletes face, including the risks of dehydration and its potential impact on kidney function and exercise-induced proteinuria. To mitigate these risks, athletes may benefit from adopting preventative strategies such as maintaining proper hydrating and allowing sufficient kidney recovery between intensive training sessions. In addition, different recommendations could be given to enhance medical guidelines and athlete preparation strategies for short-distance triathlons based on the study findings:

- Tailoring fluid and electrolyte supplementation during and after short-distance triathlons to individual athletes' specific requirements rather than relying on generalized approaches.
- Vigilantly tracking changes in athletes' body composition, particularly among those who experience substantial weight loss following training or competitions, because this can disrupt fluid and sodium balance.
- Athletes who exhibit proteinuria, urobilinuria, and severe dehydration accompanied by symptoms of post-competition hyponatremia should undergo comprehensive clinical evaluation for enhanced biochemical diagnosis and, if necessary, receive appropriate medical attention.
- It is crucial to establish educational initiatives targeting short-distance triathlon athletes, coaches, and sports leaders. These programs should emphasize safe fluid replacement practices to prevent excessive fluid intake and inadequate hydration during training and short-distance triathlon competitions.

5. Conclusions

This study provides new insights into the physiological impacts of short-course triathlons, revealing significant findings not previously reported in the literature. Specifically, these events significantly induce post-race proteinuria, urobilinuria, and dehydration. Our research uniquely documents the first case of post-race urobilinuria and ketoacidosis in short-course triathlons, highlighting novel physiological responses. These findings underscore the substantial physiological stress imposed on kidney function and hydration status by short-distance triathlons despite their abbreviated distances. They highlight the importance of monitoring urinary biomarkers and hydration levels in athletes before and after competition. Our results contribute to a better understanding athletes' unique challenges in short-course triathlons and may inform future guidelines for athlete preparation and health monitoring. These insights are critical for developing strategies to mitigate the adverse effects observed, thereby enhancing athlete safety and performance. Further research, encompassing mechanistic and longitudinal aspects, is essential for a more comprehensive understanding of the health implications of this relatively understudied yet increasingly popular sports format.

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References

- Sharma, A.P.; Périard, J.D. Physiological Requirements of the Different Distances of Triathlon. In *Triathlon Medicine*; Migliorini, S., Ed.; Springer International Publishing: Cham, Switzerland, 2020; pp. 5–17. ISBN 978-3-030-22357-1.
- 2. Suriano, R.; Bishop, D. Physiological Attributes of Triathletes. J. Sci. Med. Sport 2010, 13, 340–347. [CrossRef] [PubMed]
- Staff, H.C.; Solli, G.S.; Osborne, J.O.; Sandbakk, Ø. Long-Term Development of Training Characteristics and Performance-Determining Factors in Elite/International and World-Class Endurance Athletes: A Scoping Review. Sports Med. 2023, 53, 1595–1607. [CrossRef] [PubMed]
- 4. Millet, G.P.; Vleck, V.E.; Bentley, D.J. Physiological Requirements in Triathlon. J. Hum. Sport Exerc. 2011, 6, 184–204. [CrossRef]
- Fellmann, N. Hormonal and Plasma Volume Alterations Following Endurance Exercise. Sports Med. 1992, 13, 37–49. [CrossRef] [PubMed]
- Millet, G.P.; Vleck, V.E.; Bentley, D.J. Physiological Differences Between Cycling and Running. Sports Med. 2009, 39, 179–206. [CrossRef]
- Vleck, V.; Millet, G.P.; Alves, F.B. The Impact of Triathlon Training and Racing on Athletes' General Health. Sports Med. 2014, 44, 1659–1692. [CrossRef]
- 8. Rojas-Valverde, D.; Olcina, G.; Sánchez-Ureña, B.; Pino-Ortega, J.; Martínez-Guardado, I.; Timón, R. Proteinuria and Bilirubinuria as Potential Risk Indicators of Acute Kidney Injury during Running in Outpatient Settings. *Medicina* 2020, *56*, 562. [CrossRef]

- de Jesus Alves, M.D.; dos Santos Silva Silva, D.; Pereira, E.V.M.; Pereira, D.D.; de Sousa Fernandes, M.S.; Santos, D.F.C.; Oliveira, D.P.M.; Vieira-Souza, L.M.; Aidar, F.J.; de Souza, R.F. Changes in Cytokines Concentration Following Long-Distance Running: A Systematic Review and Meta-Analysis. *Front. Physiol.* 2022, 13, 838069.
- 10. Khodaee, M.; Saeedi, A.; Irion, B.; Spittler, J.; Hoffman, M.D. Proteinuria in a High-Altitude 161-Km (100-Mile) Ultramarathon. *Phys. Sportsmed.* **2020**, *49*, 92–99. [CrossRef]
- 11. Poortmans, J.R.; Labilloy, D. The Influence of Work Intensity on Postexercise Proteinuria. *Eur. J. Appl. Physiol.* **1988**, 57, 260–263. [CrossRef]
- Rojas-Valverde, D.; Martínez-Guardado, I.; Sánchez-Ureña, B.; Timón, R.; Scheer, V.; Pino-Ortega, J.; Olcina, G. Outpatient Assessment of Mechanical Load, Heat Strain and Dehydration as Causes of Transitional Acute Kidney Injury in Endurance Trail Runners. Int. J. Environ. Res. Public Health 2021, 18, 10217. [CrossRef] [PubMed]
- Atkins, W.C.; Butts, C.L.; Kelly, M.R.; Troyanos, C.; Laursen, R.M.; Duckett, A.; Emerson, D.M.; Rosa-Caldwell, M.E.; McDermott, B.P. Acute Kidney Injury Biomarkers and Hydration Outcomes at the Boston Marathon. *Front. Physiol.* 2022, *12*, 813554. [CrossRef] [PubMed]
- 14. Scheer, V.; Tiller, N.B.; Doutreleau, S.; Khodaee, M.; Knechtle, B.; Pasternak, A.; Rojas-Valverde, D. Potential Long-Term Health Problems Associated with Ultra-Endurance Running: A Narrative Review. *Sports Med.* **2022**, *52*, 725–740. [CrossRef] [PubMed]
- 15. Casa, D.J.; Armstrong, L.E.; Hillman, S.K.; Montain, S.J.; Reiff, R.V.; Rich, B.S.E.; Roberts, W.O.; Stone, J.A. National Athletic Trainers' Association Position Statement: Fluid Replacement for Athletes. *J. Athl. Train.* **2000**, *35*, 212–224. [PubMed]
- 16. Crandall, C.G.; González-Alonso, J. Cardiovascular Function in the Heat-Stressed Human. *Acta Physiol. Oxf. Engl.* **2010**, *199*, 407–423. [CrossRef]
- Junglee, N.A.; Di Felice, U.; Dolci, A.; Fortes, M.B.; Jibani, M.M.; Lemmey, A.B.; Walsh, N.P.; Macdonald, J.H. Exercising in a Hot Environment with Muscle Damage: Effects on Acute Kidney Injury Biomarkers and Kidney Function. *Am. J. Physiol. Renal Physiol.* 2013, 305, F813–F820. [CrossRef]
- McDermott, B.P.; Anderson, S.A.; Armstrong, L.E.; Casa, D.J.; Cheuvront, S.N.; Cooper, L.; Kenney, W.L.; O'Connor, F.G.; Roberts, W.O. National Athletic Trainers' Association Position Statement: Fluid Replacement for the Physically Active. *J. Athl. Train.* 2017, 52, 877–895. [CrossRef]
- 19. Gaitán, J.M.; Eichner, N.Z.M.; Gilbertson, N.M.; Heiston, E.M.; Weltman, A.; Malin, S.K. Two Weeks of Interval Training Enhances Fat Oxidation during Exercise in Obese Adults with Prediabetes. J. Sports Sci. Med. 2019, 18, 636–644.
- Wołyniec, W.; Ratkowski, W.; Renke, J.; Renke, M. Changes in Novel AKI Biomarkers after Exercise. A Systematic Review. Int. J. Mol. Sci. 2020, 21, 5673. [CrossRef]
- 21. Martin, D.E.; Coe, P.N. Entrenamiento Para Corredores de Fondo y Medio Fondo; Editorial Paidotribo: Barcelona, Spain, 2007; ISBN 978-84-8019-119-7.
- 22. Crouter, S.E.; Antczak, A.; Hudak, J.R.; DellaValle, D.M.; Haas, J.D. Accuracy and Reliability of the ParvoMedics TrueOne 2400 and MedGraphics VO2000 Metabolic Systems. *Eur. J. Appl. Physiol.* **2006**, *98*, 139–151. [CrossRef]
- Hoffman, M.D.; Stuempfle, K.J.; Fogard, K.; Hew-Butler, T.; Winger, J.; Weiss, R.H. Urine Dipstick Analysis for Identification of Runners Susceptible to Acute Kidney Injury Following an Ultramarathon. J. Sports Sci. 2013, 31, 20–31. [CrossRef] [PubMed]
- 24. Wyness, S.P.; Hunsaker, J.J.H.; Snow, T.M.; Genzen, J.R. Evaluation and Analytical Validation of a Handheld Digital Refractometer for Urine Specific Gravity Measurement. *Pract. Lab. Med.* **2016**, *5*, 65–74. [CrossRef] [PubMed]
- 25. Maughan, R.J. A Simple, Rapid Method for the Determination of Glucose, Lactate, Pyruvate, Alanine, 3-Hydroxybutyrate and Acetoacetate on a Single 20-Mul Blood Sample. *Clin. Chim. Acta* **1982**, *122*, 231–240. [CrossRef] [PubMed]
- Hew-Butler, T.; Rosner, M.H.; Fowkes-Godek, S.; Dugas, J.P.; Hoffman, M.D.; Lewis, D.P.; Maughan, R.J.; Miller, K.C.; Montain, S.J.; Rehrer, N.J.; et al. Statement of the Third International Exercise-Associated Hyponatremia Consensus Development Conference, Carlsbad, California, 2015. *Clin. J. Sport Med.* 2015, 25, 303. [CrossRef] [PubMed]
- Sylow, L.; Kleinert, M.; Richter, E.A.; Jensen, T.E. Exercise-Stimulated Glucose Uptake-Regulation and Implications for Glycaemic Control. *Nat. Rev. Endocrinol.* 2017, 13, 133–148. [CrossRef]
- Makhlin, N.V.; Bukaev, I.N.; Liubarskaia, S.G.; Gorodetskaia, S.B.; Ponomareva, V.I.; Rudakov, A.G. [The characteristics of proteinuria during intensive physical loading]. Urol. Nefrol. 1990, 2, 15–17.
- 29. Shephard, R.J. Exercise Proteinuria and Hematuria: Current Knowledge and Future Directions. J. Sports Med. Phys. Fit. 2016, 56, 1060–1076.
- Walzik, D.; Joisten, N.; Zacher, J.; Zimmer, P. Transferring Clinically Established Immune Inflammation Markers into Exercise Physiology: Focus on Neutrophil-to-Lymphocyte Ratio, Platelet-to-Lymphocyte Ratio and Systemic Immune-Inflammation Index. *Eur. J. Appl. Physiol.* 2021, 121, 1803–1814. [CrossRef]
- Johnson, K.B.; Connolly, C.P.; Cho, S.P.; Miller, T.K.; Sallis, R.E.; Hiller, W.D.B. Clinical Presentation of Exercise-Associated Hyponatremia in Male and Female IRONMAN[®] Triathletes over Three Decades. *Scand. J. Med. Sci. Sports* 2023, 33, 1841–1849. [CrossRef]
- 32. Bennett, B.L.; Hew-Butler, T.; Rosner, M.H.; Myers, T.; Lipman, G.S. Wilderness Medical Society Clinical Practice Guidelines for the Management of Exercise-Associated Hyponatremia: 2019 Update. *Wilderness Environ. Med.* **2020**, *31*, 50–62. [CrossRef]
- 33. Houtkooper, L. Food Selection for Endurance Sports. Med. Sci. Sports Exerc. 1992, 24, 349. [CrossRef]
- 34. Bellinghieri, G.; Savica, V.; Santoro, D. Renal Alterations during Exercise. J. Ren. Nutr. 2008, 18, 158–164. [CrossRef] [PubMed]

 Sumida, K.; Nadkarni, G.N.; Grams, M.E.; Sang, Y.; Ballew, S.H.; Coresh, J.; Matsushita, K.; Surapaneni, A.; Brunskill, N.; Chadban, S.J.; et al. Conversion of Urine Protein-Creatinine Ratio or Urine Dipstick Protein to Urine Albumin-Creatinine Ratio for Use in Chronic Kidney Disease Screening and Prognosis: An Individual Participant-Based Meta-Analysis. *Ann. Intern. Med.* 2020, 173, 426–435. [CrossRef]

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