AFRL-RH-WP-TR-2021-0018

EFFECTS OF AN AMINO-ACID GEL (AQUAGARD®) ON HYDRATION AND PERFORMANCE PARAMETERS DURING PROLONGED AEROBIC EXERCISE

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APRIL 2021
Interim Report

Distribution Statement A: Approved for public release.

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE ***(DD-MM-YY)*** 29-04-21	**2. REPORT TYPE** Interim Report	**3. DATES COVERED** ***(From - To)*** 1 Jan 2018 – 29 Apr 2021

4. TITLE AND SUBTITLE
Effects of an AMINO-ACID Gel (AQUAGARD®) on Hydration and Performance Parameters During Prolonged Aerobic Exercise

5a. CONTRACT NUMBER
FA8650-18-F-6931

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
00000F

6. AUTHOR(S)
Adam Strang1, SSgt. Roger Smith1, Kaela Hierholzer, Brenda Moore, Rebecca Crews, & Nicholas Mackowski

5d. PROJECT NUMBER
0000

5e. TASK NUMBER
00

5f. WORK UNIT NUMBER
H0S6

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Oak Ridge Institute for Science and Education, 1299 Bethel Valley Rd., Oak Ridge, TN 37830; Infoscitex Corp., 4027 Colonel Glenn Hwy., Beavercreek Township, OH 45431

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
1Air Force Materiel Command
Air Force Research Laboratory
711th Human Performance Wing
Airman Systems Directorate
Airman Biosciences Division
Performance Optimization Branch
Wright-Patterson AFB, OH 45433

10. SPONSORING/MONITORING AGENCY ACRONYM(S)
711 HPW/RHB

11. SPONSORING/MONITORING AGENCY REPORT NUMBER(S)
AFRL-RH-WP-TR-2021-0018

12. DISTRIBUTION/AVAILABILITY STATEMENT
Distribution Statement A. Approved for public release.

13. SUPPLEMENTARY NOTES
Report contains color MSC/PA-2021-0188 AFRL-2021-1455, cleared 20 May 2021

14. ABSTRACT

Studies suggest that amino acids play an important role in intracellular hydration. Based on this, amino-acid supplements have become commercially available to enhance hydration. AquaGard® is among this supplement class but is unique because it is available as a food gel. In attempt to validate this product, the current study had volunteers complete a pair of 10-mile runs at a fixed speed (5 mph) while given water (586 ml) and AquaGard® (129 g). According to most hydration measures (sans total body mass) participants were shown to have maintained a hydrated state throughout the run regardless of the substance provided. In addition, statistical trends in hydration and performance parameters observed during the run were mostly equivalent between water and AquaGard® (again, sans total body mass). Given a specified 4.5x difference in mass-to-volume ratio between the substances (i.e., comparatively more water was provided), these results indicate a potential practice advantage for AquaGard® for hydration and performance maintenance.

15. SUBJECT TERMS
Glucose, lactate, hemoglobin

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT:**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** (Monitor)
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Nathaniel Baldwin
Unclassified	Unclassified	Unclassified	SAR	28	**19b. TELEPHONE NUMBER** *(Include Area Code)* N/A

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ACKNOWLEDGMENTS

This project was performed as part of a Cooperative Research and Development Agreement (CRADA) between 711 HPW and Aqua Innovations Limited. (#18-158-SM-01CRD).

1.0 INTRODUCTION

A hydrated state is critical for the proper function of most bodily systems (e.g., cardiovascular, digestive, respiratory, and nervous) (Jequier & Constant, 2010; McDermott, et al., 2017). The need for proper hydration is amplified during physical activity to compensate for increased fluid loss - caused mostly by increased sweat volume (Korey Stringer Institute, 2019). However, both whole body and intracellular fluid loss is difficult to measure, so total body mass is commonly used as a surrogate. This led to the recommendation that for every 1-kilogram (kg) of total body mass loss, an additional 1-liter (L) of fluid is needed as replacement to maintain hydration (Casa, Clarkson, & Roberts, 2005).

This recommendation can be tough to follow since body fluid loss can be as great as 3 to 4-L per hour during intense activity (Gisolfi, 1993). To put it bluntly, it can be nearly impossible to ingest (due to discomfort) and absorb (due to biological limits) enough fluid to maintain hydration during intense and prolonged activity. This had led many who are faced with this challenge to engage in voluntary dehydration or adopt some alternative hydration strategy, such as hydration periodization, pre-activity hydration, ingesting foods with high water content, etc. (Noakes, 1993).

Unfortunately for military personnel, many of the hydration strategies mentioned above are not feasible due to inherent constraints of mission and training environments. For example, consider a 4-hour loaded (22kg) ruck march in an environment without access to water. In order to maintain hydration in this situation 12L of fluid is recommended, which equates to 54 percent (%) of the total carrying load. It is impractical to carry this much water in place of or in addition to military gear. In another example, consider a fighter pilot on a similar duration flying mission. There is limited space to store and/or cool 12L of water without significant changes to air frame design. In addition, consumption of such large water volume would likely lead to high volume excretion, which is not comfortably accommodated by the flight suite.

Recently, a limited number of studies have explored the role that amino acids play in intracellular hydration. Specifically, Di Pasquale (2008) proposed that amino acids can be transported into cells by the sodium-ion dependent pumps or transport systems. After this occurs, the system convert~~s~~ the energy of the sodium gradient across the plasma membrane into osmotically active amino acid gradients. This conversion facilitates water entering the cell and aids in its retention (*if* sufficient amino acids remain available).

The practical implication of Di Pasquale's hypothesis is that increasing the availability of amino acids could improve the effectiveness and/or efficiency of intracellular hydration. To this end, Tai et al. (2014), recently showed that an amino acid – electrolyte (AE) beverage favored rehydration of the intracellular space.

Based on this evidence, a few amino-acid based commercial supplements have appeared on the market claiming to support hydration maintenance. Most of these supplements come in the form of whole liquids (e.g., Enterade®) or water additives (e.g., Amino Vital®). These supplements are marketed for both clinical and athletic applications, with some evidence that these supplements may be effective as anti-diuretics in patients receiving and/or recovering from cancer treatment (DeFilipp et al., 2021 & Chauhan et al. 2018).

AquaGard® is among this new class of supplements but is unique because it is a stand-alone food product (gel). The developers of AquaGard® state that the specific combination of amino acids contained in the product (when used as directed) are sufficient to support human hydration. In a study performed at The Ohio State University (for additional information, contact Aqua Innovations Ltd.) researchers found that AquaGard® caused no adverse physiological or digestive effects and was effective for maintaining a hydrated state for up to 8 hours under 'normal' conditions (i.e., no exposure to excessive heat, humidity, or intense physical activity).

The current study aimed to build upon these initial findings by examining hydration (e.g., urine specific gravity, nude body mass, etc.) and performance [e.g., blood lactate, heart rate (HR), perceived exertion etc.] effects during a bout of prolonged and high-intensity physical activity when supplemented by water and AquaGard®. To accomplish this, volunteer participants completed a pair of 10-mile runs (spaced at least 7 days apart) at a fixed speed (5 mph) while provided with a limited amount of water (total of 586 ml) and AquaGard® (total of 129 g – according to manufacturer recommendations).

Given these conditions we expected that when participants were provided water they would elicit responses indicative of mild-to-moderate dehydration (e.g., decreased total body mass, increased urine specific gravity, etc.) and fatigue (e.g., increased blood lactate, perceived exertion, HR, etc.). We were less certain about what to expect when participants were provided AquaGard® since this supplement (and its dosage recommendations) has not been scrutinized under condition of intense physical activity. However, given that participants were provided less AquaGard® (129 g) as compared to water (586 ml), we posited the following:

1. Participants were likely to exhibit great body mass loss during the AquaGard® condition[1]. This is simply because less substance was provided to combat body mass loss (likely attributed to sweating) that was likely to occur during the run.
2. If there were no significant differences in the other hydration or performance parameters observed between the water and AquaGard®, this could indicate a practical advantage for AquaGard®.
3. If AquaGard® was found to maintain hydration status better than water, this would indicate a practical and physiological advantage for this product.

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[1] According to the developers of AquaGard® this product is designed to limit additional body mass while providing effective hydration.

2.0 METHODS

2.1 Experimental Design

This study represented a 2 × 2 × 2/7 within subjects design. The first within-subjects factor was Supplement, with two levels (Water vs. AquaGard®). The second within-subjects factor was Lab Team/Location (T/L), with two levels (STRONG lab vs. Human Performance Satellite)[2]. The third within-subjects factor was Time, with either two (pre-run vs. post-run) or seven (pre-run, +20min, +40min, +60min, +80min, +100min, and 120min) factors.

Dependent variables included body mass (kg)[3], urine specific gravity (USG)[2], urine volume (mL)[2], urine mass (g)[2], urine PH[2], heart rate [HR; beats per minute (bpm)][4], blood glucose (mg/dL)[3], hematocrit (%)[3], hemoglobin (g/dL)[3], lactate(mmol/L)[3], tympanic temperature (ºF)[3], and Borg scales ratings of perceived exertion (RPE; 6 to 20)[3].

2.2 Participants

This study contained twelve participants (10 male; 2 female) who were free of illness or musculoskeletal injury at the time of participation. All participants completed the study without incident of injury or illness – including no reports of adverse digestive or other symptomatic effects following exposure to the exercise protocol or AquaGard®. All procedures were approved by the Air Force Research Laboratory's (AFRL) Internal Review Board (IRB).

2.3 Procedures

Initial Screening. An initial screening visit was used to obtain consent from participants and determine their eligibility. Upon arrival for screening, participants were provided the informed consent document for review, given a verbal explanation of the experimental procedures and provided an opportunity to ask questions. Following written consent, participants had their height, mass and body fat percentage (DEXA) recorded (Table 1). Then, participants completed the Ebbeling single-stage submaximal treadmill exercise test to estimate maximal aerobic capacity (i.e., $\dot{V}O_{2max}$). For this test participants walked on a treadmill for 4 minutes between 2.0 – 4.5 miles per hour in order to achieve a HR between 50 – 70% of an age-predicted maximal HR. Following the initial 4 minutes, the treadmill incline was increased to 5% for 4 minutes. Heart rate was recorded for the last 30 seconds of the second 4-minute bout and used to estimate $\dot{V}O_{2max}$. Participants exhibited a projected maximal aerobic capacity ($\dot{V}O_{2max}$) above 45 $ml/kg^{-1}/min^{-1}$.

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[2] Data collection for this study was disrupted by a 711 HPW reorganization of personnel and facilities. Because of this, the study was executed by two independent research teams working at two separate locations [i.e., Signature Tracking for Optimized Nutrition and Training (STRONG) lab (Bldg. 840, Area B) and the Human Performance 'Satellite' lab (Wright-Field Fitness Center, Area B)]. It was important for us to confirm that this reorganization did not influence study results so these changes were explored as a study factor.

[3] Two-level variable

[4] Seven-level variable

Table 1. Participant Demographics

Demographic Variable	Mean	Standard Deviation (SD)
Age (years)	29.50	5.05
Height (cm)	177.75	7.74
Body Mass (kg)	80.86	12.29
Estimated VO_{2max} ($mL/kg^{-1}/min^{-1}$)	53.41	4.90
Body Fat (%)	16.51	5.73
Body Mass Index	25.47	2.62

Experimental sessions. Each participant completed two experimental sessions separated by at least seven days. The procedures of these two sessions were identical - with the exception of the oral hydration product consumed. The order of the oral supplement examined in the two sessions was randomized for each participant to account for the potential influence of order effects.

Four hours prior to each session participants were instructed to consume 0.47 – 0.59 L of water and abstain from ingesting any supplements (including caffeine). Upon arriving to the laboratory, participants underwent a series of baseline (pre-run) assessments. These assessments included urine (i.e., volume, mass and PH), capillary blood (i.e., glucose, lactate, hematocrit, and hemoglobin), body mass (nude), temperature (tympanic), and perceived exertion (i.e., Borg) measurements (Figure 1). After these baseline assessments were completed, participants donned a chest HR monitor (Polar V1) and consumed 86 ml (or g) of either water or Aqua-Gard®. Participants were then instructed to rest for twenty minutes in order to promote digestive absorption of each substance.

Upon completion of the absorption period, participants began the exercise protocol. This protocol required participants to run at 5 miles per hour with 0% incline for a total of 120 minutes (equivalent to 10 miles). The run itself was broken into a set of six, 20-minute intervals. At the completion of each interval, participants ceased running for a brief period of time (less than 5min) while capillary blood, HR, and RPE samples were gathered. Following 60 minutes of exercise, participants were provided an additional 250 ml of water or 43 g of Aqua-Gard®. At completion of the exercise protocol (+120 minutes), participants replicated baseline assessments and were provided 250 ml of additional water for recovery.

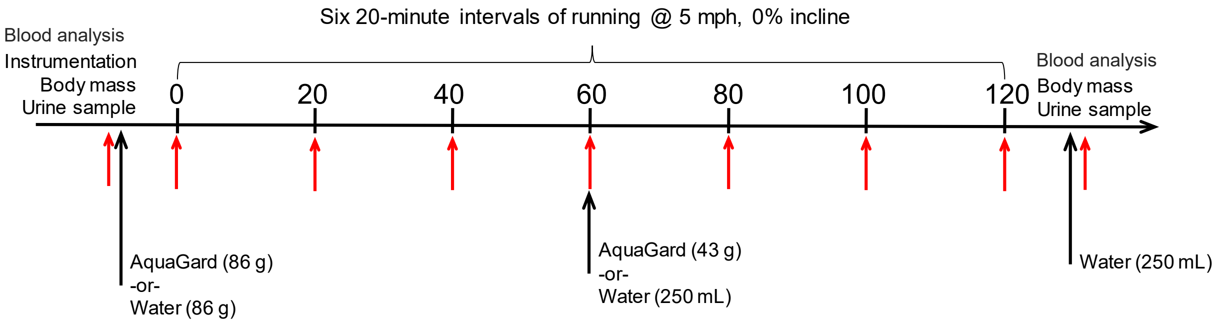

Figure 1. Study Visit Timeline. Red arrows indicate measurement of RPE, HR, tympanic temperature, and blood analysis via finger puncture at the end of each 20-minute exercise interval. Black arrows indicate providing hydration product to participant.

Capillary blood samples and processing. Capillary blood was obtained via finger puncture using a 23 gauge needle and analyzed "on the spot" to determine glucose (Abbot Diabetes Care FreeStyle glucometer), lactate (Nova Biomedical Lactate Plus), hemoglobin (EKF Diagnostics HemoPoint H2), and hematocrit levels. Hematocrit was measured by filling heparinized capillary tubes (Carolina Scientific) and placing them in a centrifuge for 5 min at a minimum of 11,500 revolutions per min to separate red blood cells from plasma (Grafco 410E Hematocrit Centrifuge). An analog hematocrit reading scale (Gemmy Industrial Corp.) was used to quantify hematocrit. Relative changes in blood volume and plasma volume were calculated using hemoglobin concentration and hematocrit values.

Urine samples and processing. Urine samples were collected in disposable vessels. The research team measured the volume (Nalgene graduated cylinder) and mass (Carolina Science OHAUS balance) of the urine samples. Further, urine was analyzed for color (Marine comparison chart), pH (Micro Essential Laboratory Hydrion paper pH strips), and urine specific gravity (USG, ATAGO Pen Refractometer). USG was performed in triplicate for each urine sample and the average was used for data analysis. Bottled water served as the control liquid for USG.

2.4 Statistical Analysis

Inferential statistics for all dependent variables were executed using a set of 2 × 2 × 2/7 within-subjects ANOVAs (critical-$p < .05$) and follow-up paired t-tests (if applicable). All statistics were performed utilizing the statistical package for the social sciences (SPSS) (v18, IBM, Armonk, NY).

3.0 RESULTS

Ratings of Perceived Exertion. A main effect of Time was detected, F (1, 6) = 80.04, $p < .01$, $\mu_p^2 = .889$, indicating that as the run progressed RPE increased for both groups (Figure 2). A main effect of T/L was also detected, F (1, 10) = 6.60, $p < .05$, $\mu_p^2 = .397$, indicating that participants who performed the experiment in the STONG lab (M = 11.98) reported a higher overall RPE as compared to those in the Human Performance Satellite lab (M = 9.67).

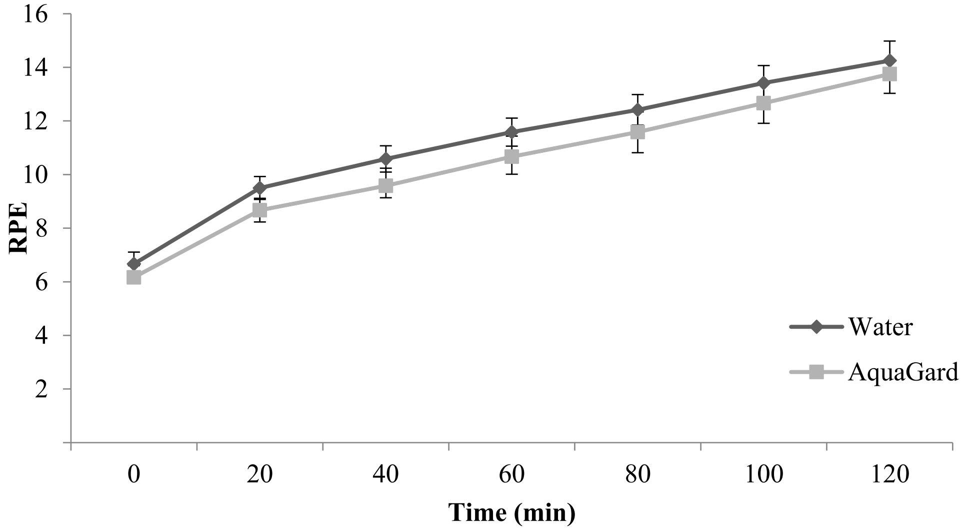

Figure 2. Rating of Perceived Exertion Response for Water and AquaGard® over Time.

Heart rate. A main effect of Time was detected, F (1, 6) = 241.36, $p < .01$, $\mu_p^2 = .960$, indicating that as the run progressed participants elicited increased HR under both supplement conditions (Figure 3)[5].

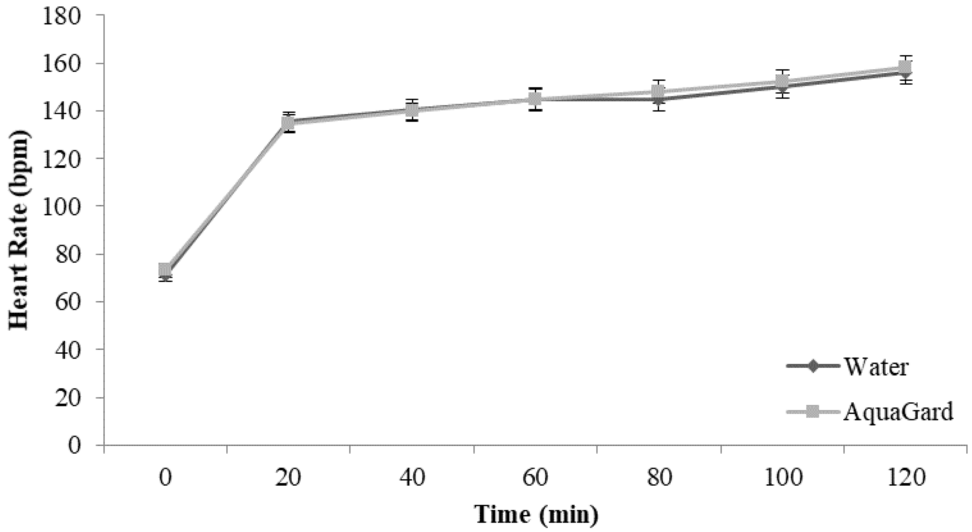

Figure 3. Heart Rate Response for AquaGard® and Water over Time.

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[5] It is noteworthy that this exponential trend is indicative of the ‘classic’ HR drift phenomenon for which the cause is still debated (Coyle & Gonzalez-Alonso, 2001).

Blood Lactate. A Supplement × Time interaction was detected, $F\ (1, 6) = 4.12$, $p < 0.5$, $\mu_p{}^2 = .292$ (Figure 4). This interaction was driven by changes in blood lactate at baseline between the two conditions, $t\ (20) = 2.57$, $p < .05$. This difference subsided by completion of the run (i.e., no difference in Blood Lactate at +120 min), indicating no critical change in Blood lactate between the two conditions over time.

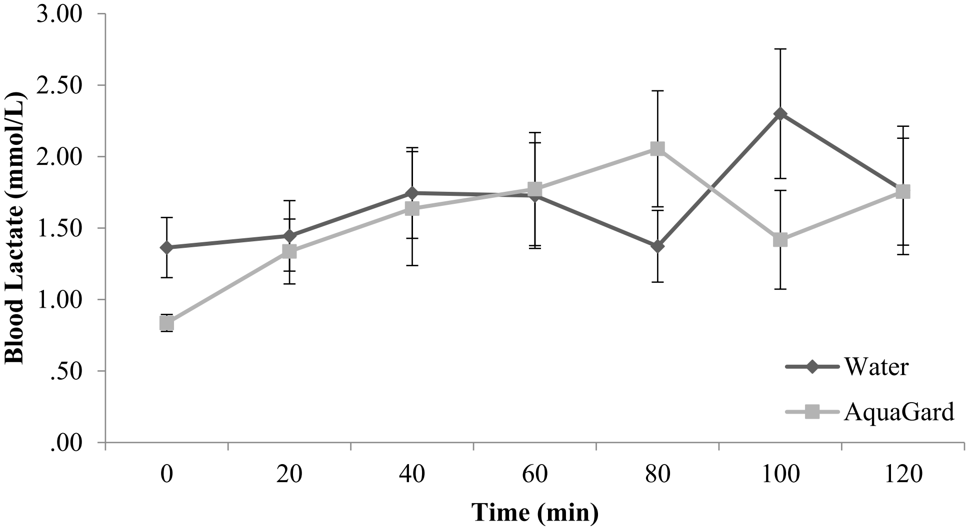

Figure 4. Blood Lactate Response for AquaGard® and Water over Time.

Blood Glucose. A main effect of Time was detected, F (1, 10) = 3.85, $p < .01$, $\mu_p^2 = .278$, indicating a decrease in blood glucose levels under both conditions over time (Figure 5).

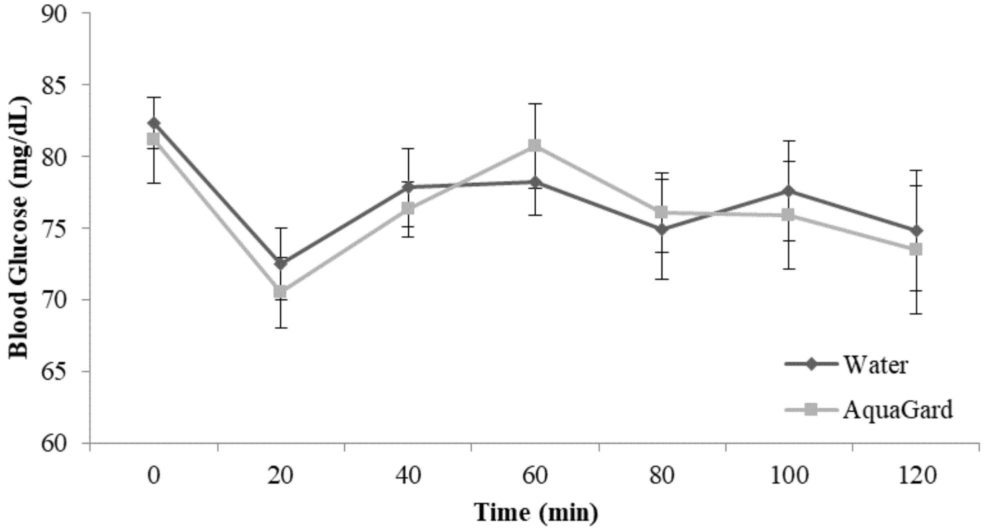

Figure 5. Blood Glucose Response for Water and AquaGard® over Time.

Tympanic Temperature. A main effect of Time was detected, F (1,10) = 2.31, $p < .01$, $\mu_p^2 =$ .266, indicating an increase in tympanic temperature over time under both conditions (Figure 6).

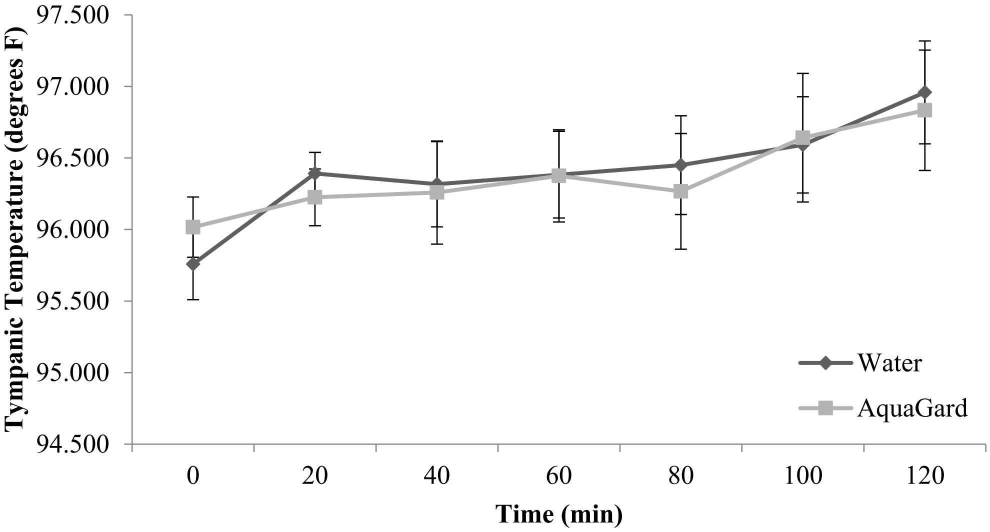

Figure 6. Tympanic Temperature Response for AquaGard ®and Water over Time.

Hematocrit. No main effects or interactions were detected (Figure 7). According to clinical thresholds for Hematocrit, participants remained in a hydrated state throughout the run for both conditions (Figure 7b & c).

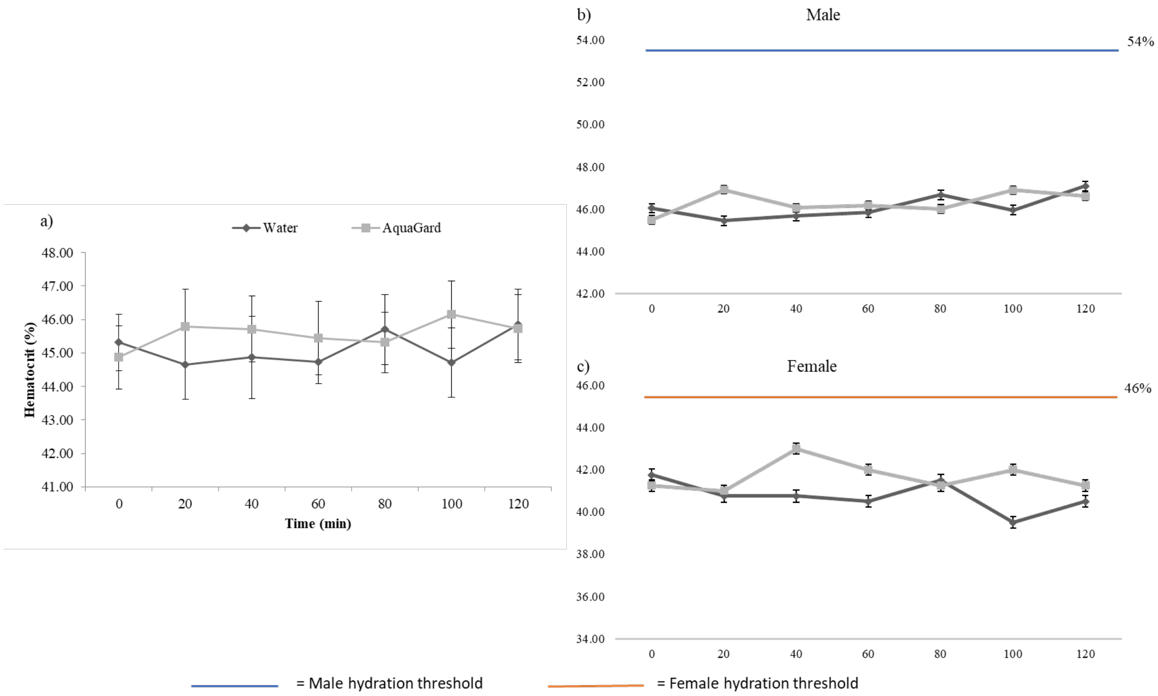

Figure 7. Hematocrit Response for AquaGard® and Water over Time.

(a). Hematocrit Response for AquaGard® and Water over Time broken down by Male (b) and Female (c) participants. Orange and blue lines indicated hydration thresholds, above which Hematocrit levels indicate a dehydrated state.

Hemoglobin. Main effects of Supplement, F (1, 10) = 5.49, $p < .05$, $\mu_p^2 = .354$, and Time, F (1,10) = 2.40, $p < .05$, $\mu_p^2 = .194$, were detected (Figure 8). These findings indicate that Hemoglobin levels were higher overall in the AquaGard® condition and that Hemoglobin levels increased over time under both conditions. According to clinical thresholds for Hemoglobin, participants remained in a hydrated state throughout the run for both conditions (Figure 8b & c).

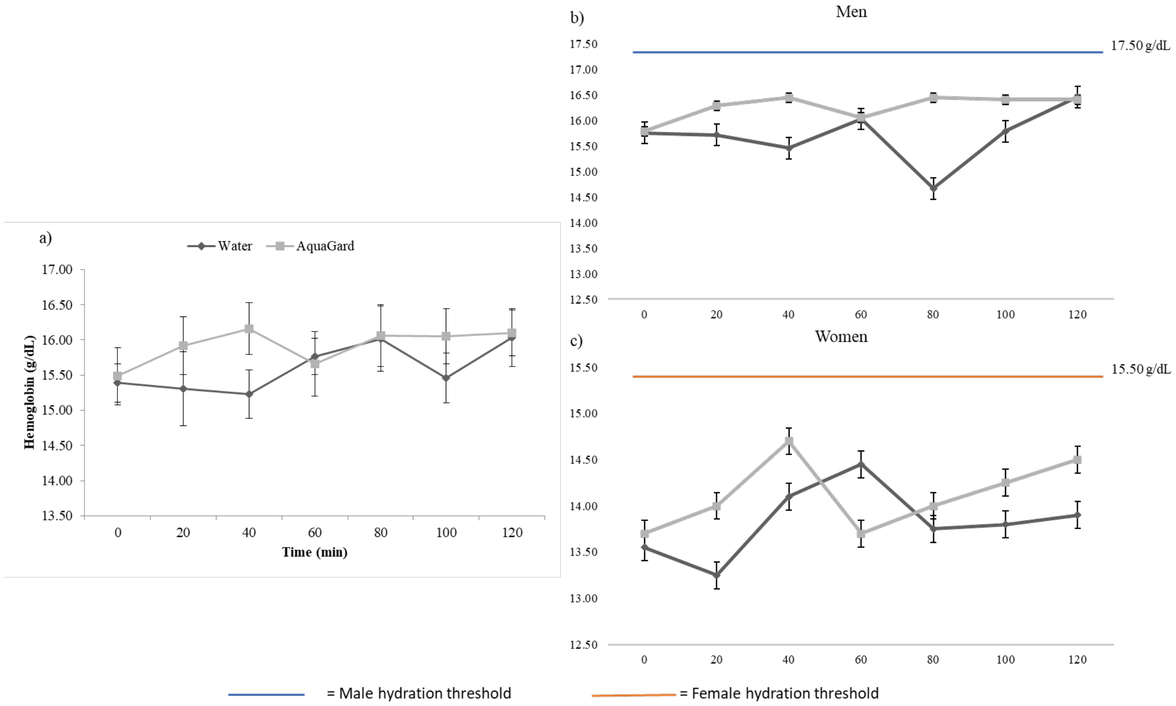

Figure 8. Hemoglobin Response for AquaGard® and Water over Time .

(a). Hemoglobin Response for AquaGard® and Water over Time broken down by Male (b) and Female (c) participants. Orange and blue lines indicated hydration thresholds, above which Hemoglobin levels indicate a dehydrated state.

Body Mass. Main effects of Time, F (1, 10) = 128.83, $p < .05$, $\mu_p^2 = .354$), Supplement, F (1, 10) = 5.35, $p < .05$, $\mu_p^2 = .354$, and a Time × Supplement interaction, $F(1, 10) = 7.42$, $p < .05$, $\mu_p^2 = .354$, were detected (Figure 9). Together these findings indicate that significant body mass loss was detected under both conditions at post-test, but that the post-test loss was greater under the AquaGard® condition. Note that, on average, both conditions resulted in an approximate 2% body mass loss.

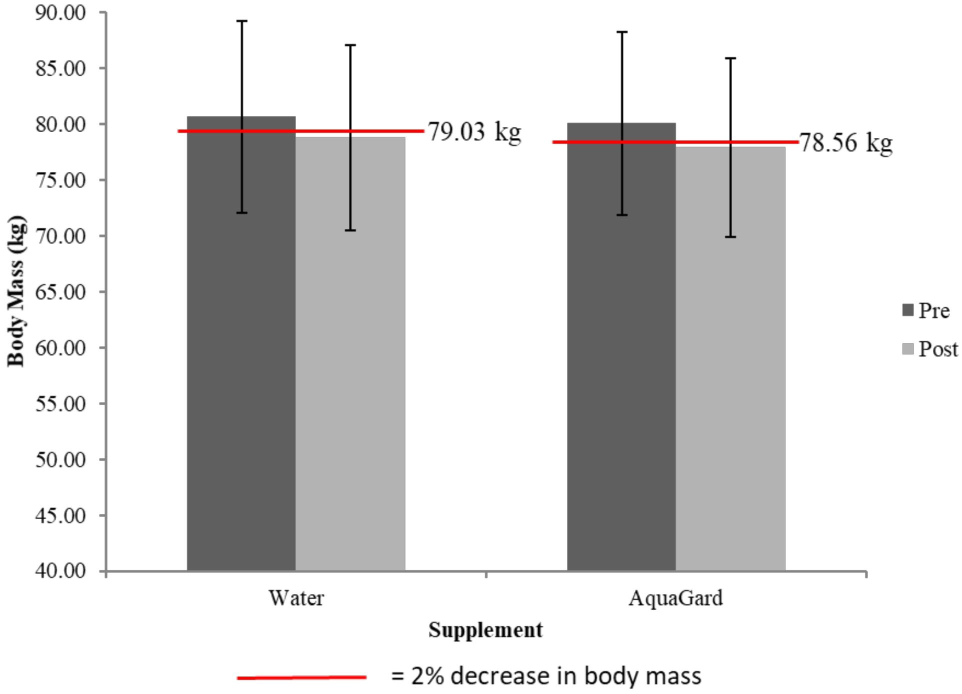

Figure 9. Pre and Post-test Body Mass for Water and AquaGard®.
The red line indicates a hydration threshold for body mass, below which a dehydrated state is indicated.

Urine Specific Gravity. A main effect of Time was detected, $F\ (1,10) = 5.77$, $p < .05$, $\mu_p^2 = .366$, indicating an increase in USG over time for both conditions (Figure 10). According to the clinical USG threshold, subjects remained in a hydrated state throughout the run for both conditions.

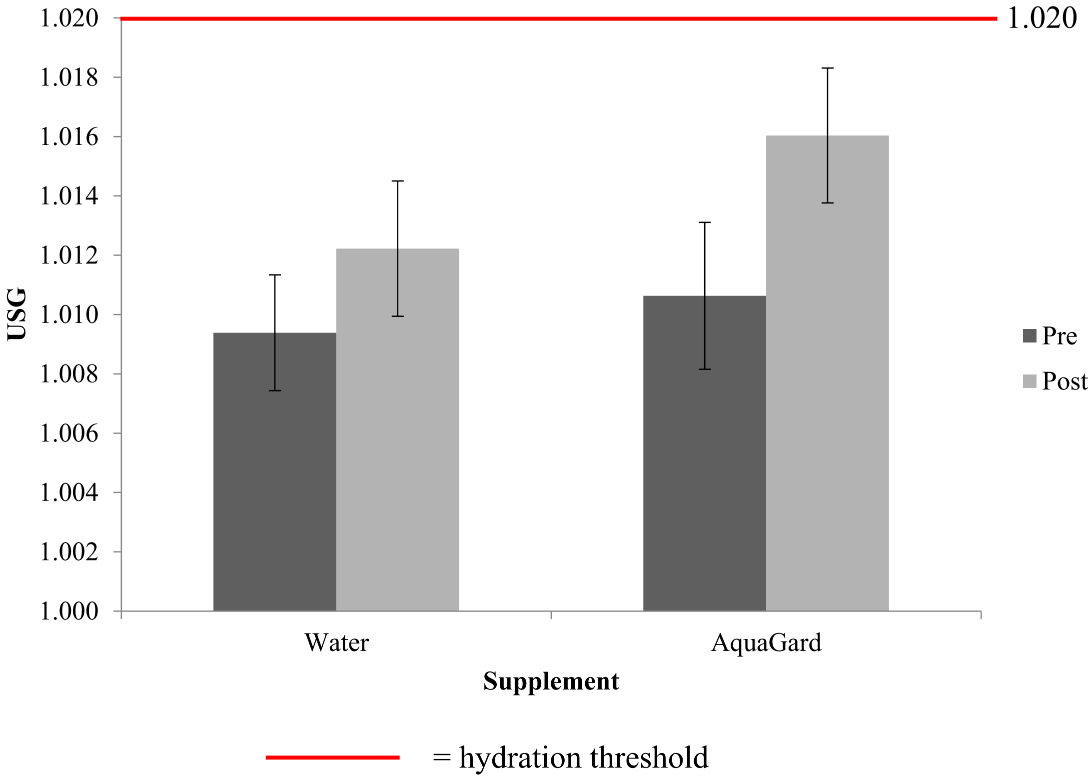

Figure 10. USG Pre- and Post-test for AquaGard® and Water.
The red line indicates a hydration threshold for USG, above which a dehydrated state is indicated.

Urine pH. No main effects or interactions were detected indicating no change in urine pH for either condition (Figure 11). According to the clinical pH threshold, participants remained in a hydrated state under both conditions.

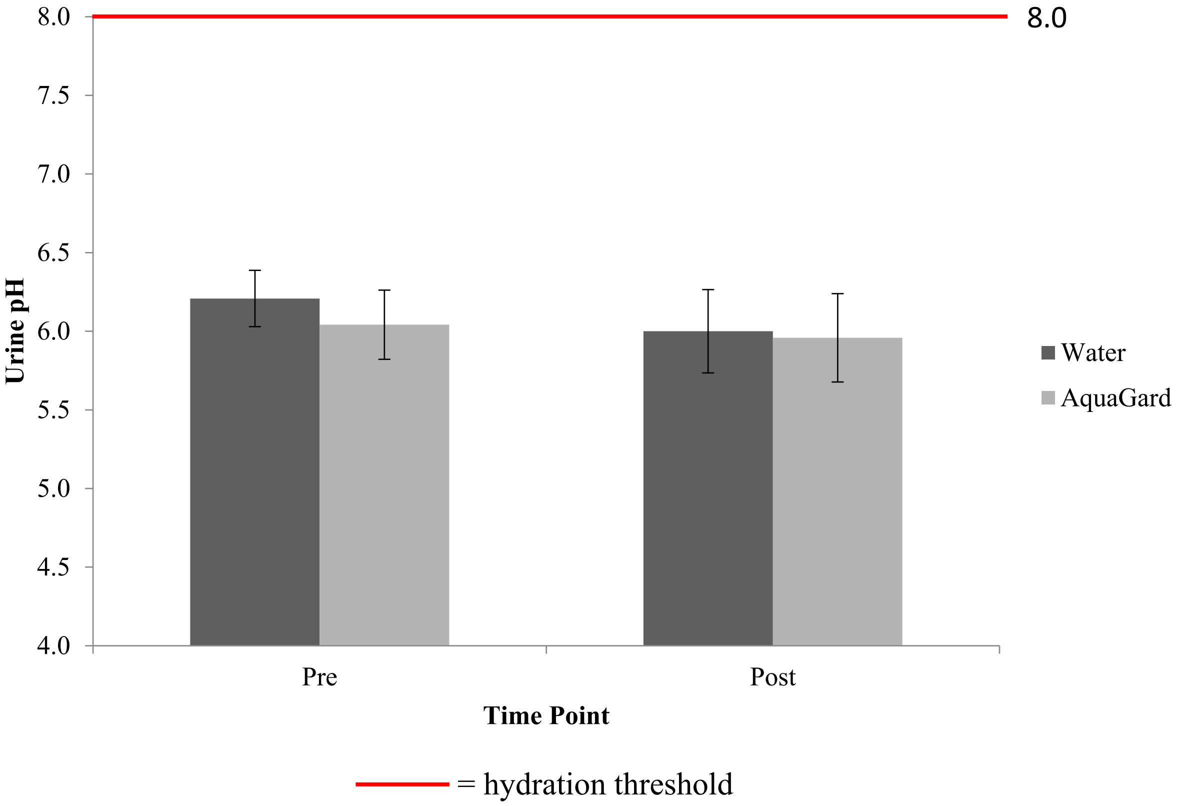

Figure 11. Urine pH for both supplements at Pre- and Post-test
The red line indicates a hydration threshold for pH, above which a dehydrated state is indicated.

Urine Volume. No main effects or interactions were detected - indicating no change in urine volume following the run (Figure 11).

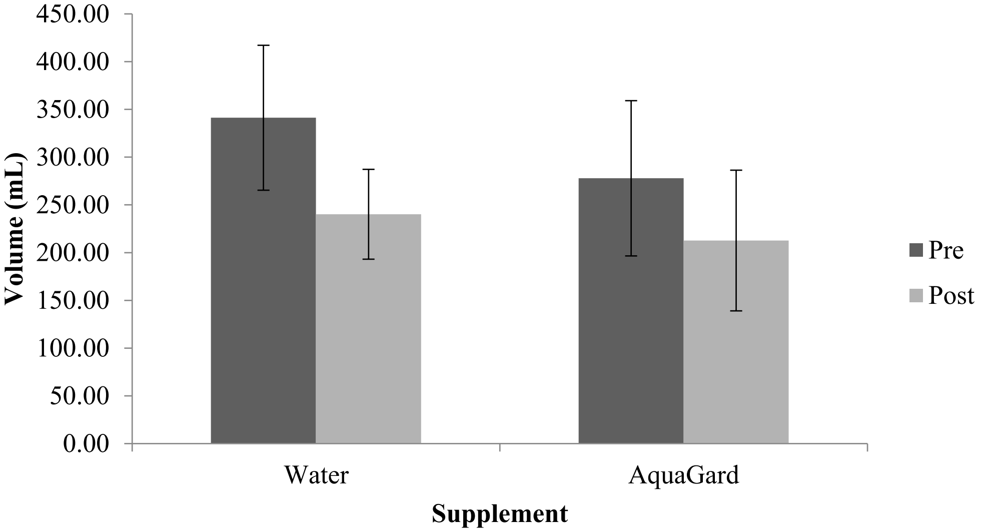

Figure 12. Urine Volume for both supplements at Pre- and Post-test.

Urine Mass. No main effects or interactions were detected. This indicates no significant change in urine mass regardless of the supplement consumed.

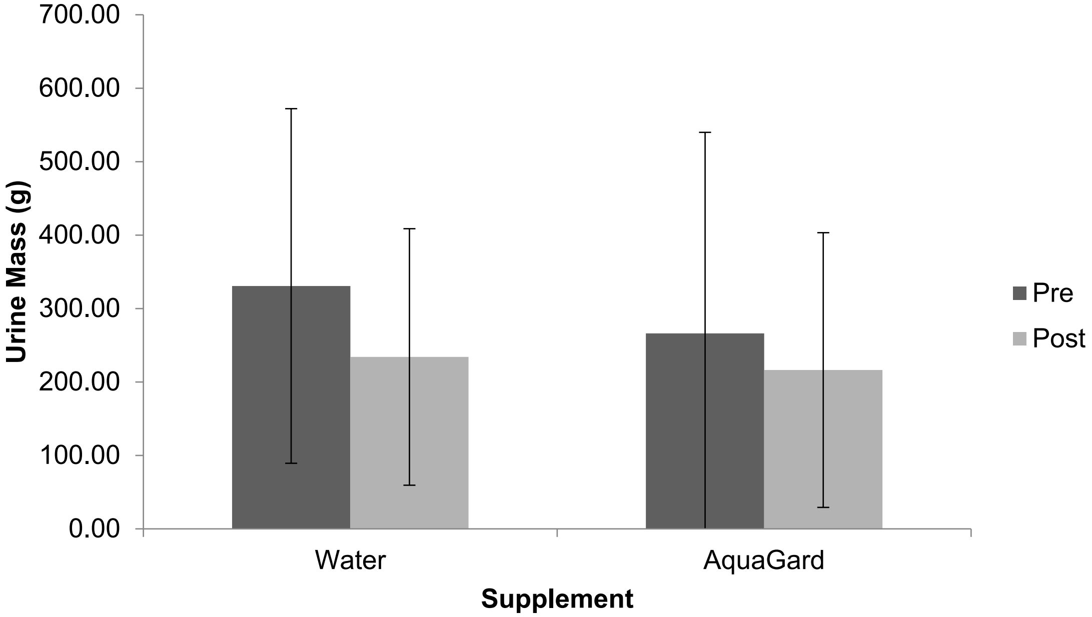

Figure 13. Urine Mass for both supplements at Pre- to Post-test.

4.0 DISCUSSION

The purpose of this study was to examine the effects of AquaGard® vs. water on parameters of hydration and performance during a bout of intense and prolonged physical exercise (10 mi run). Given the amount of water provided and the exercise protocol we expected that participants would elicit responses indicative of mild-to-moderate dehydration and fatigue during the run. While participants did exhibit signs of mild-to-moderate fatigue and exertion (e.g., increased lactate, RPE and HR; decreased glucose), they largely did not cross thresholds indicative of clinical dehydration for most hydration measures (sans body mass) even though significant changes from baseline were identified.

Interestingly, participants elicited nearly identical responses when provided AquaGard® - except for an expected greater loss in body mass (Table 2). Together, these findings indicate that the changes in hydration and performance measures observed over the run were generally equivalent between water and AquaGard®.

Table 2. Summary of Results.

Hydration & Performance Measures	Expected Trend for Water	Observed Trend (Water)	Observed Trend (AquaGard®)	Interaction (Rate of Change Diff.)	Dehydrated State?
Urine Specific Gravity	↑	↑	↑	None	No
Body Mass	↓	↓	↓	Yes	Yes (-2% for both)
Hemoglobin	↑	↑	↑	None	No
Hematocrit	↑	↑	↑	None	No
Urine pH	↓	-	-	None	No
Urine Mass	↓	-	-	None	-
Urine Volume	↓	-	-	None	-
Blood Glucose	↓	↓	↓	None	-
Blood Lactate	↑	↑	↑	None	-
Heart Rate	↑	↑	↑	None	-
Tympanic Temperature	↑	↑	↑	None	-
RPE	↑	↑	↑	None	-

As stated in the introduction, we posited that *if* AquaGard® elicited similar (i.e., equivalent) responses to water during the run this may indicate a practical advantage for AquaGard® since it has a 4.5x greater volume-to-mass ratio (i.e., 129 g of AquaGard® vs. 586 ml of water). Our current findings support this conclusion.

While these findings are encouraging, they are not definitive. In fact, both the design and outcomes of this initial investigation raise a number of important questions about AquaGard®

and other amino-acid supplements that should be addressed. For example, it is unknown what would have happened if researchers had adhered to existing fluid replacement recommendations (i.e., 1L of water for every 1kg weight loss during activity) for hydration maintenance. Would an attempt to adhere to this recommendation compromise performance, comfort and/more digestive absorption? More importantly, what corresponding dosage(s) of AquaGard® would be necessary to reach equivalent (or improved) hydration maintenance (if possible)?

This study also begins to call into question the appropriateness of total body mass as a gold standard indicator of hydration state – at least in the evaluation of this type of product. This is because changes in total body mass tell us very little about intracellular water availability, which is critical to the proposed mechanism of action for amino-acid supplements. To account for this, the current study included a battery of hydration measures, including USG, Hematocrit, Hemoglobin, and Urine pH. And while none of these measures directly assess intracellular water either, they did provide a more comprehensive assessment of hydration state from which to draw conclusions. Future studies should consider additional (and more definitive) metrics for assessing body water content, such as plasma osmolality (Hew-Butler et al., 2018) or bio-electrical impedance (Powers et al., 2009).

Finally, it may be important for future studies to compare this general class of supplementation (and/or specific products like AquaGard®) to other commercial sports drinks that promise hydration and performance maintenance/enhancement. Under such circumstances, researchers would be advised to examine more direct performance variables, such as metabolic efficiency, biomechanical efficiency, and/or event completion times.

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LIST OF ACRONYMS, ABBREVIATIONS, AND SYMBOLS

%	percent
AE	acid – electrolyte
HR	heart rate
kg	kilogram
RPE	ratings of perceived exertion
USG	urine specific gravity
L	Liter
ml	milliliter
mph	miles per hour
g	gram
mg	milligram
dL	deciliter
mmol	milimole
F	Fahrenheit