6-21-2013

Changes in Markers of Salivary Immunity, Stress, and Muscle Damage Following an Ironman Triathlon and During Recovery

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Purpose: Examine physiological and immune responses in triathletes during a competitive race and determine if cold water immersion (CWI) will attenuate these responses. Methods: 39 triathletes (age:44±11yrs; height:174±7cm; body mass:68.6±8.3kg; % body fat:10.7±4.2%) competing in the 2012 Ironman World Championships volunteered. Measurements of body mass (%BML), urine specific gravity (USG), salivary immunoglobulin A (SIgA), salivary cortisol (Scort), and salivary α-amylase (Sαam) were taken at baseline (BASE), prior to the race (PRE), following the race (POST), and one and two days after the race (+1 DAY and +2 DAY, respectively). Measurements of blood cortisol (CORT), creatine kinase (CK), and myoglobin (MYO) were taken at BASE, POST, +1 DAY, and, +2 DAY. Subjects were randomly assigned into a cooling group ((COOL) 12-minutes CWI following the race) or a control group ((CONT) 12-minutes of passive sitting following the race). Measurements of gastrointestinal temperature (∆Tgi) and heart rate (HR) were taken at 0-minutes (0MIN), 6-minutes (6MIN), and 12-minutes (12MIN) during the intervention. Results: USG was greater POST (p<0.001) and remained elevated +1 DAY (p<0.001) compared to PRE. %BML POST was significant compared to PRE body mass (p<0.001). ∆Tgi was greater in COOL vs. CONT (p=0.021) at 12MIN. HR was lower in COOL vs. CONT at 6MIN (p<0.001) and 12MIN (p=0.001). No interaction occurred for any blood or saliva variable. CORT was elevated POST from BASE (p<0.001) and returned to BASE by +1 DAY. CK peaked +1 DAY and was greater than BASE at all time points (p<0.001). MYO peaked POST and remained elevated +1 DAY (p<0.001). Scort was greater PRE compared to BASE (p=0.001), and greater POST compared to all other time points (p<0.001). SIgA was lower POST compared to PRE (p=0.015), but not different than BASE. SIgA returned to PRE values by +1 DAY. No differences were observed at any time point for Sαam. Conclusion: Despite resulting in lower Tgi and HR, 12-minutes of CWI did not attenuate the heightened
immune, stress, and muscle damage responses, as measured in blood and saliva, following an Ironman Triathlon. **Word Count: 332**
Changes in Markers of Salivary Immunity, Stress, and Muscle Damage Following an Ironman Triathlon and During Recovery

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A Dissertation
Submitted in Partial Fulfillment of the Requirement for the Degree of Doctor of Philosophy at the University of Connecticut 2013
Doctor of Philosophy Dissertation

Changes in Markers of Salivary Immunity, Stress, and Muscle Damage Following an Ironman Triathlon and During Recovery

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2013
Acknowledgements

Dr. Anderson - Thank you for your support over the last 6 years especially the last year through comps and dissertation. Despite your crazy schedule you always have a smile on your face and a positive attitude.

Dr. Armstrong - During my time at UConn I have never enjoyed classes as much as yours. Your passion for teaching and exercise science in general is admirable and thank you not only for your classroom instruction but your guidance in the lab as well. I am thankful that I have been able to work with you over the past 6 years.

Dr. Maresh - I cannot even begin to thank you for help with this project. From day 1 you immersed yourself in this study and strived to make it the best it possibly could be. I cannot begin to thank you for your support, dedication, and sacrifice to this project. You are one of the greatest leaders I have ever met, and clearly value the importance of the working relationships with the faculty and students.

Dr. Casa - When I came to UConn for my Master’s 6 years ago I never would have imagined staying for my PhD. But from the minute I got here, your passion and love for athletic training and research rubbed off on me and I instantly fell in love with the program. Over the course of the next 4 years you have provided me with enough knowledge and mentorship to last a lifetime, and I can never repay you for the lessons you have taught me. From working medical tents of major races, to speaking engagements at conferences, to KSI, I am beyond grateful for the opportunities you have provided, and can only hope to continue to share them with you in the future.
Family-Thank you for your understanding and unconditional support and encouragement not only during my time at UCONN but in everything I do and everything I have always done.
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REVIEW OF THE LITERATURE

Immunology

Role of Immunoglobulins

Immunoglobulins are proteins that are part of the immune system and found throughout the body. Specific to saliva, these immunoglobulins primarily include immunoglobulin A (SIgA), immunoglobulin M (SIgM), and immunoglobulin G (SIgG) and are one of the many components of “whole saliva”. Of these, SIgA is the most abundant and is considered to be the main immune mechanism in the oral cavity. More specifically, SIgA is the main protective mechanism against upper respiratory tract infections (URTI) which are a common occurrence in both the general population and the athletic population. This is important because URTI’s can impede activities of daily living as well as athletic performance. The degree of these effects is largely related to the type of exercise being done, specifically the duration and intensity. Additionally, factors such as fitness level, total volume of training, and overtraining syndrome can influence this response as well. Therefore, many researchers have sought to study the relationship between SIgA concentration and the occurrence of URTI’s in the athletic population. Regardless of the specific cause, results of these studies have found that there is a greater incidence of URTI in individuals with lower SIgA concentrations and/or low saliva flow rate. The specific etiology of lower SIgA concentrations and/or saliva flow rate can vary, and the regulation and control of this response can be quite complex, as seen in Figure 1 below.
Most of the protein components of saliva, including immunoglobulins, are actively secreted by salivary glands. These glands include the parotid glands, the submandibular glands, and the sublingual glands. Specifically, SIgA is produced by plasma cells within these glands which distinguish them from other protein components of the saliva. The receptor for SIgA is found within the cell membrane and is known as poly-immunoglobulin receptor (pIgR). Before SIgA can be secreted, it has to go through a two-step process beginning with the binding of SIgA to the pIgR so it can form the IgA-pIgR complex. Once this complex is formed, it can then be translocated to the surface of the secretory cells in the salivary glands. Second, cleavage of the complex must occur which results in the secretion of SIgA. Once secreted, SIgA can then carry out its anti-viral functions. However, several factors are capable of altering the normal secretion of SIgA as well as the flow rate of the saliva itself. For example, acute stress such as a bout of exercise or hypohydration, or chronic stress such as overtraining syndrome can affect the secretion of SIgA thereby affecting the salivary immune protection against URTI’s (see Figure 2 below):
**SIgA as a protective mechanism against URTI**

SIgA can provide anti-viral defense via three different functions. First, it can prevent the pathogen from adhering and penetrating the mucosal cells; second, it can neutralize viruses within the cells and therefore prevent viral replication; and third, it can transport the virus outside the cell via transcytosis where it can then be neutralized\(^7\). The intracellular virus-neutralizing activity is unique to SIgA with respect to the other immunoglobulins\(^8\).

The regulation of synthesis and secretion of SIgA is largely under neuroendocrine control via the autonomic nervous system (ANS). Secretion of SIgA and the secretion rate involve both parasympathetic and sympathetic stimulation. Under resting conditions, parasympathetic nerves are responsible for salivary fluid secretion (via acetylcholine) and sympathetic nerves are responsible for protein secretion (via norepinephrine)\(^7\). These responses are summarized in Figure 3 below (rat model)\(^7\):
Generally speaking, both SIgA secretion by the plasma cells and the availability of the pIgR for translocation are the rate-determining steps in the secretion of IgA, and therefore the potential for URTI defense. However, many factors can affect these two processes and therefore the change in SIgA concentration can be quite complex. One way in which SIgA concentration can increase is through any increase in autonomic nerve-mediated reflex stimuli. These reflex stimuli can include anxiety, chewing, taste, and the site of food. The effect of chewing was nicely shown by Proctor et al. in which subjects were found to have an increase in the transcytosis of SIgA and increases in the secretion of SIgA into the saliva when samples were taken after chewing compared to resting conditions. Additionally, sympathetic stimulation such as stress and moderate exercise can also cause an increase in salivary flow rate. While less frequently studied as stress and exercise, hydration status, dietary intake, and the environment have also been considered to affect both the concentration and secretion rate of SIgA.

Variables altering secretion/flow rate (non-exercise variables)

Several potential mechanisms by which acute and chronic exercise affects immunity have been proposed, and can be seen in Figure 4 below:
While the etiology of each factor is quite different, all affect the concentration of SIgA by altering the activation of the SAM/HPA axis. In other words, any factor that is deemed as a stress to the body has the potential to result in altered salivary immune function. More specifically, these factors often include exercise, hydration status, nutritional intake, supplements, and environmental conditions.

**General Stress Response:** Changes in SIgA can be influenced by the release of catecholamines from the adrenal medulla as well as the release of cortisol from the adrenal cortex. More specifically, epinephrine has been shown to increase the transport of SIgA into saliva through an increase in the mobilization of the pIgR receptor. Therefore, it is clear that an activation of the “fight or flight” stress response and the associated stimulation of the SAM axis and HPA axis can have a profound effect. Li and Gleeson proposed that there may be a threshold of sympathetic nervous system activity in which saliva flow rate will be affected. Similarly, with respect to the individual stress responses that are activated via “fight or flight”,

**Figure 4:** Effects of acute and chronic exercise on salivary immunity
Bishop\textsuperscript{10} and Allgrove\textsuperscript{12} both agree that the SAM axis is more important than the HPA axis, indicating that the effects of epinephrine and norepinephrine are greater than the effects of cortisol.

**Hydration Status:** Dehydration has preliminarily been shown to be a major contributor to observed responses of SIgA. The two most common populations in which this is seen are in exercising athletes and the elderly. For example, Walsh et al.\textsuperscript{13} suggested that dehydration may be more responsible for the reduction in saliva flow rate than neuroendocrine regulation following prolonged strenuous exercise. In this study, reductions in SIgA were only observed when subjects were dehydrated beyond 2\% body mass loss, as seen in Figure 5 and 6 below\textsuperscript{13}:

(saliva flow rate, osmolality, and total protein concentration during progressive acute dehydration (dark squares) and with sufficient fluids to offset fluid losses (open squares)).
Figure 5: The effects of plasma epi and norepi concentration during progressive acute dehydration (dark square) and with sufficient fluids to offset fluid losses (open squares)
In a follow up study, Oliver and Walsh et al. observed the effect of a 48 hour fluid restriction protocol on the effect of SIgA secretion rate\textsuperscript{14}. This study showed that 48 hours of fluid restriction caused a decrease in SIgA concentration. As a result, it was suggested that the decrease in SIgA observed during the fluid restriction was secondary to the associated increase in plasma osmolality\textsuperscript{14}. These results are summarized in Figure 7 below:
Figure 7: (effects of 48h fluid restriction (upside down triangle), energy restriction (triangle), fluid and energy restriction (diamond) compared with control (square)).

Recently, the effect of exercise-induced dehydration and subsequent overnight fluid restriction on salivary immunity was observed\textsuperscript{15}. In this study subjects exercised in the heat, either with fluids or without fluids to promote a body mass loss of 1\%, 2\%, and 3\% with subsequent overnight fluid restriction. Measures of hydration and salivary immunity were
recorded and it was found that dehydration decreased saliva flow rate, but only at 3% BML\textsuperscript{15} (Figure 8):

![Graph showing saliva flow rate and albumin concentration over BML during exercise and Day 2](image)

Figure 8: progressive exercise heat induced dehydration with subsequent overnight fluid restriction (dark circles) and rehydration to offset overnight fluid loss (open circles).

Additionally, SIgA concentration was increased with no effect on SIgA secretion rate. However, dehydration did not affect alpha amylase concentration, but did decrease the secretion rate of alpha amylase at 3% BML. Collectively, these results can be seen in the figures below\textsuperscript{15}:  

10
Figure 9: Effect of body mass loss on saliva protein content
These results mirrored that of the previously described study\textsuperscript{13} in that dehydration likely is a major contributor to changes in saliva flow rate and subsequent concentrations of salivary proteins, however only when levels of dehydration become moderate (2-3% BML).

**Environmental Factors:** It has been suggested that due to the close relationship between sympathetic activation and exercise-associated changes in SlgA that any additional stimulation of these pathways via exercise in adverse environments would have an additive effect\textsuperscript{10}. However, it is difficult to determine the true effects of the environment in these studies as the confounding variable of the exercise bout itself plays a major role. For example, Walsh et al.\textsuperscript{16}
observed a lower SIgA concentration following 2 hours of cycling at a moderate intensity in
-6°C conditions compared to 19°C conditions. However, this effect was actually related to a
higher flow rate of saliva because there was no effect of the cold on SIgA secretion rate\textsuperscript{16}.

\textit{The effect of exercise on secretion/flow rate}

When examining the effects of exercise on salivary immunity, other markers such as
alpha-amylase and cortisol are often expressed in addition to SIgA. These two markers both are
indicative of the stress response induced by exercise, with alpha amylase representing the effect
of the SAM axis and cortisol representing the effect of the HPA axis. More specifically, alpha
amylase is considered to reflect changes in plasma norepinephrine (therefore is highly dependent
on exercise intensity) and cortisol has been suggested to play an important role in inhibiting
SIgA production\textsuperscript{8}. The effects of exercise on these variables are extremely variable in the
literature mainly due to differences in the exercise protocol used. As previously discussed, Li and
Gleeson proposed that there may be a threshold of sympathetic nervous system activity in which
saliva flow rate will be affected\textsuperscript{11}. Based on this proposal, Kimura et al. suggested that there may
be a second threshold of sympathetic stimulation, above which the pIgR complex becomes
down-regulated\textsuperscript{17}. While this is highly speculative, it certainly provides a logical explanation for
why there is so much variation in the literature pertaining to the effects of SIgA on exercise
protocols of varying duration and intensity. Additionally, this suggestion was shown to explain
the results found in a study by Allgrove in which short duration high intensity exercise increased
the secretion of SIgA but long duration exercise resulted in a decrease in SIgA (Figure 11)\textsuperscript{12}:
Exercise Intensity: As mentioned, the effects of an acute bout of exercise are extremely variable. It is generally well accepted that moderate physical activity (via the enhanced sympathetic stimulus) enhances SIgA output therefore benefiting the mucosal immune defenses. However, any deviation from “moderate physical activity” can result in negative immune effects. When only considering an acute bout of exercise (irrespective of training status and other potential confounding variables), exhaustive exercise (high intensity) has been shown to increase the secretion rate of SIgA\textsuperscript{12,18}. Blannin et al.\textsuperscript{18} was the first to examine this relationship and found that SIgA secretion rate, as well as SIgA concentration, increased during exercise to exhaustion. However, these changes in SIgA were accompanied by a decrease in saliva rate which could explain the increase in the total concentration of SIgA. On the other hand, when Allgrove observed the influence of exercise intensity on salivary immunity, it was found that
while SIgA secretion rate increased, there was no change in saliva flow rate and the increase in SIgA concentration post-exercise was independent of exercise intensity (Figure 12)\(^2\):

![Figure 12: Effect of exercise intensity on salivary IgA concentration and secretion rate](image)

<table>
<thead>
<tr>
<th></th>
<th>Pre exercise</th>
<th>Post exercise</th>
<th>1 h post exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Saliva flow rate</strong> (ml·min(^{-1}))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50%</td>
<td>0.48 (0.09)</td>
<td>0.40 (0.07)</td>
<td>0.45 (0.07)</td>
</tr>
<tr>
<td>75%</td>
<td>0.41 (0.08)</td>
<td>0.43 (0.09)</td>
<td>0.50 (0.09)</td>
</tr>
<tr>
<td>Exhaustion</td>
<td>0.41 (0.10)</td>
<td>0.42 (0.09)</td>
<td>0.45 (0.09)</td>
</tr>
</tbody>
</table>

It is important to note that there were increases in salivary osmolality in all trials, in which it would be assumed that saliva flow rate would decrease throughout exercise. However, it was reported that dehydration was minimal (<0.5% BML at all time points) which likely explains the
unchanged flow rate and supports the conclusions that results were due to exercise intensity with no confounding influence from hydration status. While dehydration level was not reported in the Blannin study\(^{18}\) this could explain the inconsistent findings with SIgA concentration and saliva flow rate.

As previously mentioned, Allgrove\(^{12}\) associated these finding with changes in sympathetic activity (SAM axis) instead of changes in cortisol (HPA axis). Unfortunately, these are the only two published studies that successfully controlled for exercise intensity. Since both protocols involved exercise to exhaustion, the exercise duration was relatively short. Therefore, it seems as though the general conclusion that intense exercise has a whole will result in an increase in SIgA cannot be made, but rather only those that involve a limited increased activation of the sympathetic nervous system. When intense exercise is sustained for too long, eventually there will be a dramatic increase in the cortisol response as well as other stress markers such s inflammatory and muscle damage markers. When these variables are chronically elevated, there will be a resulting suppression of the immune system.

**Exercise Duration:** While a single bout of high intensity exercise will generally increase salivary immunity via increases in SIgA, an acute bout of long duration exercise will decrease SIgA. This was consistently shown in several studies involving endurance or ultra-endurance events\(^{2,3,4,19}\). All of these studies showed a decrease in SIgA secretion rate following the exercise bout. Furthermore, Pacque\(^{19}\) found that this value further decreased 2 hours after an 82km mountain run. Additionally, Peters\(^4\) and Nieman\(^2\) showed the inter-relationship between SIgA with those of other immune markers and inflammatory cytokines. These results were not surprising when assuming the associated prolonged elevation of cortisol resulting from the ultra-endurance exercise. So, while cortisol does not seem to play a major role in the regulation of
S1gA secretion in response to acute exercise, it likely plays a crucial role in long duration endurance exercise mostly due to the increased activity of the HPA axis during long duration exercise.

Chronic Exercise: Decreased secretion of S1gA can also occur during periods of intensified exercise training, overtraining, and when insufficient recovery is not allotted between exercise bouts. All of these scenarios are related to a chronic stress response (primarily sustained elevated cortisol) that can have an inhibitory effect on S1gA. Furthermore, these times of chronic stress are associated with increased risk of URTI. Figure 13 below shows the effect of repetitive training sessions on salivary immunity:

![Figure 13: Effect of repetitive training sessions on salivary immunity](image)

Figure 13: Effect of repetitive training sessions on salivary immunity

Obviously, this can be detrimental to athletic performance if an URTI occurs at the time of competition. In these cases, it is thought that there is an “open window” in which an individual is at greater risk of acquiring an URTI due to the suppression of the immune system following endurance exercise. However, this suppression is known to resolve with proper rest
and recovery, and therefore should not pose an extreme threat as long as athletes are not overtraining. For example, Akimoto showed the beneficial effects of an increase in SIgA concentration and secretion after an endurance training program (in unfit elderly subjects) (Figure 14):

![Figure 14: Effect of training status on salivary IgA concentration and secretion rate](image)

Figure 14: Effect of training status on salivary IgA concentration and secretion rate

**Physiology**

*Water Turnover and Bioenergetic requirements*
Euhydration is important to maintain normal physiological processes and maintaining a state of euhydration during exercise has been shown to optimize performance\textsuperscript{22-24}. Short duration, high intensity exercise is often associated with dehydration while long duration low intensity exercise can be associated with hyponatremia. The Ironman triathlon is unique in that both dehydration and hyperhydration are probable to occur. With respect to exercise performance, dehydration during exercise can result in a reduction in plasma volume and stroke volume. As a result, heart rate must increase and cardiac output will decrease because of the inability of heart rate to compensate for the reduced stroke volume. This could result in a decrease in performance due to premature fatigue and/or an increase in core body temperature. On the other hand, fluid overload can result in a decrease in serum sodium concentration that can lead to hyponatremia, again resulting in a decrease in performance. Fluid needs are often not met during exercise and a resulting increase in plasma sodium can occur.

Fluid balance during exercise is often measured by a change in body mass during the activity. While this measurement is often times used in the field, laboratory, and clinical settings for its convenience, it may not reflect total body water loss/gain in all situations. However, it can be used as a predictor to indicate the fluid lost or gained during the event. In the case of an Ironman, fluid input involves that from fluids consumed as well as food consumed during the race. Fluid output includes fluid lost via sweating, urinating, and bowel movements. However, since the Ironman involves exercise of 8-17 hours, there is an assumed degree of water lost from the oxidation of substrates which may not be accounted for when simply measuring pre and post-event body mass.

The oxidation of glycogen is the prominent substrate contributing to metabolic water loss since water is stored as a component of glycogen. This may provide an endogenous water source
which could mean that total body water may not change despite a decrease in body mass (or at least not to the extent of the decrease in body mass). This endogenous water source could be especially helpful due to the limited intake that can be withstood in these long distance events, due to limitations of fluid consumption, gastric emptying, and gastrointestinal irritation (Figure 15)\textsuperscript{25}. 

![Figure 15: Effect of carbohydrate consumption on fluid absorption during exercise](image)

However, it has also been suggested that the water gain through substrate metabolism likely does not contribute substantial amounts of fluid\textsuperscript{25}. While metabolic fluid losses/gains are difficult to directly study in the field, it may be of importance to consider these variables when calculating water turnover, especially during prolonged endurance events.

When exercise is prolonged such as during an Ironman triathlon, energy requirements can exceed 10,000 kcal\textsuperscript{26-28} and successful completion of the event is partly reliant on the ability to sustain a high rate of energy expenditure. Therefore, it is obvious that the high energy requirements of this event place great dependence on the metabolic processes of the body to keep up with the high energy demand.
In one of the first studies to diligently track nutritional intake during an Ironman, Kimber et al.\textsuperscript{26} assessed energy balance (energy expenditure vs. energy intake) and reported that their subject’s exogenous fuel intake equated to approximately 40\% of their total energy requirement, leaving an approximate 5,600 kcal deficit during the race. Similarly, Hew Butler\textsuperscript{27} also found a significant energy deficit following and Ironman triathlon. These large deficits are likely due gastrointestinal tract dysfunction during competition.

Data is scarce in the realm of gastrointestinal dysfunction during ultra-endurance events, however laboratory studies indicate that the importance of GI function may increase as duration of exercise increases. Generally speaking, gastric emptying can limit the rate at which fuel and fluids become available for use. Moreover, there is an upper limit at which the stomach can handle fluids, beyond which discomfort and intolerance can occur. Jeukendrup showed that this intolerance becomes more extreme as exercise duration continues, and also becomes heightened when adding carbohydrates to the drink (Figure 16)\textsuperscript{28}: (open bars=water; shaded bars=glucose; solid bars=glucose+fructose).

![Figure 16: Effect of exercise duration on perception of stomach fullness](image)

Figure 16: Effect of exercise duration on perception of stomach fullness
As the current literature shows, it does not appear that current drinking habits among triathletes approach this upper limit of failure of gastric emptying. Two studies\textsuperscript{29,30} reported an average or just over 700ml of fluid intake per hour during an Ironman. As this data is scarce, it is important to consider all factors associated with fluid balance during an Ironman event, including environmental conditions, amount of aid stations, individual considerations, etc.

As previously described\textsuperscript{26,27}, exogenous fuel intake is not able to keep up with the amount of fuel needed to complete an Ironman triathlon. Therefore, it is obvious that endogenous fuel sources are also critical in their ability to successfully complete the triathlon despite this deficit. Muscle glycogen and blood glucose are the primary sources of energy during the initial phases of this event, and depletion of these sources is associated with premature fatigue. While blood glucose and glycogen are primarily responsible especially in the onset of an Ironman, it has been shown that the initial high carbohydrate oxidation will plateau after the first few hours of exercise, even if exogenous carbohydrate intake is high (Figure 17)\textsuperscript{31}:

![Figure 17: Exogenous glucose oxidation during exercise](image)

Figure 17: Exogenous glucose oxidation during exercise
This transition of substrate utilization from carbohydrates to fats is not surprising, and can even be enhanced with proper training. During training, especially for ultra-endurance athletes, it is important to train long distances because this will deplete carbohydrate stores and emphasize the use of fat as a fuel substrate. Ultimately, this will preserve glycogen stores and theoretically allow the athlete to perform exercise longer.

Training also allows for fast oxidation of macronutrients to maximize oxidative phosphorylation. This will induce a signal cascade that will cause an upregulation of the enzymes needed to quickly breakdown these macronutrients. Coffee and Hawley\textsuperscript{32} showed that this upregulation results in an increased rate of energy production from aerobic pathways, tighter metabolic control, and an increased exercise economy which ultimately leads to less fatigue during exercise.

Some research among the Ironman population has focused on hyponatremia by trying to identify a link between body mass loss and serum sodium concentration after an Ironman. In this cohort of studies, results were mixed as to the relationship between percent body mass loss, serum sodium concentration, and plasma volume\textsuperscript{27,29,33-37}. Average percent body mass loss ranged between 2-5%, while serum sodium concentrations were found to increase, decrease, or remain the same when compared to pre-race values, and plasma volume shifts were also variable. In those studies attributing sodium levels to extra cellular volume, it was concluded that plasma volume was maintained despite decreases in body mass, which agrees with the idea that the body will protect plasma volume with a contribution from the intracellular fluid\textsuperscript{27,33,36}.

It is important to understand that body mass changes, plasma volume shifts, and serum electrolyte concentrations after an ultra-endurance event depend more on simply that amount of
fluid consumed during the event. For example, factors such as sweat rate, hydration programs
during training, heat acclimatization, and environmental conditions play key roles in the total
amount of fluid lost and gained during competition. These variations highlight the importance
of individualized training and competition regimens. Therefore, specific to hydration plans
during competition, universal recommendations can almost never be made, but rather individuals
should develop a plan specific to their needs during training in order to be successful.
Additionally, these plans may have to be altered based on varying environmental conditions of
their respective races.

Thermoregulation

Thermoregulation is the body’s ability to maintain a safe range of core body temperature.
When metabolic heat production exceeds the body’s ability to dissipate heat the result is
hyperthermia. Conversely, hypothermia results when heat dissipation exceeds that of heat
production. Just as triathletes can become dehydrated or hyperhydrated during an Ironman, they
are also at risk for both hyperthermia or hypothermia. While thermoregulation during exercise
has been extensively studied, the majority of the data has been obtained from laboratory studies
in which factors such as exercise intensity, duration, environmental conditions, and hydration are
controlled. Moreover, field studies have largely been conducted on shorter duration events, with
the exception of recent reports of thermoregulation and pacing strategy during a marathon.
Thermoregulation is especially important to consider in the Ironman population due to the
integration of other confounding variables such as cardiovascular, respiratory, metabolic, and
nervous system responses, environmental conditions, and hydration status.
Two of the primary factors that affect thermoregulation with any form of exercise are the
intensity of the exercise and the environmental conditions present during the exercise bout.
Mitigating the rate of rise of core temperature is primarily dependent on the body’s ability to
dissipate endogenous heat production, which is a direct result of exercise intensity. Heat
dissipation can be maximized when a high 1) core temperature to skin temperature ratio, and 2)
skin temperature to air temperature ratio exists. Therefore, it is obvious that skin temperature is
extremely important. The primary heat dissipation mechanism during exercise is sweating and
the resulting evaporation of the sweat off the skin. When a high humidity is present, the
evaporation mechanism will be severely blunted causing skin temperature to remain high and no
cooling to occur, resulting in an increase in core temperature. If dehydration is also present,
cardiovascular strain will be heightened putting the body in a physiological deficit. During
exercise, it is assumed that some degree of cardiovascular strain will be present, as it is necessary
for heart rate to increase to compensate for decreased stroke volume resulting from blood being
shunted away from the body core to the periphery. When this is present, the following responses
are likely to occur: 1) a decrease in intensity to maintain a given workload, or 2) a voluntary
decrease in work output to minimize cardiovascular strain. In both circumstances, a decrease in
performance will occur either voluntarily or due to premature fatigue. One of the main factors
deciding these responses is the environmental conditions that are present during the exercise
bout.

Studies have reported on “optimal conditions” for athletic performance, however most of
these reports pertain to the marathon. Generally, this optimal range has been reported as
between 5-20 degrees C (first reported by Frederick et al. in 1983\textsuperscript{38}). In this study, an inverse
“V” was plotted to show the relationship between winning marathon time and ambient
temperature. Additionally, when examining over 100 years of the Olympic marathon, Maughan\textsuperscript{39} showed that the percentage of finishers was highly correlated with ambient temperature. Furthermore, this study found a 25% drop in successful finishers when ambient temperature rose above 25 degrees C. Similarly, when studying the relationship between exertional heat stroke and environmental conditions at the Falmouth Road Race, it was found that the incidence rate was highly correlated with both ambient temperature and heat index \((r^2>0.7)\)\textsuperscript{40}.

A confounding variable that plays a primary role in the relationship between environmental conditions and change in core body temperature is exercise intensity. First established by Nielsen in 1938, it was proposed that an increase in core temperature is proportional to metabolic rate and almost independent of environmental conditions over a wide range. Sawka and Pandolf later showed that altering intensity level in different environmental conditions will also alter sweat rate, and therefore thermoregulation (Figure 18)\textsuperscript{41}:

![Figure 18: Effect of environment on sweat rate at different running speeds](image)

Figure 18: Effect of environment on sweat rate at different running speeds
These initial proposals promoted further research to identify modifications and internal defense mechanisms against a dramatic rise in core temperature. Since then, much research has focused on anticipatory regulation and pacing strategies to prevent these dangerous increases in core body temperature\textsuperscript{42-44}.

With regards to the Ironman population, the extreme duration of the race makes it a unique event with regards to exercise intensity. Due to the long duration, absolute intensity is often relatively low. Furthermore, the adjustments to intensity level that are often made for varying environmental conditions in a team sport setting are often not made during an Ironman race due to this relatively low intensity level. In other words, it is likely that approximately the same intensity will be utilized during these races regardless of whether it is 80 degrees or 50 degrees. Therefore, in cool conditions the absolute rise in core temperature is likely dependent on intensity because heat loss is able to balance metabolic heat production. However, in warm conditions (especially if coupled with high humidity) heat loss will be restricted so the environment may also contribute to heat gain. Furthermore, fatigue associated with hyperthermia will likely be more pronounced in high humidity conditions, especially if the heat is uncompensable due to the exaggerated effects of cardiovascular drift. With that being said, hyperthermia is not the only problem threatening thermoregulation during an Ironman. The long duration, moderate intensity level, and varying environmental conditions also poses great concern for hypothermia.

\textit{Muscle damage}

Muscle damage during an Ironman triathlon is exceedingly high. The extent of muscle damage is often measured via blood markers such as creatine kinase and myoglobin. All phases
of the Ironman triathlon likely contribute to muscle damage, however the most significant contributer is the run phase which requires a significant eccentric muscle contraction component. Depending on the layout of the course (with regards to elevation changes) the extent of eccentric muscle contraction may vary. Furthermore, overall performance in an Ironman is highly correlated with performance in the run phase. While higher trained athletes may actually endure more muscle damage during the race due to a faster pace, higher trained athletes will adopt specific adaptations that can reduce overall damage due to improved recovery/repair mechanisms.

There are currently two published studies that examined markers of muscle damage following an Ironman triathlon\(^\text{45,46}\). Not surprising, both studies found dramatic increases in both creatine kinase and myoglobin following the race, while Suzuki et al. found CK to peak 1 day post-race\(^\text{45}\). Additionally, Neubauer et al. showed that this response remained elevated for 19 days following the race (Figure 19)\(^\text{46}\):
Figure 19: Muscle damage after an Ironman triathlon

In both cases, it is obvious that the repair process remains active during an extended time during recovery.

Other factors such as muscle pain, delayed onset muscle soreness (DOMS), and muscle function are also of interest in addition to biomarkers of muscle damage. DOMS typically peaks between 24 and 48 hours post-exercise and involves pain, discomfort, swelling, and decreases in motion and function\textsuperscript{45,47-49}. DOMS can be variable based on many factors such as familiarity to the exercise, intensity and duration of the exercise, and type of muscle contraction. The exact physiological mechanism of DOMS is not yet fully understood, however it likely relates to the degree of mechanical damage induced by muscle cells during exercise, especially resulting from eccentric muscle activity\textsuperscript{47}. The degree of mechanical damage induced by the exercise will indicate the degree of damage to the muscle tissue, namely the amount of inflammatory response and the release of intracellular enzymes such as creatine kinase (CK)\textsuperscript{50}. These responses are thought to be the primary contributor to the pain, swelling, and limited muscle function associated with DOMS, and therefore the two are oftentimes directly linked.

Muscle damage with or without DOMS can further lead to decreases in muscle function and strength\textsuperscript{45,49}. The etiology of decreases in muscle strength and function appear to be multifactorial, including associations with muscle fiber damage, muscle fatigue, and muscle pain/soreness. Suzuki et al.\textsuperscript{45} showed that following an Ironman triathlon, significant decreases were found with maximal isometric strength of the knee extensors, squat jump height, and counter movement jump height 1 day following the race. Coincidentally, this time point also produced the highest CK values of subjects (Figure 20)\textsuperscript{45}:
Figure 20: Effect of an Ironman triathlon on muscle force

Muscle damage is also associated with inflammation and immune responses. More specifically, there is an ensuing systemic inflammatory and immune response that continues during the repair process after exercise. In addition, hyperthermia, oxidative stress, and metabolic stress are inherent of ultra-endurance exercise and are capable of releasing inflammatory cytokines. Part of this stress response includes stimulation of the hypothalamic pituitary adrenocortical axis which causes an increase in cortisol release. Moreover, the cortisol response is partly mediated by cytokines, the degree of which will partly determine the extent of the systemic inflammatory response. When exercise-induced tissue damage occurs, it induces rapid repair and adaptation responses. More specifically, muscle damage will cause inflammatory cells such as phagocytes to rush to the injured muscle tissue. These cells accumulate with the task of cleaning up the injured area and allowing the inflammatory process to continue. In addition, this inflammatory response also contains an immune component which allows cells such as macrophages and neutrophils to migrate to the injured tissue. This inflammatory response is beneficial and necessary to repair and adaptation, however repeated
damage from heavy training could result in a chronic cytokine response, in which case the healing process will get not be able to proceed from the inflammatory phase. This adverse outcome is a direct result of overstimulation of cortisol that will result in suppression of the immune system and the halting of the inflammatory response. This response can often times occur in overtraining syndrome.

**Cold Water Immersion and Recovery**

*Thermoregulation*

Cold water immersion is the most effective way to reduce core body temperature\(^5\). In addition to its effects on thermoregulation, it has also been shown to positively affect cardiovascular, metabolic, and perceptual responses. Many athletes use post-exercise strategies in hopes of enhancing recovery. While cold water immersion has long been used for both prevention and treatment of exertional heat illness, it recently has become a popular post-exercise recovery strategy as well. While this is becoming a popular post-exercise recovery strategy, there is little research to back the proposed physiological and perceptual benefits of cold water immersion on recovery. Moreover, even fewer studies have examined these effects in the ultra-endurance population.

Several mechanisms have been proposed to explain the potential recovery benefits from cold water immersion including decreased metabolic rate (resulting in decreased tissue temperature and decreased pro-inflammatory cytokines), increased venous return, decreased inflammation and damage of muscle tissue, and decreased muscle soreness\(^4\). From a biochemical standpoint, cold water immersion may provide an attenuation of the general stress response, oxidative stress, and cytokine (i.e. pro-inflammatory’s) that are prevalent and
extremely high after an ultra-endurance exercise bout such as an Ironman triathlon. While methodology and ensuing results from these studies are highly variable, anecdotally cold water immersion could provide a great benefit to Ironman athletes.

Instead, research has focused on the effect of cold water immersion on core body temperature, cardiovascular response, and perception of muscle soreness/pain. Unfortunately, much of this research has focused on anaerobically based exercise (i.e. power output, force, speed). Despite this, the theoretical basis for the benefits of cold water immersion on recovery remain the same, namely attenuating muscle soreness (including delayed onset muscle soreness (DOMS)), decreasing core body temperature (and local tissue temperature), and promoting venous return to normalize cardiovascular function.

The use of cold water immersion to decrease the amount of muscle damage and DOMS is theoretically attributed to two mechanisms: 1) decreasing the metabolic rate and inflammation associated with strenuous exercise (and therefore decreasing tissue temperature and core body temperature), and 2) restoring cardiovascular function via an increase in venous return.

Several factors may affect the effectiveness of cold water immersion on recovery such as when the cooling is initiated post-exercise, duration of cooling, and the water temperature of the bath. One of the first studies to compare different water immersion temperatures showed that cooling rates were nearly identical between cold water immersion (14°C) and ice water immersion (5°C), while both provided significantly faster cooling rates vs. no cooling. However, when rectal temperature was continued to be monitored post-immersion, the ice water immersion bath provided lower body temperatures during the post-immersion recovery period (Figure 21):
Figure 21: Effect of cold water immersion and ice water immersion on rectal temperature after exercise

In a similar study, immersion water temperatures of 2, 8, 14, and 20°C were compared after subjects exercised at a moderate intensity until rectal temperature reached 40°C\(^{64}\). The cooling rates of the 8, 14, and 20°C baths showed similar cooling rates, all of which were significantly slower than the 2°C bath (Figure 22)\(^ {64}\):

Figure 22: Effect of different water temperatures on rectal temperature after exercise
A growing area of interest among researchers is the apparent close association between heat stress and immune function. Currently, there is little research to show clinically significant outcomes related to immune responses and infection incidence among athletes who train and/or compete in hot and humid conditions, or the potential benefit of a cooling intervention post-exercise to mitigate the inherent immune suppression following an ultra-endurance bout of exercise. Currently, there is an ongoing controversy on whether the immune system is involved in the etiology of exertional heat illnesses, especially exertional heat stroke. First proposed by Shephard\textsuperscript{65} and most recently studied by Lim and Mackinnon\textsuperscript{66}, this relationship is primarily based on the resulting gastrointestinal ischemia occurring from exercise in the heat. This occurs because during exercise, blood is shunted away from the body core (including the GE tract) in order to sufficiently supply the working muscles with blood as well as the skin (to promote sweating). Furthermore, when exercise is performed in the heat, this response is exaggerated. This can result in damage to the intestinal mucosa and leakage of lipopolysaccharide (LPS) into the circulating blood\textsuperscript{67}. One way in which LPS is removed from the circulating blood is from help by monocytes and macrophages. However, during intense exercise, these immune defenses are often overwhelmed and cannot adequately perform these tasks. As a result, a sequence of events can ensue resulting in a further increase in the systemic inflammatory response that in extreme cases may lead to intravascular coagulation and multi-organ failure\textsuperscript{67}. This response lends possible explanation to cases of exertional heat stroke that often times lack a distinguishable physiological etiology (Figure 23): (Classical and immune pathways of EHS (solid arrows indicate likely links in the pathway; broken arrows indicate unsubstantiated in EHS etiology)
A question that remains unanswered is whether or not the leakage of LPS from the gut, the resulting effect on cytokine release and immune suppression, and altered thermoregulation and cardiovascular instability during exercise-heat stress can be attenuated after the cessation of exercise.

Some research exists on the effects of passive cooling on immune function. These results show that mild decreases in core temperature may have a stimulatory effect on immune function\textsuperscript{68,69} while modest to severe decreases in core temperature have depressive effects on immune function\textsuperscript{70,71}. This is likely due in large part to the associated activation of the neuroendocrine and immune responses. The effect of post-exercise body cooling on immune function remains to be unstudied. Based on the aforementioned mechanisms of the association between immune function and thermoregulation, it is possible that a bout of cooling during recovery from exercise may limit immune suppression following endurance exercise.
The purpose of this study is to (1) assess the physiological and immunological changes following an Ironman triathlon; (2) track the physiological and immunological changes through two days of recovery; and (3) determine if cold water immersion immediately following an ironman triathlon attenuates these responses. Our hypotheses were that (1) the Ironman triathlon would lead to a high degree of physiological stress including suppressed salivary immunity; (2) physiological stress and muscle damage would remain present during recovery, while immune function would return to baseline the day following the race; and (3) cold water immersion would attenuate the effects of the race compared to a control condition.

METHODS

Participants

39 healthy triathletes (29 males, 10 females) who participated in the 2012 Ironman World Championships volunteered as subjects for this study. (Table 1)

<table>
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<tr>
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<th>N</th>
<th>AGE (Y)</th>
<th>HEIGHT (CM)</th>
<th>BODY MASS (KG)</th>
<th>BODY FAT (%)</th>
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<td>Males</td>
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<td>45 ± 10</td>
<td>176 ± 6</td>
<td>71.8 ± 6.5</td>
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<tr>
<td>Females</td>
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<td>43 ± 12</td>
<td>168 ± 6</td>
<td>59.1 ± 5.1</td>
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<td>39</td>
<td>44 ± 11</td>
<td>174 ± 7</td>
<td>68.6 ± 8.3</td>
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Table 1: Subject demographic information

Participants were excluded from participation if they had 1) a known chronic health problem, 2) a previous history of exertional heat stroke within the 3 years prior to the race, 3) a history of
cardiovascular, metabolic or respiratory disease, 4) a history of suspected obstructive disease of the gastrointestinal tract, and/or 5) a current musculoskeletal injury limiting them from normal physical activity. All qualifying individuals were required to complete a medical history questionnaire prior to participation. All participants read and signed an informed consent and the study protocol was approved by the University of Connecticut Institutional Review Board.

**Design Overview (Experimental Approach to the Problem)**

To assess the physiological and immunological changes following an Ironman triathlon, investigators recorded measurements related to salivary immune function, stress, muscle damage, and hydration status. To assess the effect of cold water immersion on recovery, subjects were randomized by gender and finish time into either a cold water immersion group or a control group. All measurements with the exception of blood samples were taken at baseline (1 day during the week leading up to the race), immediately prior to the race, immediately following the race, 1-day following the race, and 2-days following the race. Blood samples were taken at all time points except for immediately prior to the race. All samples were collected at similar times of the day, with the exception of race day, to control for diurnal variation.

**Testing Protocol**

*Baseline (BASE) Measurements*

Initial testing of participants began during the week prior to the Ironman race. All participants were required to report to the testing site for 2 hours during this time. Upon arrival, subjects were seated for a 10-minute period prior to collection of a saliva sample for measurements of salivary immunoglobulin A (SIgA), cortisol (S_cort), and alpha amylase (S_amyl). Subjects rinsed with bottled water and were instructed to sit quietly for the 10-minute waiting
period while a researcher demonstrated the proper technique for saliva collection. Additionally, it was ensured that subjects had not consumed any food or fluid other than water nor did they brush their teeth in the previous 30-minutes, as this could affect the saliva collection. After the 10-minute waiting period, participants were asked to produce a 1mL sample of saliva via passive drool technique into a 1.5 mL clear plastic epindorf tube. Time to complete the 1mL volume sample was noted by a researcher to enable calculation of saliva flow rate.

Following the saliva sample, subjects were asked to provide a urine sample for a baseline hydration measure via urine specific gravity (USG) while voiding their bladder prior to body mass (BM) assessment. Body mass to the nearest 0.1kg and body composition was performed via InBody720 analyzer. This required subjects to stand on the unit and hold sensors for approximately 2 minutes. Height was also recorded during this time for demographic identity. Subject demographic information can be seen in Table 1.

<table>
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<td>44</td>
<td>174.0</td>
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Table 1: Demographic information for all subjects.

Blood samples were obtained on all subjects via single stick butterfly needle. Subjects were positioned in a supine position with their trunk slightly elevated and arm resting.
comfortably. 33mL of blood was taken from a superficial vein in the antecubital fossa for biochemical analyses of cortisol (CORT), creatine kinase (CK), and myoglobin (MYO).

Following these measurements, participants then continued baseline testing to have their sweat rate calculated and be fitted with a heart rate monitor and GPS watch. This instrumentation served to record metrics of their race performance such as heart rate (HR), distance, and speed. All participants were fitted with the heart rate monitor and GPS watch prior to exercise of their choice (either biking or running) for 30-60 minutes. Sweat rate was calculated via body mass loss during this exercise bout, and expressed as liters per hour.

Lastly, participants filled out several questionnaires which included: 1) Training History Questionnaire (Appendix A), 2) Dietary Record (Appendix B), 3) Sleep Record (Appendix C), 4) Environmental Symptoms Questionnaire (Appendix D), and 5) Delayed Onset Muscle Soreness Scale (Appendix E). Prior to leaving the testing area, participants were given instructions to prepare for race day, as well as given an ingestible temperature thermistor for race day tracking of gastrointestinal temperature ($T_{gi}$). Subjects were instructed to take the temperature sensor immediately before going to sleep the night before the race, to give ample time to pass into the small intestine.

*Pre-Race (PRE) Measurements*

Participants arrived to the testing site 1-2 hours prior to the start of the race for their pre-race measurements. Upon arrival, all subjects were fitted with their heart rate monitor and GPS watch, provided a urine sample, and were weighed to the nearest 0.1kg. Participants then were seated to fill out questionnaires and provide a pre-race saliva sample. During this time, researchers checked $T_{gi}$ to ensure the sensor was properly reading. If a reading failed to be
produced, subjects were asked to swallow another thermistor. Following pre-race measurements, serial measurements of web bulb globe temperature (WBGT) were taken throughout the course of the day.

*Post-Race (POST) Measurements*

Immediately upon completion of the race, subjects reported to the testing area near the finish line. $T_{gi}$, HR and BM were recorded and subjects were randomized to either the intervention group (cold water immersion (CWI) or the control group. For the intervention group, subjects were seated in a CWI tub with water up to their shoulders for 12 minutes. Water temperature was kept constant at approximately 10°C, and water was stirred every 2 minutes. For the control group, subjects were seated on a bench next to the immersion tub for 12 minutes. During this time, $T_{gi}$ and heart rate readings were taken at 0 minutes, 6 minutes, and 12 minutes. After the 12 minute period, participants were then escorted by a researcher to the indoor laboratory space for the remainder of the post-race procedures. Upon arrival to the laboratory, subjects provided a urine sample, provided a saliva sample, had their blood drawn, had their body weight recorded, and completed several questionnaires. Prior to leaving, participants signed up for recovery testing for the following two days.

*Recovery Measurements*

On the day following the race (REC1) and two days following the race (REC2), subjects reported back to the testing laboratory for recovery measurements. Time of day was kept consistent to limit variability in blood and saliva samples due to diurnal rhythms. During these visits, subjects provided a saliva sample, urine sample, blood sample, had their body weight recorded, and completed several questionnaires.
Biochemical Analyses and Instrumentation

**Blood Analyses:** All blood samples were taken via single stick butterfly needle into clear vacutainers. Whole blood was drawn into tubes pretreated with EDTA vacutainer for the analyses of CORT and MYO, and into Serum tubes for the analysis of CK. Blood was then centrifuged at 3,000 rpm for 15 minutes at 4°C. Samples were then aliquoted to pre-labeled clear plastic epindorf tubes and frozen via dry-ice to be shipped back to the human performance laboratory. Frozen samples were then stored at -80°C until analysis.

Individual samples were thawed prior to biochemical analysis. Cortisol and myoglobin were assessed in duplicate via enzyme-linked immunosorbent assay (ELISA) (Calbiotech, Spring Valley, CA). Inter-assay variation for the assays were 8.9% and 5.9%, respectively. Intra-assay variability for the assays were 7.4% and 8.5%, respectively. Creatine kinease was measured in duplicate using assay procedures from Genzyme Diagnostics (Charlottetown, PE, Canada). Biomate spectrophotometer from Thermo Scientific (Waltham, MA) was used to determine the appropriate absorbance values used in calculations. All analyses were completed based on manufacturers’ instruction. The CK analyses yielded an inter-assay coefficient of variation of 8.9% and an intra-assay coefficient of variation of 6.5% for all samples.

**Saliva Analyses:** Saliva samples were collected into 2mL clear salivettes (Salimetrics, State College, PA). After collection, samples were frozen via dry ice and shipped to the Pediatric Exercise and Genomics Research Center at the University of California Irvine, where they were stored at -80°C until analysis. After thawing, saliva samples were centrifuged at 1500 g for 15 minutes to obtain the clear supernate from the sample. Samples were aliquoted into 96
well non-binding plates for each assay. For each sample, 3 aliquots were separated for the determination of concentrations of SIgA, Scort, and Sαam.

SIgA concentrations were determined using an indirect competitive immunoassay method using a commercially available kit (Salimetrics, State College, PA). Six plates were utilized for this assay in order to run all samples in duplicate. Based on the standard values, Inter-assay variation averaged 5%. The Intra-assay variation averaged 2.1%. Scort concentrations were determined using a high sensitivity salivary cortisol enzyme immunoassay kit (Salimetrics, State College, PA). Six plates were utilized for this assay in order to run all samples in duplicate. Based on the standard values, Inter-assay variability averaged 3.8%. The Intra-assay variability averaged 2%. Sαam activity was determined using a kinetic salivary α-amylase assay kit (Salimetrics, State College, PA). All analyses were completed based on manufacturers’ instruction.

Urine Analyses: Urine samples were collected into individually assigned clear plastic urine cups. Urine samples were analyzed for urine specific gravity using a refractometer (A300CL, Atago Co., Tokyo, Japan).

Demographic measurements: Body mass and percent body fat was measured via InBody720 body composition analyzer (Biospace, Seoul, South Korea). Height was recorded via standard tape measure.

Individual race characteristics: Subjects were given an ingestible temperature sensor in order to obtain core body temperature readings (HQ inc., Palmetto, FL). Subjects wore a Timex Global Trainer GPS watch (Timex Group USA, Middlebury, CT) during the sweat rate analysis during baseline testing and for the entirety of the race. In addition, they were fitted with a Timex
Flex-Tech Digital heart rate monitor (Timex Group USA, Middlebury, CT). These devices continually recorded heart rate and GPS variables, including distance covered and speed. At the completion of data collection, all individual information was downloaded to TrainingPeaks computer software (Peakware, Lafayette, CO) for analysis.

**Statistical Analysis**

All data is presented as mean ± standard deviation of the mean. A one-way analysis of variance (ANOVA) was used to determine changes in urine specific gravity and percent body mass loss over time (Pre-race, Post-race, +1 Day post-race, and +2 Days post-race). A one-way ANOVA was also used to determine changes in heart rate and delta gastrointestinal temperature during the 12-minute intervention. Post-hoc testing via bonferroni corrections were implemented when significance was noted. All blood variables were analyzed via linear mixed model ANOVA (Group (2) X Time (4)) and independent samples t-tests were utilized when a significant interaction was observed. All saliva variables were analyzed via linear mixed model ANOVA (Group (2) X Time (5)). SPSS 19.0 (SPSS Inc., Chicago, IL) was used for all analyses and significance was set *apriori* at p<0.05.

**RESULTS**

*Hydration:* Urine specific gravity was significantly greater post-race (1.021 ± 0.006; p<0.001) and remained significantly elevated at the +1Day time point (1.022 ± 0.008; p<0.001). USG returned to pre-race values (1.012 ± 0.007) at the +2Day time point (1.017 ± 0.006; p=0.083). Percent body mass loss post-race (-3.49 ± 2.29%; p<0.001) was significant compared to pre-race body mass, however returned to pre-race levels by 1 day post-race (Figure 24).
Physiological: A significant interaction (Time X Group) occurred for delta core body temperature (p=0.02). While Tgi change was not significantly different between groups during the first 6 minutes of cooling (0.348 ± 0.292°C vs. 0.194 ± 0.292°C; p=0.169), significant differences were observed at the 12-minute time point (0.693 ± 0.495°C vs. 0.282 ± 0.385°C; p=0.021) (Figure 25).
A significant interaction (Time X Group) also occurred for heart rate during the 12-minute intervention (p=0.016). HR at the 0-minute time point was not significantly different between groups (p=0.147). The cooling group exhibited a significantly lower HR than the control group at both the 6-minute mark (77 ± 10 bpm vs. 92 ± 11 bpm; p<0.001) and the 12-minute mark (75 ± 12 bpm vs. 88 ± 10 bpm; p=0.001) of cooling (Figure 26).
Saliva: No significant interaction (Group X Time) occurred for SIgA (p=0.982); however, a significant main effect for time was observed (p=0.015). At PRE, SIgA was higher than all other time points (332.6 ± 261.0 µg/mL), with significantly higher differences occurring at the BASE (234.1 ± 175.4 µg/mL; p=0.020), +1 Day (226.1 ± 204.2 µg/mL; p=0.003) and +2 Day (202.3 ± 119.3 µg/mL; p=0.003) time points. No significant difference was observed with SIgA at POST (261.5 ± 172.1 µg/mL) compared to any other time point (Figure 27).
No significant interaction (Group X Time) was observed for Sαam (p=0.34); however, a significant main effect for time was observed (p=0.006). Sαam was significantly elevated at POST (328.8 ± 304.0 IU/mL) compared to all other time points, with no other significant interactions occurring between time points (Figure 28).
To account for the variability in saliva flow rate, concentrations of SIgA and Sαam were also expressed as a secretion rate. No significant interaction (Group X Time) occurred for saliva flow rate (p=0.728); however, a significant main effect for time was observed (p=0.019) with the +1 Day time point exhibiting significantly greater flow rates than all other time points (Figure 29).
This analysis revealed no significant interaction (Group X Time) for secretion rate of SIgA (p=0.889) or secretion rate of Sαam (p=0.315); however, a significant main effect of time was observed for secretion rate of SIgA (p=0.026). Secretion rate of SIgA was significantly higher at PRE compared to BASE (92.0 ± 92.8 μg/min vs. 51.4 ± 34.8 μg/min; p=0.017). The POST time point (49.8 ± 45.8 μg/min) was significantly lower than PRE (92.0 ± 92.8 μg/min; p=0.015) and +1 Day (86.4 ± 105.4 μg/min; p=0.035) (Figure 30).
No significant interaction (Group X Time) was observed for Scort (p=0.808); however, a significant main effect for time was observed (p<0.001). Scort was significantly elevated at PRE (0.736 ± 0.298 µg/dL; p=0.001) compared to BASE (0.181 ± 0.088 µg/dL). Scort was significantly greater at POST (1.62 ± 1.27 µg/dL) compared to all other time points, and Scort PRE was significantly greater than BASE, +1 Day (0.324 ± 0.795 µg/dL), and +2 Day (0.137 ± 0.056 µg/dL) (Figure 31).
Blood: No significant interaction (Time X Group) occurred for CORT (p=0.35). However, a significant (p<0.001) main effect of time occurred where CORT was significantly elevated post-race compared to baseline values (586 ± 242 nmol/L vs. 99 ± 58 nmol/L; p<0.001). CORT returned to baseline values by the +1 Day time point (90 ± 35 nmol/L), with continued normal levels at the +2 day time point (87 ± 40 nmol/L) (Figure 32).
No significant interaction (Time X Group) occurred for CK (p=0.63). However, a significant main effect of time (p<0.001) and significant main effect of group (1802 ± 1848 IU/L vs. 1312 ± 1384 IU/L; p=0.03) were observed. CK was significantly elevated post-race compared to the baseline value (1980 ± 1793 IU/L vs. 235 ± 186 IU/L; p<0.001), and remained significantly elevated through the +2Day time point (1358 ± 896 IU/L; p<0.001), with values peaking 1 Day post-race (2619 ± 1827 IU/L; p<0.001) (Figure 33).
There was a significant interaction (Time X Group) for MYO (p=0.32); however, post-hoc testing revealed no significant differences between groups at any time point. A significant (p<0.001) main effect of time was observed for MYO, with values peaking post-race (755 ± 373 nmol/L). MYO remained significantly elevated 1 Day post-race (192 ± 177 nmol/L) and returned to pre-race levels (26 ± 19 nmol/L) 2 Days post-race (54 ± 59 nmol/L) (Figure 34).
No significant interaction (Time X Group) occurred for DOMS (p=0.89); however, a significant main effect for time was observed (p<0.001). DOMS peaked post-race (61 ± 23) and was significantly higher compared to pre-race values (13 ± 16). DOMS remained significantly elevated at the +1 Day (46 ± 24) and +2 Day (38 ± 26) time points compared to baseline, however were significantly lower than post-race values.

**DISCUSSION**

The purpose of this study was to (1) assess the physiological and immunological changes following an Ironman triathlon; (2) track the physiological and immunological changes through two days of recovery; and (3) determine if cold water immersion immediately following an ironman triathlon attenuates these responses. Ultra-endurance exercise such as an Ironman triathlon is inherent of acute responses involving hydration status, core body temperature, stress,
immune, and physiological variability. Previous research has shown that such events can cause extreme rises in the systemic stress and inflammatory responses\textsuperscript{45,46,72}, a large degree of muscle damage\textsuperscript{45,46}, and fluctuations in core body temperature and cell volume\textsuperscript{27,29,30,33-37}. Recently, the effect of ultra-endurance exercise on the innate immune system has been of interest\textsuperscript{2-4}, however these responses have yet to be studied in the Ironman population.

Hydration status during physical activity is of primary importance not only for health and safety but also for optimal performance. Dehydration often plagues athletes who participate in high intensity exercise in the heat, and in extreme cases can predispose those individuals to exertional heat illness. In an Ironman triathlon athletes are also at risk for dehydration, but due to the long duration and relatively low intensity of the activity, they are also at risk for hyponatremia.

Several studies have been published (both laboratory and field) showing a clear relationship between hydration status and core body temperature\textsuperscript{22,23,73-75} and hydration status and exercise performance\textsuperscript{22-24}. Recently, other studies have tried to discount that aggressive hydration strategies are not important during practical activities (as opposed to laboratory-based exercise protocols) and do not significantly contribute to hyperthermia or general exercise performance. Many of these studies have been completed on Ironman athletes\textsuperscript{27,29,33-37}, with highly variable degrees of \%BML, core body temperature, and ambient temperature. In the present study, average \%BML was \(-3.49 \pm 2.29\%\) with a range of \(+1.98\) to \(-7.77\%\) (Figure 1). While the large range of body mass change was not surprising, overall the \%BML was higher than anticipated and higher than previous reports.
Another observation was that male subjects lost a much higher percentage of their pre-race BM than did females (Figure 12):

![Diagram](image_url)

**Figure 12: Varying degrees of %BML by gender.**

More specifically, 90% of female subjects had a 4% BML or less with only 1 female losing more than 4% BM. On the other hand, more than 50% of male subjects had more than 4% BML. Due to naturally higher sweat rates and higher absolute intensities, it would be assumed that male athletes would typically have a higher %BML than female athletes; however, a discrepancy of this magnitude is of great interest for future research.

One factor that may explain the high degree of BML is the rather harsh environmental conditions that were observed during the present study. Compared to previous similar research, this study exhibited a higher ambient temperature as well as relative humidity. As environmental conditions can have a primary role in sweat rate, it is likely that the high conditions of this race resulted in higher sweat rates in our subjects, ultimately resulting in a greater BML.
Additionally, this race was the Ironman World Championships in which the most elite competitors were present. Therefore, it is possible that the large BML of our subjects were due to comparatively higher exercise intensities than those of previous studies.

Given the multiple variables that contribute to rate and overall amount of BML, it is important for athletes to have individualized hydration plans. One effective way to do this is to calculate individual sweat rate to estimate the total water loss during a given exercise bout. Key factors to consider in this calculation are the type of exercise (i.e. bike vs. run), exercise time, exercise duration, exercise intensity, environmental conditions, and fluid consumed during the exercise bout. This method may assist athletes in maintaining a state of euhydration, limiting adverse physiological effects of both dehydration and hyperhydration, and ultimately improving exercise performance.

Studying the effect that exercise has on the immune system has gained much attention over the past 20 years, both with research interest and practical implications. A non-invasive and relatively simple method to assess immune function is through salivary analysis, specifically through SIgA, Sαam, and Scort. While SIgA is the primary protective mechanism against URTI in the oral cavity, analysis of Sαam and Scort are also of interest as they provide representation of the systemic stress response throughout the body. Just as stress has been shown to affect the concentration of SIgA, other variables such as hydration status, intensity and duration of exercise, and recent food and fluid intake can greatly affect the concentration of SIgA. With regards to exercise, it can generally be stated that an acute bout of high intensity exercise will increase SIgA concentration (and therefore acutely improve salivary immunity), while an acute bout of long duration activity will decrease the concentration of SIgA. Given this, it was anticipated that SIgA would decrease significantly after completion of the race.
As previously mentioned, any stimulus inducing a stress response can affect not only the concentration of proteins in the saliva, but also the flow rate of the saliva into the oral cavity. Therefore, as exercise is a form of stress, salivary flow rate must be considered when studying the concentration of SIgA in the saliva. Accompanying measurements of Scort and Sαam are helpful in providing information on the stress response. Will responses of Scort and Sαam are considerably variable in the present literature, it appears as though the primary influence of both variables is the degree of sympathetic nerve activity\textsuperscript{11}. Moreover, Scort has been shown to similarly mirror cortisol levels found in the blood, while Sαam appears to be more variable. In the present study, SIgA concentration was highest immediately prior to the race, with a non-significant decrease immediately following the race (Figure 4). However, when considering salivary flow rate and presenting SIgA as a secretion rate, there was a significant decrease in SIgA secretion rate from pre to post-race. This value returned to pre-race levels by +1DAY. However, it is important to note that while the POST time point exhibited lower values than PRE, there were no differences between BASE and POST (Figure 7).

Largely these data are consistent with previous reports\textsuperscript{2-4,19}. Nieman\textsuperscript{2,3}, Pacque\textsuperscript{19}, and Peters\textsuperscript{4} all showed SIgA secretion rate to be significantly lower after an ultraendurance exercise bout compared to pre-race values. Moreover, Nieman\textsuperscript{2,3} also found absolute concentration of SIgA to be unaffected (absolute concentration of SIgA was not reported in the other studies\textsuperscript{4,19}). While the Nieman studies\textsuperscript{2,3} only considered pre and post-race values, additional monitoring by Pacque\textsuperscript{19} and Peters\textsuperscript{4} further supported our results. More specifically, Pacque also found SIgA secretion rate to be higher immediately prior to the race compared to a baseline measurement. Additionally, it was reported that the decreased SIgA secretion rate post-race did not return to pre-race values when measured the morning after the race (however was similar to baseline.
values). This result was different than what was found in the present study as SIgA secretion rate had returned to PRE values at +1DAY, which also agrees with the results of Peters$^4$. A compilation of these previous results, as well as those of the present study, can be seen in Table 2.

<table>
<thead>
<tr>
<th></th>
<th>Concentration of SIgA</th>
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<th>Secretion Rate of SIgA</th>
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<tbody>
<tr>
<td></td>
<td>BASE</td>
<td>PRE</td>
<td>POST</td>
<td>+1DAY</td>
<td>BASE</td>
<td>PRE</td>
<td>POST</td>
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<td>Nieman, 2003$^2$</td>
<td>N/A</td>
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<td>$\downarrow$</td>
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<tr>
<td>Nieman, 2006$^3$</td>
<td>N/A</td>
<td>$\leftrightarrow$</td>
<td>N/A</td>
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<tr>
<td>Pacque, 2007$^9$</td>
<td>N/A</td>
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<td>N/A</td>
<td>$\downarrow$</td>
<td>$\downarrow$</td>
<td>$\downarrow$</td>
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<tr>
<td>Peters, 2010$^4$</td>
<td>N/A</td>
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<td>$\downarrow$</td>
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<td>DeMartini, 2013</td>
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While most of these results were similar and generally expected, significant differences between the BASE and PRE values of SIgA secretion rate warrant further discussion (Figure 7). A probable explanation for this phenomenon is the time of day that the saliva samples were collected. While most samples were collected at similar times of day by design, the PRE sample was collected very early in the morning before the race began. As SIgA shows diurnal variation in which levels are known to peak in the morning$^7$, it is likely that the significant elevations observed at the PRE time point were simply due to the time of collection.

As previously mentioned, Scort and Sαm are often measured in addition to SIgA for the purposes of describing the general stress response. Unfortunately, the few studies previously mentioned$^{2-4,19}$ examining salivary immunity in ultra-endurance events did not include Scort or
Sαam in their analyses. In our study, we found Scort to mirror those levels of cortisol measured in the blood (Figures 8 and 9). More specifically, Scort was significantly elevated immediately following the race but returned to baseline levels by +1DAY (Figure 8). In addition, we were able to have an additional data point for Scort (PRE). This was important because Scort was found to be significantly elevated immediately prior to the race when compared to all other time points, except for POST which showed further significant elevations (Figure 8). These results were not surprising, and the significant increase observed at PRE was likely due to the time of collection, as well as possible influence from the anticipatory response to beginning the race.

While Scort and Sαam have not been studied in the ultra-endurance population, general stress has been shown to increase saliva protein concentration including Sαam activity. Two studies involving high intensity exercise have shown that the exercise-induced stress resulted in significantly higher secretion rates of Sαam post-exercise, with Walsh reporting that those effects remained up to 5-hours post-exercise. Interestingly, Sαam activity was not significantly different, further highlighting the importance of considering salivary flow rate when examining salivary proteins. With regards to the present study, we found no significant difference at any time point for secretion rate of Sαam.

The exhaustive nature of the Ironman triathlon led to an expected increased systemic stress response. Exercise induced changes in cortisol are known to affect the immune system, with exercise serving as a model of temporary immunosuppression that occurs after severe physical stress. Many factors are associated with this exercise-stress model that can cause interactions between the nervous, endocrine, and immune systems. As plasma concentrations of cortisol increase only in relation to exercise of long duration, the Ironman triathlon serves as a main avenue for extreme elevations. In our study, CORT was significantly elevated post-race.
compared to baseline values (586 ± 242 nmol/L vs. 99 ± 58 nmol/L; p<0.001) and returned to baseline values by the +1Day time point (Figure 9). This data suggests that the race provided a stress response capable of producing a system stress response. Similar to a previous report, despite the dramatic increase in CORT post-race, the body was able to recover within one day after the event.

In addition to the heightened metabolic and hormonal responses, structural damage to muscle tissue was also evident. In addition to increases in cortisol, levels of muscle damage as measured by CK and MYO were substantially elevated (Figures 10 and 11, respectively). As the Ironman triathlon involves a large inherent degree of mechanical stress and damage, especially in the run portion of the race, it was expected to observe large increases in muscle damage markers immediately following the race. This hypothesis is consistent with three prior studies that examined muscle damage following an Ironman triathlon. In these studies, it was consistently shown that both CK and MYO were significantly elevated following the triathlon. It was also consistently shown that while MYO peaked immediately post-race, CK values continued to rise and ultimately peaked 1 day following the race. While monitoring time during recovery was variable in each study, both CK and MYO was reported to remain significantly elevated compared to pre-race values during the days following the race.

Muscle damage results from the present study closely mirrored those found previously. More specifically, both CK and MYO concentrations were significantly elevated POST compared to BASE, with further rises occurring in CK at +1 DAY (Figures 10 and 11). However, while trends of varying levels were similar, our results revealed that concentrations of both CK and MYO were lower than those that were previously reported. Additionally, while previous studies have shown both CK and MYO to remain significantly elevated up to 19 days
post-race, our results showed that MYO returned to baseline values just 2 days post-race (Figure 11).

Comparatively, these results highlight two important inferences. First, while published data in this population is scarce, it appears that competing in an Ironman triathlon results in a generalized muscle damage response with respect to time course of damage and associated recovery. Second, while timing trends appear to be consistent, there is likely great variability in the degree of muscle damage endured, as well as rapidity of recovery in the days following the event. This variability is assumingly dependent on individual characteristics such as fitness level, training level, age, gender, etc., as well as potential variability in course layout which may involve differences in biomechanics such as increased requirement of eccentric muscle contraction. While these suggestions are primarily hypothetical, one factor that may be supported is that the current study involved the Ironman World Championship race, therefore participants were elite and potentially superior in fitness and training level than those of previous studies. This could be key in explaining the lower concentrations of both CK and MYO in the present study, as well as potentially explaining the seemingly faster recovery with respect to MYO.

In addition to biochemical analysis of circulating markers of muscle damage, measurements of perceived soreness and pain are often used to describe symptomatic responses to muscle damage. A popular means of doing this is through the Delayed Onset Muscle Soreness (DOMS) visual analogue scale. In the present study, DOMS was found to peak POST and remain significantly elevated from pre-race values through 2 days following the race. This again was similar to previous research involving ultra-endurance exercise, where both DOMS and muscle pain were collectively found to be highest both immediately post and 1 day following the event.
As previously mentioned, muscle damage, stress, and immune responses are inherent of many athletic events, especially those that are high in intensity and/or duration. In addition to the perceived side effects, activities of daily living and subsequent training may also be affected. Therefore, methods to attenuate these adverse effects are often implemented. One method to do this is through cold water immersion, and its benefits theoretically occur through several mechanisms. First, the cold water has the ability to mitigate the heightened metabolic rate at the muscle and tissue. Second, the water itself has a hydrostatic effect that allows for a faster return of central blood volume and subsequent restoration of cardiovascular functioning. Enhancing recovery via cold water immersion has long been used as a recovery tool in many athletic settings, however few reports are given with its use in the Ironman population.

With the exception of core body temperature and heart rate during the intervention (CWI vs. control), we found no effect of cold water immersion on any variable. While the effect of CWI on general exercise recovery is controversial, it is quite likely that the nature of the Ironman triathlon is simply so extreme that a single bout of CWI is unable to promote any measureable effects on recovery. Additionally, as subjects only exhibited moderate degrees of hyperthermia, multiple bouts (or one of longer duration) of CWI were not possible. However, multiple bouts of warm water immersion may be considered. While this would not allow for the attenuation of metabolic effects seen with CWI, it would allow for potential heightened effects of the hydrostatic pressure. Theoretically, this could have a positive effect on the markers of muscle damage as it could promote a faster healing response at the tissue level.
CONCLUSIONS

The unique cohort of athletes provided ample opportunity to study the physiological, biomechanical, and immune effects resulting from an acute bout of ultra-endurance exercise. Our results paralleled the few similar previous reports in that an Ironman triathlon results in moderate levels of hyperthermia and dehydration, as well as elevated systemic stress, muscle damage, and salivary immunity responses. However, we showed that most of these elevations returned to normal by 2 days following the race. Furthermore, we found no effect of cold water immersion on the attenuation of these responses.

From a practical standpoint, these results can be extremely beneficial to the ultra-endurance athlete as they greatly affect the recovery process following an acute bout of exercise as well as potentially limit future training and activities of daily living. Additionally, emphasis should be paid to recovery strategies that may attenuate the physical, biomechanical and immunological damage following a race, as well as during training sessions. Future research should focus on examining the effect of multiple bouts of cold water immersion following a triathlon, as well as alternative recovery strategies. Additionally, tracking these athletes throughout their competitive season on a regular basis is of great interest.

Limitations

Given the nature of this event, certain limitations were inherent throughout data collection. First, given that this was a field study, not all confounding variables (i.e. environmental conditions, nutritional intake, etc.) could have been controlled. Additionally, while blood draws and saliva samples were intended to be collected at similar times of day across testing days, the collection times immediately before and after the race could not be
controlled for both across days and between subjects. Therefore, diurnal variation may have affected some samples. Finally, we were only able to track the athletes for 2 days following the race. If we were able to monitor them for a longer period of time during their recovery, we may have seen significant results through that extended period.

References


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