Methods of Weight Cutting in Mixed Martial Arts with Specific Interest in Hot Salt Baths

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Thesis Submitted for the Award of PhD

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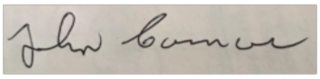
Submitted to Dublin City University
August 2022

Declaration:

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of Doctor of Philosophy is entirely my own work, and that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge breach any law of copyright, and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

Signed:  ID No: 16213712 Date: 31/08/22

Acknowledgements

Thank you to my family, especially my wife Fiona who gave me so much help throughout so that I could actually have time to work on this. Thank you.

Thank you to Owen Roddy, Paddy Holohan, John Kavanagh, Chris Fields and Tom King for the access to their athletes and convincing some of them to take part in what is never an easy process.

Thank you to all the fighters. Weight cutting is by far the hardest part of fighting and you guys did it for me on several occasions.

To some of my close friends; Paul O'Connell and Philip Clarke. Thank you to Paul for his advice on the writing process. I really needed it. Thank you to Phil for his help on the statistics, by far my weakest area.

Thank you to everyone in Dublin City University, a PhD is never a solo effort and I'm grateful for all your help and hard work. Thank you to Adam Shelley, Ciaran Clarke, Alannah Hedderman, Mark Germaine, and Conor Gibson for your help during data collection.

Finally, to Dr. Brendan Egan, my PhD thesis supervisor, thank you for making sense of of the absolute gibberish that nearly all my first drafts were and not resigning as my supervisor due to my severe lack of understanding of the written English language. I would never have completed this PhD without your constant, and mostly encouraging, feedback. Thank you so much! I owe you.

Peer-reviewed Publications Arising from this Thesis

Connor, J., & Egan, B. (2019). Prevalence, Magnitude and Methods of Rapid Weight Loss Reported by Male Mixed Martial Arts Athletes in Ireland. *Sports (Basel)* 7(9), 206. https://doi.org/10.3390/sports7090206

Connor, J., Shelley, A., & Egan, B. (2020). Comparison of hot water immersion at 37.8°C with or without salt for rapid weight loss in mixed martial arts athletes. *Journal of Sports Sciences* 38(6), 607–611. https://doi.org/10.1080/02640414.2020.1721231

Connor, J., Egan, B. (2021). Comparison of hot water immersion at self-adjusted maximum tolerable temperature, with or without the addition of salt, for rapid weight loss in mixed martial arts athletes. *Biology of Sport* 38(1), 89-96. https://doi.org/10.5114/biolsport.2020.96947

Connor, J., Germaine, M., Gibson, C., Clarke, P., & Egan, B. (2022). Effect of rapid weight loss incorporating hot salt water immersion on changes in body mass, blood markers, and indices of performance in male mixed martial arts athletes. European Journal of Applied Physiology, 10.1007/s00421-022-05000-7. https://doi.org/10.1007/s00421-022-05000-7

Conference Presentations Arising from this Thesis

Connor, J. Prevalence and Methods of Rapid Weight Loss Reported by Mixed Martial Artists. Poster Presentation at European Congress of Sport Science 2018, Dublin (4th-7th July 2018).

Connor, J. Comparison of Hot Water Immersion With or Without Salt on Rapid Weight Loss in Mixed Martial Arts Fighters. Poster Presentation at Faculty of Sports and Exercise Medicine, Hosted by Royal College of Surgeons in Ireland, Dublin September 14th 2019.

Connor, J. Comparison of hot water immersion at self-adjusted maximum tolerable temperature, with or without the addition of salt, for rapid weight loss in mixed martial arts athletes. American College of Sports Medicine: Combat Sports Online Conference, May 4th 2020.

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Abstract

Methods of Weight Cutting in Mixed Martial Arts with Specific Interest in Hot Salt Baths by John Connor MSc

Rapid weight loss (RWL) means the manipulation of body mass in the last several days preceding a competition through different methods of training, and modifications in dietary and fluid intakes. Many studies describe the prevalence of methods of RWL, but there has been little empirical research into whether the methods athletes employ for RWL are effective.

My literature review examined the prevalence, magnitude and methods of RWL in sports with weight classes, but with specific interest in combats sports and MMA. The review also explored mechanisms by how the various approaches produced RWL and how these approaches affect health and performance.

Study 1 was a survey study employing the previously-validated Rapid Weight Loss Questionnaire to investigate current trends of RWL in Irish Mixed Martial Arts athletes. A novel finding was that hot (salt) baths were implemented by a high percentage of respondents, despite this method of RWL not being previously studied for its effectiveness in the weight cutting process.

Study 2 investigated body mass losses during RWL using a hot bath protocol with salt in the form of Epsom salt (1.6%wt/vol) or without salt. In a crossover design, MMA athletes performed a 20-min immersion at a fixed temperature of 37.8°C followed by a 40-min wrap. This bath and wrap was performed twice per visit. The body mass loss in salt water baths (SWB) was similar to fresh water baths (FWB).

Study 3 used the same protocol to investigate body mass losses during hot water immersion with or without salt, but this time with the temperature commencing at 37.8°C and self-adjusted by participants to their maximum tolerable temperature. Again the body mass lost in SWB was similar to FWB.

Study 4 investigated the effects of a higher salt concentration (5.0%wt/vol Epsom salt) using the hot bath protocols in Studies 2 and 3 on body mass, blood-based markers, and indices of performance. The magnitude of body mass lost in SWB was similar to FWB, and there was no difference between conditions on blood markers or in the performance tests.

Future research should focus on how to optimise the hot bath process in order to aid RWL. This may include investigating the ideal temperature of water, salinity or osmolality of water, and the duration of bathing.

Chapter 1 - Introduction

Weight Class Sports and The Process of Making Weight

Weight classes in competitive sports were introduced as a means of increasing participation in a sport and to make it fairer or create an "even playing field" (Reale et al. 2017a). Competitors are therefore matched with competitors of similar size and weight. Boxing was the first sport to introduce weight categories in 1909 as uneven matches were dangerous and unappealing to spectators. In time, other combat sports also introduced weight classes. Titles (national and world) could only be recognised if there were standardised weight classes (McCarson, 2017). Many of these weight class sports include combat sports such as the traditional Olympic sports, Wrestling, Judo, Boxing and Tae Kwon Do, it also includes other mainstream sports such as Horse Riding and Rowing.

With the advent of weight classes inevitably came the practice of 'making weight' i.e. the intentional loss of body mass in order to weigh-in below the threshold of a given weight class. A feature of the process of making weight is the process of "weight cutting" in close proximity to the weigh-in, where athletes are weighed prior to competition or a fight (Liebling, 2004). Weight cutting describes the manipulation of body mass in the final days preceding a competition or fight through the combination of different manipulations of exercise, diet and fluid intake. Intentional weight loss in order to reach a desired target body weight is frequently practiced in sports that have weight class restrictions (Crighton et al., 2016; Hillier et al., 2019). This process can be called the "weight cut", "cutting" or simply the "cut" colloquially, but in scientific terms is known as Acute Weight Loss (AWL) or Rapid

Weight Loss (RWL). Throughout this thesis, I will refer to the process as RWL unless the authors of referenced works have specifically referred to AWL.

Athletes in weight class sports have used weight cutting as a means of gaining an advantage over their opponents as the assumption is that being larger is an advantage (Reale et al. 2017a). It has also been noted by the same authors that athletes are aware that RWL can have a detrimental affect on their performance. However, once the consequences of RWL diminishes their opponents performance to a greater extent than their own performance, the the athlete is generally happy to undertake the process of RWL (Reale et al. 2017a). Moreover as Pettersson et al. (2013) noted in a study of 14 Swedish combat sport athletes, there are positive psychological aspects to the experience of RWL: "*...Practicing weight regulation mediates a self-image of being "a real athlete." Weight regulation is also considered mentally important as a part of the pre-competition preparation, serving as a coping strategy by creating a feeling of increased focus and commitment. Moreover, a mental advantage relative to one's opponents can be gained through the practice of weight regulation."*

Athletes in combat sports such as boxing and Mixed Martial Arts (MMA) are required to compete under specific weight classes. The timeline between weigh-ins and fight time can vary depending on the organisation sanctioning the fight. While all professional organisations have weigh-ins the day before the fight, weigh-ins are at least 24 hours before the fight and can be up to 36 hours beforehand.

The regain/recovery window (i.e. time from weigh-in until fight/competition time) and the practices involved to regain the athlete's weight prior to competing is often termed the Rapid

Weight Gain (RWG) period. Practices during RWG can be just as important for performance as the RWL period (Coswig, et al. 2018, Slater et al., 2007), with the time from weigh-in to the competition affecting the methods and the magnitude of RWL athletes implement.

For example, rowing only has two weight classes (lightweight and above) and has a 2-hour window from weigh-in until racing. In horse racing, jockeys must weigh-in immediately before and after a race. In amateur boxing, competitors need to weigh-in on the morning of competition, and if it is a multi-day tournament weigh-ins are repeated on the morning of each bout.

Professional boxing and MMA have day before weigh-ins, and thus the processes of RWL and RWG are heavily influenced by nutrition practices, and can potentially be exploited for the benefit of the athlete if evidence-based practices are implemented (Coswig et al., 2018). However, when work commenced on this thesis in 2017, such an evidence-base was lacking to a large extent.

There are two distinct phases of making weight in combat sports in preparation for a fight or competition. The first phase is general weight loss, which can last for several weeks (e.g. 6-12 weeks) prior to competition. This phase is most obviously marked by a period of reduced energy intake to target a loss of tissue mass, ideally in the form of fat mass (Langan-Evans et al. 2011). The second phase is the weight cut or RWL phase. This typically lasts approximately 7-14 days and culminating immediately prior to weigh-in. RWL has been broadly considered to be the loss of up to ~10% of body mass in the 1-2 weeks (sometimes even days) before competition (Artioli et al., 2010). The main methods of manipulating body mass in this phase is achieved by reducing body water stores through dehydration and carbohydrate restriction. This also leads to glycogen depletion, and reducing gut contents by following a low residue diet is added too (Reale et a., 2017b).

Adverse Outcomes and Concerning Practices with RWL

As mentioned above, while some athletes view the weight making process as a net positive (Pettersson et al. 2013), and the use of weight cutting leading up to competition in combat sports starts at an early age and continues for advanced level athletes (Yarar et al. 2020), there remains scant evidence that processes of RWL and RWG confer performance advantages. This literature to evidence this point will be considered in greater detail, but briefly, more of an advantage may be evident in grappling based sports for weight cutting when the weight lost is regained. The same effect of a weight based advantage is however is not as evident in striking sports. For example, an advantage of weight cutting was shown in Judo where the weight regain of medal winners was significantly greater than in non-medal winners in both males and females (Reale et al. 2016). There was however no notable advantage from weight regain in boxing (Reale et al, 2016; Daniele, 2016).

Whether advantageous or not, the worst-case scenario in weight cutting and RWL is a loss of life. There have been several deaths across the various combat and weight class sports. Khodaee et al. (2015) have cited a Centre for Disease Control and Prevention (CDC) article that reported the deaths of three collegiate athletes in the mid-nineties as the extreme example of what can go wrong in sports with weight classes (all of which mortalities occurred during strenuous weight-loss workouts). There was also the death (in 2013) of Leandro "Feijao" Souza in MMA, who died just before weigh-in. In this example Mr Souza had taken a fight with one week's notice and attempted to cut 33lbs (more than 20% of body mass) to make the flyweight limit of 126lbs (Jenness, 2017). Another weight cutting related death (from severe dehydration) in MMA occurred to Yang Jian Bing, a flyweight from China competing in Asia's ONE Championship MMA promotion in 2015. Finally the death of the female Muay Thai fighter, Jessica Lindsay occurred who collapsed during extreme training related to weight cutting (Campbell, 2017).

One of the major reported causes for these extreme outcomes is severe dehydration used for RWL (Crighton et al. 2015). Other likely causes will be covered in further detail in the literature review.

Murugappan et al. (2018) presented a case study that highlighted the particular risks of extreme RWL in those with sickle cell traits in which this combination of dehydration and weight loss can causes severe exertional rhabdomyolysis, and in some cases, has led to death.

In the past decade, numerous survey studies have been undertaken in an effort to describe the weight making practices of weight class and combat sport athletes in terms of magnitudes and methods, while also describing the sources of information these athletes utilise to inform their practices (Berkovich et al., 2019; Coswig et al., 2018; Hillier et al., 2019). These survey studies recorded that coaches, fellow athletes and online media are generally the most relied upon sources of information, whereas doctors and dieticians are less relied upon as sources of information and advice on weight loss prior to a competition or fight (Berkovich et al., 2019). Notably, in one study, those athletes who received their nutrition and weight cutting advice from social media used a wider variety of methods of weight cutting. In contrast those that sourced their information from a registered dietitian or nutritionist reported using the least amount of methods for weight cutting (Park et al., 2019).

Ultimately, knowledge about methods and the sources of information is useful for increasing awareness of these issues and safety precautions about RWL methods. Improved nutrition education is critical for reducing the magnitude and misuse of RWL methods (Berkovich et al., 2019). To do so however, much more research is required on understanding the response to various methods of RWL, as opposed to research that simply notes what the various methods of RWL are being used. This thesis attempts to address one particular knowledge gap around the use of hot water immersion as one of the most common methods of RWL used today by MMA athletes.

Mixed Martial Arts: An Overview

The sport of MMA has been in existence for a little over a quarter of a century. The Ultimate Fighting Championship (UFC) held its first event on November 12, 1993. This event is considered the genesis of the sport of MMA even though the first event was pitched as a "styles versus styles" event to show which was the most potent martial art. Some people consider MMA to be older than this however, with "Vale Tudo" (literally translating as "anything goes") matches/fights occurring in Brazil and elsewhere throughout the 20th century (McCarthy & Hunt, 2011). It was not until later UFC shows that fighters would train in multiple fighting disciplines and the sport of Mixed Martial Art was born.

Nevertheless, in its early days the UFC was considered to be more "spectacle than sport" but increasing revenue, participation and modifications to its rules have changed it into an elite worldwide sport with athletes of the highest calibre competing (McCarthy & Hunt, 2011). MMA now combines the fighting styles and martial arts of Boxing, Judo, Karate, Wrestling, Brazilian Jiu-Jitsu (BJJ) and Muay Thai among several other martial arts into one complete fighting system.

Professional MMA is made fights of up to 3 x5-minute rounds. If the fight goes the full 3 rounds (otherwise known as the "distance") then the fight is decided by judges score cards. Like in boxing, there are three judges and they score the rounds the same as boxing on the "10-point must system". This means a judge must score one fighter with a 10 so that rounds can be 10-10 (a drawn round), 10-9 (one fighter has marginally won the round) and 10-8 (where one fighter has clearly won the round and/or the fight has almost been stopped). The judge's scorecards are tallied at the end of the fight to declare the winner. Fights can be stopped before this if there is a knockout, a submission, a stoppage by a medical professional, a referee stoppage, or the match is conceded ("towel is thrown in") by one of the corners. When it is a title fight (or sometimes a main event) fights are 5 rounds of 5-minute duration and the same rules in relation to scoring as outlined above apply.

In its earliest incarnations, MMA and the UFC did not have any weight classes and weigh-ins were only performed to gather the fighters anthropometric information for the "tale of the tape" or official fight record, before fights. Since those early days, the UFC introduced several weight classes with the first occurring at "UFC 12" on February 7, 1997 (McCarthy & Hunt, 2011). This has now developed to the current situation in professional MMA where there are several weight classes for men (125lbs, 135lbs, 145lbs, 155lbs, 170lbs, 185lbs, 205lbs and 205-265lbs). In amateur MMA there is one extra weight class (265+lbs).

Similar to what occurred in other weight class and combat sports, the introduction of weight classes was the beginning of weight cutting and RWL in MMA. Despite MMA being one of the fastest growing international sports, only recently have reports begun to emerge on the weight making practices of these athletes. At the time of this PhD research programme commencing there were only 5 studies of this nature relating to the sport of MMA. (Andreato et al., 2014; Coswig et al., 2015; Crighton et al., 2016; Jetton et al., 2013; Mendes et al., 2013).

In the interim, while other reports have emerged, (Anyżewska et al., 2018; Barley et al., 2019; Hillier et al., 2019) survey data remains the majority of the information gathered so far on RWL in MMA and as stated previously, few studies have undertaken scientifically controlled RWL investigations. That aside, the survey data has provided some data on what the established means RWL is usually achieved. The surveys have shown MMA athletes employing one or all of the following methods: water loading, fluid restriction, diuretics, complete fasting, low residue, or low carbohydrate diets in the final 3 to 5 days prior to weigh-in (Barley et al., 2018). The practice of water loading has been studied by Reale et al (2018) which in this case, refers to an increased fluid intake of 100ml/kg for 3 days and then consuming 15 ml/kg for day 4 followed by a rehydration protocol on Days 5-6. Reale et al. (2018) have shown that water loading can increase the magnitude of RWL 3.2 v 2.4% of body mass without any noted negative consequences.

The depletion and repletion of glycogen which is a known effect of RWL has not been studied in MMA athletes but work on other combat sports has shown that it can be depleted and replenished without an effect on performance (Tarnopolsky et al, 1996). This however is dependent on the athlete having sufficient time between the weigh-in and fight time. The main processes that MMA athletes employ for dehydration, such as exercise, saunas and hot baths have little or no research in this specific population.

Positionality: My Professional Experience and Practice

Positionality describes the understanding that research is a process, not a singular act or event, and that what we bring from our own experiences and background both shapes and, dialectically, is shaped by the ongoing research process (Bourke, 2014). Jafar, 2018 aligns positionality with reflexivity i.e. *"an act of self-reflection that considers how one's own opinions, values, and actions shape how data is generated, analysed and interpreted".* This approach is salient given my background first and foremost as an applied practitioner with combat sport athletes, and with positionality increasingly recognised as important in quantitative and clinical research (Jafar, 2018), for these reasons, I will describe my professional experience and practice in this section.

I have been involved in working in applied sports science support with MMA athletes for over 10 years in various guises. When I first started, my primary focus was to manage and advise the MMA athletes on their strength and conditioning training. This progressed to then being recruited to provide information and support to the athletes about their diet and became the athletes' nutritionist.

At the start of my work with MMA athletes in 2009, there had only been one Irish athlete (Tom Egan) to reach the UFC. At this time MMA was still in its infancy as a sport in Ireland . Even though there were 'professional' fighters having 'professional' fights, these were professional in name and rule, but very few of these athletes were paid as fully professional athletes. In other words, while the rule set was professional, the training, scientific and financial support was not. In my experience fighter's knowledge in the area of nutrition was extremely limited. One anecdote I was once told by a reigning national champion was that he "avoided all carbs", just as he was consuming a potato crisp sandwich. This points to the dearth of knowledge and understanding about the impact and value correct nutrition can bring to the sport and to the athlete's performance. In the intervening years I have witnessed how fighters knowledge of nutrition has increased, but how there can still be major gaps in this knowledge, as their sources for this information can be reliant on anecdotal sources or information from social media.

My role initially as a nutritionist was 'simply' to get the athletes as lean as possible in the weeks prior to the fight, while maintaining lean body mass to a large extent. At the early stages of my involvement it was the athletes who then managed their own weight cuts in the days and final weeks preceding the fight. I observed that the information they used to inform their weight cuts was a combination of coaches and fellow athletes/teammates experience combined with whatever information they could gather on weight cutting from the internet (this is consistent with the survey data described in the scientific literature). My view was that their approaches were lacking an evidence base, and therefore unsurprisingly were haphazard and frankly dangerous. However, when I searched the literature for that evidence base there was at the time, there was very little research conducted that could directly inform the RWL phase of the weight making process.

After noting the dearth of research in this area, it motivated me to pursue an MSc in Exercise and Nutrition Sciences, and to later move on to undertake this PhD. During the entire period, I continued my applied practice with athletes, and in an effort to find the most effective and safest methods to weight cut, we ran small pilot trials on alternative methods of weight cutting that were being used by athletes. These were competed without necessarily the published literature to back up their use in RWL. These pilots included several methods that have since been backed up in research (Foo et al., 2022) such as the low residue diet (which we colloquially called the "chicken and nut butter diet") and the aforementioned water loading. Traditionally, the use of hot baths and saunas was already a major component of the MMA athletes' protocol in the last few days to dehydrate. I found in my experience that fighters would go to whatever lengths necessary to make weight using these methods, with often their only guiding principle, beyond making the required weight, being "the more suffering the better", which is also echoed in the findings of Pettersson et al. (2013).

In my experience, combat sports athletes usually like to hit a certain target weight to start the weigh cut from. How they get there within the first phase diet is an area that has several approaches. The approach the athletes take can depend on how much time they have to hit their target. In MMA especially, fighters can take fights on very short notice (2-4 weeks in some cases with extreme cases of fighters taking fights with one week notice). The approach to the diet (or first phase) can be completely dictated by how much time. the athlete has before their fight.

It has been my goal to use the experience and knowledge that I have gained over the years to make my athletes' weight cuts safer and easier for them. I have worked with a UFC Champion, several other world champions in MMA, and also with several world champions in boxing across several weight classes. Even with this experience and remaining abreast of the latest research, many knowledge gap remains in the scientific research and best practice when it comes to the final RWL phase, and although beyond the scope of this thesis, the Rapid Weight Gain phase too.

It is therefore clear that my positionally to complete this Phd was influenced by my prior experience working with athletes and my commitment to ensure both optimum levels of performance and safety. Furthermore, as both a practitioner of martial arts and a sports science professional, I understand the desires that drives fighters to win and to give their all to competing. What immersion in the literature and rigorous studies with participants as part of this PhD journey has highlighted for me, time and again, however are the dangers associated with unscientific practices inherent with weight cutting. As such my desire to scrutinise and illuminate the most effective and safest methods for fighters, whether amateur or professional, to cut weight safely and effectively has driven and sustained my interest throughout the research process. I fully expect that this same desire will drive me forward to explore this area further in the future.

Thesis Aims and Research Questions

My PhD studies commenced with an evaluation of self-reported prevalence, magnitude and methods of RWL using a validated questionnaire in a sample of competitive MMA athletes . These were comprised of both amateur and professional fighters based in Dublin, Ireland. This study revealed an unexpectedly high prevalence of the use of hot water immersion as a method of RWL, colloquially termed “hot baths”, most often comprising of salt water by the addition of salt in the form of magnesium sulfate (Epsom salt).

As a result of these survey findings (Chapter 3), I developed a standardised hot bath protocol, and undertook a series of studies to systematically investigate the effect of hot water immersion, with or without salt, as a method of RWL. My specific aims in developing this protocol was to:

i. To investigate the magnitude of body mass losses in MMA athletes using a standardised hot bath protocol at *fixed* water temperature, with or without the addition of Epsom salt to a concentration of ~1.6% (Chapter 4);

   Hypothesis (stated as a null hypothesis): That the addition of salt to a hot bath will not result in greater loss of body mass compared to the same hot bath protocol without the addition of salt.

ii. To investigate the magnitude of body mass losses in MMA athletes using a standardised hot bath protocol at *self-adjusted* water temperature, with or without the addition of Epsom salt to a concentration of ~1.6% (Chapter 5);

   Hypothesis I: That bathing in a protocol at *self-adjusted* water temperature will result in a greater loss of body mass compared to a fixed water temperature.

   Hypothesis II (stated as a null hypothesis): That the addition of salt to a hot bath will not result in greater loss of body mass compared to the same hot bath protocol without salt.

iii. To investigate the magnitude of body mass losses in MMA athletes using a standardized hot bath protocol at self-selected water temperature, with or without the addition of Epsom salt *to a concentration of ~5%* (Chapter 6);

   Hypothesis: That the addition of a salt at a high concentration to a hot bath will result in greater loss of body mass compared to the same hot bath protocol without salt.

iv. To investigate the effects of a standardised RWL process combined with an ecologically-valid recovery period and process on blood biomarkers and indices of performance(Chapter 6).

   Hypothesis: Change in blood-based biomarkers induced by RWL will return to baseline after the recovery period, and indices of performance will be unaffected by the RWL process given this adequate recovery period.

Chapter 2
Literature Review

Making Weight in Weight Class Sports

As briefly described in Chapter 1, there are two distinct phases of making weight in combat sports in preparation for a fight or competition: (i) the general (or gradual) weight loss phase lasting weeks to months, and (ii) the RWL phase occurring sometime in the last 7 to 14 days immediately prior to weigh-in. This section of the literature review will first describe the general weight loss phase and how this approach to a sustained energy reduction targeting a reduction in tissue mass affects performance measures, and whether a fast or slow approach to fat loss influences these outcomes. The next section of the literature review will describe the different methods that fighters (and other athletes) use to "cut" weight during the RWL phase, which largely focus on the manipulation of total body water, glycogen and the contents of the gastrointestinal tract (Burke et al., 2021).

The General/Gradual Weight Loss Phase in Weight Class and Combat Sports

Body composition is important in both combat and non-combat weight class sports. For example, among MMA athletes with an average age of 30 (SD± 4) years body fat was negatively correlated with lower body power ($r = -0.75$) and strength endurance performance ($r = -0.67$) measured using standing broad jump and flexed arm hang, respectively (Marinho et al, 2012). Similarly, success in lightweight rowing, another weight class sport is related to lower body fat and greater total muscle mass (Slater et al., 2005). Among male and female rowers, gradual dieting, fluid restriction (this is considered more RWL than body mass management), and increased training load were identified as the most popular methods of body mass management (Slater et al., 2005).

There is relatively little published data regarding the approaches (rapid vs. slow) used by weight class and combat sport athletes during general/gradual weight loss phase of training. Among male track and field sprinters and jumpers, 4-weeks of a high weight reduction diet involving 750 kcal/day energy restriction resulted in a 2.2±1.0 kg loss in total body mass compared to a 0.4±1.2 kg reduction following consumption of low weight reduction diet involving an energy restriction of 300 kcal/day (Huovinen et al. 2015). Fat-free mass, bone mass, testosterone, cortisol, and sex hormone binding globulin did not change in either group. Countermovement jump performance and 20-m sprint time improved consistently ($p \leq 0.05$) in the HWR group, by 2.6±2.5 cm and 0.04±0.04 seconds, respectively.

Researchers at the Norwegian Olympic Sport Center compared a fast rate (FR) of weight loss involving 1.4% body mass/week with a slow rate (SR) involving a weight loss of 0.7% body mass/week among athletes involved in the following individual and team based sports; football, volleyball, cross-country skiing, judo, jujitsu, tae kwon do, waterskiing, motocross, cycling, track and field, kickboxing, gymnastics, alpine skiing, ski jumping, freestyle sports dancing, skating, biathlon, and ice hockey (Garthe, et al., 2011). A desirable level of body fat was set for each athlete by a group of nutritionists. The duration of the dietary intervention ranged from 4 to 12-weeks and was dependent on the weight loss required by each athlete to achieve their target body fat. There was a similar loss in body mass in SR (5.6%) and FR (5.5%). In contrast, lean body mass increased by 2.1% in SR and did not change in FR. Similarly, there was an improvement in countermovement jump performance and 1RM max for the squat, bench press and bench pull in SR and no change in performance in FR. The SR group spent longer on the intervention than the FR group (8.5±2.2 and 5.3±0.9 wk, respectively). These results therefore suggest having enough time to follow a slow rate of weight loss is more beneficial for athletes.

Gradual weight loss however may also have negative consequences. Among healthy male University judokas, a 4.5 MJ/day energy restricted diet for 15 days that resulted in a 1.7% reduction in body mass and 1.5% reduction in fat free mass was found to negatively impact performance in the Judo Fitness Test (Chrara et al., 2019). Sustained energy reduction may also result in dietary deficiencies. Papadopoulou et al. (2017) evaluated macro- and micronutrient intake among male and female Tae-Kwon-Do athletes in the month leading up to a national competition and found that the male and female athletes were obtaining only 60.3% and 48.6% of their daily energy requirements, respectively. The majority of macro- and micro-nutrients were lower than the recommended values with carbohydrate and protein intakes at the lowest levels of the recommended values. Vitamin A, vitamin E, biotin, calcium, iron, magnesium, and potassium intake in women were lower than the recommended values. In men, lower intakes were observed for vitamin A, vitamin E, biotin, magnesium, and potassium.

A number of case studies that have provided detailed accounts on the general weight loss phase in preparation for competition among combat sport athletes (Kasper et al, 2019; Langan-Evans, 2018; Matthews, 2020; Morehen et al., 2021). The athletes studied, all maintained a high level of protein intake ranging from 1.6 to 2.5 g/kg/day and achieved a energy deficit largely by reducing carbohydrate intake. For example, Langan-Evans (2018) reported on an athlete who limited daily carbohydrate intake to 3.4 g/kg LBM. The range of energy restriction varied greatly. Kasper et al. (2019) and Langan-Evans (2018) reported on combat sport athletes who consumed between 1500 – 1900 kcal per day. Matthews (2020) reported an energy deficit 900 kcal/day whereas the athlete in the case report by Morehen et al. (2021) targeted an energy intake equivalent to resting metabolic rate.

Case studies suffer from a publication bias that reflects extreme scenarios. The data from the intervention studies largely supports the safe use of gradual weight loss through sustained energy restriction combined with sports-specific training. Performance decrements or suboptimal adaptations to training during a period of gradual weight loss tend to be associated with interventions that are associated with fast rates of weight loss and/or lead to the loss of lean body mass during this period.

The Rapid Weight Loss Phase in Weight Class and Combat Sports

There is currently no consensus for best practices during the RWL phase of preparation among weight class and combat sport athletes. In the literature RWL has a broad scope of meaning. For the sake of this thesis, RWL will be defined as the loss of substrates that can be lost and replaced over the course of 2-5 days such as glycogen stores, gut content, body fluid, and does not include the loss of body fat in any significance.

The guidelines suggested by Reale et al. (2017a; 2017b & 2018) remain the most informative in the field. These guidelines are based on the 'weigh-in' being undertaken on the day before competition, and a need to decrease 5-8% body mass as safely as possible. In such scenarios, the majority of RWL can be achieved through reducing the contents of the gastrointestinal tract through a low residue diet, and the manipulation of total body water via carbohydrate restriction resulting in glycogen depletion, combined with fluid restriction to produce dehydration. Several other methods can used be to compliment and/or enhance the effectiveness of the above techniques including, but not limited to, water loading, sodium depletion, passive and active sweating, and increased exercise. For the purposes of this chapter, each of the methods used for RWL will be described in the order in which they tend to be used in practice, starting approximately one week prior to 'weigh-in'. Their prevalence and the magnitude of their effects will be considered in later sections.

Water Loading

Water loading is the practice of consuming well above recommend daily fluid intakes for 3 d and then decreasing intake the levels before completely restricting fluids in the final 24 h. When used, water loading starts five days before 'weigh-in' (Reale et al., 2018). Presently, the volumes researched (Reale et al., 2018; Cho & Han, 2020) for effective use are 100 mL/kg for 3 d followed immediately by 15 mL/kg for 1 d. The 15 mL/kg is ingested on the day prior to ''weigh-in' and must be consumed 24 h before the official 'weigh-in'. For example, if the 'weigh-in' is scheduled for 1300 hours on Friday then all fluid should be consumed by 1300 hours on the Thursday). Water loading four days before 'weigh-in' reduces body mass more than simply restricting water for 24 h. Among college wrestlers, weight loss is 8% to 11% greater in response to weight loss plus water loading compared to weight loss only (Cho & Han (2020), Reale et al. 2018). The water load phase used by Reale et al (2018) was for 3 d with a day of 15 mL/kg followed by a day of fluid restriction. Participants in the Cho & Han et al., study (2020) water loaded for 7 d followed by a gradual reduction of fluid intake over 6 d followed by cessation of fluids for a day. Hydration levels and fluid restriction in response RWL will be discussed in later sections.

Salt

Removal of dietary salt for up to 5 d may lower body mass by 1-2% through the loss of body water retention (Reale et al., 2017a). In untreated hypertensive individuals, short-term dietary salt restriction was found to have no negative consequences as measured by blood pressure, urine levels and plasma creatinine (He et al., 2001). To date, the effects in RWL have not been studied in weight class athletes.

Low carbohydrate- Low residue diet

This dietary manipulation effectively involves consuming foods that contain only proteins and fats - lean meats, full fat dairy products, and high fat foods such as avocado, olives and nut butters. This diet means keeping fibre less than 10 g per day (Foo et al., 2022) and carbohydrates below 30 g per day. Carbohydrate restriction facilitates the reduction in glycogen and associated water content. Assuming that 8% of liver and 1-2% of skeletal muscle weight consists of glycogen granules, a 75 kg male would have approximately 462 g of stored glycogen and 1665-3610 g of bound water (Reale et al., 2017a). The purpose of the low residue diet is to empty the contents of the gastrointestinal tract, which may be as much as 1.0 kg (Burke et al., 2021; Reale et al., 2017a; Reale et al., 2017b). A low carbohydrate, low residue diet is normally consumed 2-3 full days before 'weigh-in' (Reale et al., 2017a). The 2-3 day window is partly due to the fact that the risk of constipation increases in some individuals on a low fibre diet.

Sweating

Active sweating and/or passive sweating routines are also used as part of a weight reduction strategy among weight class and combat sport athletes. Active sweating involves sweating during exercise, whereas passive sweating is induced by saunas, heated rooms or hot baths. A limitation of active sweating involves the use of exercise potentially leading to fatigue. In contrast, passive sweating can result in a similar reduction in body mass without the same level of fatigue as active sweating. Importantly, plasma volume is maintained in response to active sweating whereas it is reduced following passive sweating. The reduction in plasma volume can negatively impact exercise performance (Reale et al., 2017a). The effects of RLW methods on markers of health and performance will be reviewed in later sections.

Sweat Gland Fatigue (Anhidrosis)

One critical aspect of sweating is of course the sweat gland. This gland (the glandular component) is located in the dermis or between the dermis and the subcutaneous fat and has a tubular duct that emerges at the skin. There are two major types of sweat gland, the apocrine and the eccrine. The eccrine are the type that has the greatest influence on sweat rate. There are between 2 and 4 million of these glands in the body and are related to temperature control. Water comes in to the glandular component and then flows through the duct to the surface of the skin. Then heat from the body then evaporates the sweat. The eccrine sweat glands have lots of mitochondria to provide energy for prolonged intensive sweating. (Boron & Boulpaep, 2016)

The size of a sweat gland can vary 5-fold between individuals (Sato et al., 1989). The size of the gland correlates with the sweat rate. Heat acclimatisation training can increase the size of the sweat gland. Heat acclimatisation training also lowers the sodium content of sweat as the body reabsorbs the sodium better during the sweating process (Ogawa et al., 1982)

According to Baker (2017) “primary sweat is nearly isotonic with blood plasma (e.g. approximately 135–145 mmol/L Na^+, approximately 95–110 mmol/L Cl^-, and approximately 4–5 mmol/L K^+). As sweat flows through the duct, Na^+ is passively reabsorbed via epithelial

Na+ channels (ENaCs) on the luminal membrane and actively reabsorbed via Na+/K+-ATPase transporters primarily on the basolateral membrane".

There are several causes of decreased sweat rate of which many are medical that do not concern the context of this thesis. This thesis is concerned with sweat gland fatigue i.e. the decline in sweat rate that occurs over time during prolonged heat exposure and/or high rates of sweating, and the mechanisms that are associated with this phenomenon.

Excessive dehydration is considered one of the major components of sweat gland fatigue. This is related to the thesis as the subjects will be suffering from dehydration as a result of the body mass drop experienced. As noted by Baker (2017), sweat gland fatigue can occur by blockage of the sweat gland in humid conditions. It has been proposed that the decrease in sweating rate is due primarily to fatigue of the secretory mechanism of the glands (Thaysen & Schwartz, 1955). An individual who is not heat acclimatised loses >30 g of salt per day. The increased salt content of the hot baths might prolong the sweat rate of the sweat gland by somehow having an effect on the secretory gland. The mechanism for this, if true, is unknown. Several of the hot bath studies have shown that the sweat rate decline is less during hot water immersion than when the skin is exposed to air. However, this does not explain differences seen in other hot bath studies that have included salt (Whitehouse et al, 1932; Hertig et al., 1961). The largest water deficit (2 % of body wt.) in the Hertig et al. (1961) study was recorded for a subject in 15 % salt water. The rate of sweating did not decline over time in this exposure.

Prevalence, magnitude and methods of RWL

The majority of the data regarding prevalence of use, magnitudes of body mass lost and methods employed for RWL has been based primarily on questionnaire studies. Central to this work has been the Rapid Weight Loss Questionnaire (RWLQ) initially described by Artioli et al. (2010a) for use on Judokas to explore the methods used in the 7 d (RWL) phase prior to competition when 'making weight'. Initial validation work that was conducted with a relatively large (n=822) heterogeneous sample, including competitors of both genders, across a wide range of competitive levels and ages (Artioli et al. 2010b) demonstrated good reliability and discriminant validity. For example, no statistical differences were found between the RWLQ score obtained in the test and the retest, the proportions of athletes who recorded the same response or who disagreed by only ± 1 point in the 5-point scale questions were >80% (Artioli et al. 2010a; Artioli et al. 2010b). Although the RWLQ questionnaire was originally developed for the assessment of RWL in judo athletes, it has been modified and validated for other combat sports (Barley et al., 2018). Subsequently, the questionnaire has been modified and utilised for MMA athletes (Andrea et al., 2014; Matthews & Nicholas, 2017; Coswig et al., 2018) and other combat sports (Reale et al., 2018).

The RWLQ provides a rapid weight loss score (RWLS). A higher score indicates the use of use of a number of methods (saunas, dieting, fluid restriction, etc) to induce a more severe RWL. The first study to use the RWLQ found that 86% of Judo competitors had lost weight to compete, more than half (53%) had experienced weight cuts of 5% of body mass and more aggressive weight cutting behaviours were influenced by competitive level (Artioli et al., 2010a).

Prevalence of Weight Cutting in Combat Sports

The prevalence of RWL is high, although variable among combat sports. Using the RWLQ, with some modifications, different combat sports have reported a high prevalence of athletes lowering their muscle mass by > 5%. Among 580 male Judo, Jujitsu, Karate and Tae Kwon Do athletes surveyed by Brito et al., (2012), reductions in body mass ranged from 6-10 kg in the pre-competition week. The sport with the highest percentage of body mass lost during the competition week was Judo (5.6%), followed by Tae Kwon Do (4.3%), Jiu-Jitsu (4.1%) and Karate (3.6%). Jiu-Jitsu has same day weigh-ins, whereas Judo, which has day before weigh-ins. The increased recovery time may provide judokas with time to participate in more extreme RWL (>5% body mass) (Burke et al., 2021).

Using a modified RWLQ, Reale et al (2018) examined the RWL practices among Australian Wrestlers, Boxers, Judokas and Tae Kwon Do athletes. No effects were found for sport (despite different weigh-in times), sex or weight division group on RWL score. Athletes who had medaled at international and national competition(s) were classified as high and moderate caliber, respectively and all others were classified lesser caliber. The higher the caliber of athlete the greater the RWL score. Others have reported that international level and professional athletes adopt weight management behaviours that are more aggressive than national or regional athletes and amateurs (Hillier et al., 2019, Malliaropoulos et al., 2018).

A very high proportion (92.5%) of Malaysian Karate, Boxing and Tae Kwon Do athletes self-reported using RWL methods and 58% of Polish martial artists (Judo, Kickboxing, BJJ, MMA, and Boxing) reported using RWL in the 2-3 days before competition (Anyzewska et al., 2018). In Brazil, 63% of from a sample of 580 Judo, BJJ, Karate and Tae Kwon Do athletes lose weight for competitions (Brito et al., 2012). RWL is a common practice across all combat sports with elite level athletes reducing body mass by as much as10%.

Methods of Rapid Weight Loss According to RWLQ

According to the Rapid Weight Loss Questionnaire, increasing activity and a reduction in energy intake are the most popular methods of RWL. In 62 professional Polish combat sport athletes (Judo, Kickboxing, BJJ, Mixed Martial Arts MMA, Boxing) surveyed, reduced energy intake was used by 61% and increased activity by 39% (Anyzewska et al., 2018). Artioli et al. (2010b) reported increased activity in Judos at 61.7%, while reduced energy intake was 67% in Judo, Jujitsu, Karate and Tae Kwon Do Brazilian athletes (Brito et el., 2012). Fluid restriction is another very popular technique with its usage being reported at 68% (Anyzewska et al., 2018), 51% (Artioli et al.; 2010a), and 58% in 256 British judokas (Malliaropoulos et al., 2018). Training with plastic or rubberised suits and/or training in a heated room are also common for RWL [Artioli et al. (2010b) = 40% & 65%; Ng et al. (2017) = 62.2% & 56%; and Malliaropoulos et al. (2018) = 28% & 30%]. Reale et al (2018) noted that in a group of combat sport athletes, active sweating was used more by Boxers (90% of boxers used active sweating as to compared to ~70% of Tae Kwon Do and Wrestling athletes) whereas passive sweating was used more by wrestlers (60%). The use of water loading, as described in an earlier section, is also growing in popularity as a method of RWL (Crighton et al., 2015; Reale et al., 2018).

The RWLQ has however also offered some worrisome results in relation to RWL. In elite kick-boxers (61 males; age = 24.2±4.6 yr; weight = 73.9±12.8 kg; height = 179.2±7.9 cm) a relatively high percentage of athletes were using drastic weight reduction methods (i.e., laxatives 13.1%, diuretics 11.5%, diet pills 14.8%, vomiting 3.2%) (Dugonjić et al., 2018). Of those questioned 40% of the athletes surveyed usually lost 2-5% of their body mass, while ~30% lost 6-8% and almost 30% reported cutting 10% of BW or more at some time during their kickboxing career.

An area of concern that has been raised from many of the questionnaires is the source of information for RWL that the athletes use. Hillier et al. (2019) reported that MMA athletes cited coaches as their primary source of weight loss information (professionals=22%) with dieticians at 14.2%. In interviews conducted by Berkovich et al. (2019) with combat sport coaches and trainers 90% reported supervising their athletes through the weight cut however less than 50% of them stated they had received their information used to guide the athletes' weight cut from a dietician. Coswig et al. (2018) also reported that coaches were the primary source of weight loss advice for 15 professional MMA athletes. Lastly, 61.6% of Judo athletes surveyed described dieticians as "Not influential" on their weight management (Malliaropoulos et al., 2018).

Effects of RWL on Biomarkers and Performance

There have been no studies to my knowledge to show an improvement on various biomarkers on health and performance during and in response to RWL. There is, however, an ample body of research that points to the negative effects of RWL as illustrated by the following section.

In a comparison of short term weight loss (a drop of body mass of 4-5% over 10 days) to fast weight loss (a drop of 4-5% of body mass in 24 hours) on the free testosterone and cortisol levels of 14 young elite wrestlers (age = 17.79±0.75 y, height= 172.06±4.61 cm, weight = 70.04±8.7kg, BMI= 23.21±2.09 and years of experience =5.49±0.59 y), there were no significant changes in hormonal variables within groups or between groups (Moghanlou et al., 2019). However, there was a significant reduction in aerobic performance (an increase from 8.13±0.58 to 8.41±0.57 mins on the Rockport test) in the fast weight loss group compared to pre-test.

A study by Nascimento-Carvalho et al in 2018 on eight male (21.62±1.49 y, 71.25±3.54 kg, 1.74±0.03 cm) fighters (MMA, BJJ, & Muay Thai), showed that after 14 days of weight loss (reducing body mass by 5%) resulted in an increase in resting heart rate of 11 beats per minute. The methods used to reduce weight in the athletes studied were restriction of carbohydrates (34%), fats (20%) and liquid (20%), and an increase in training volume (13%). The higher resting heart rate was interpreted as increased cardiac sympathetic modulation meaning that the fighters may be at a higher cardiovascular risk if this level is maintained for competition (Nascimento-Carvalho et al., 2018).

RWL has also been shown to have an impact on markers of muscle damage. Levels of myoglobin, creatine kinase, aldolase, hemoglobin, and hematocrit were measured for seven consecutive days (4 'normal' days, 3 days of RWL) in eighteen male Judokas (mean body weight 85.3±8.1 kg, mean age 25.3±5.4 y, mean height 179±6.7 cm). These showed increases in several of those variables during RWL (Roklicer et al., 2020). Creatine kinase (CK) levels increased rapidly exceeding reference values (444.72±266.13 U/L, upper limit is 308 U/L). Serum Mb increased significantly, although values remained within the reference range (85.37±46.34 µg/L, reference range 0–73 µg/L). Aldolase levels increased but remained within normal levels (4.16±0.70 U/L, typically range 0–7.6 U/L). These authors suggested that this indicated that RWL results in muscle damage, although it must be said that changes in plasma volume were not accounted for. Thus, in the absence of a mechanism for how RWL results in muscle damage, the impact of haemoconcentration on muscle damage cannot therefore be excluded (Harrison, 1985).

One study conducted by Motevalli et al (2015) may provide some insight into a mechanism of muscle damage during RWL. In a comparison of well-trained male wrestlers (N=30; 22.5±1.7 y, 78.3±8.2 kg, 12.1%±2.7% body fat) in two groups with one group (n=15) on a 12-day gradual weight loss diet and the other group (n=15) on a 2-day RWL diet (548±110 kcal) there were no significant differences in body mass, fat mass, lean mass or body fat (Motevalli et al., 2015). Both groups decreased body mass by ~4%. Serum myostatin was significantly increased (mean 5.34 to 5.71 ng/mL) and serum follistatin was significantly decreased (mean 1.99 to 1.75 ng/mL), resulting in a significant increase in the serum myostatin-to-follistatin ratio (mean 2.82 to 3.35 ng/mL) in the RWL group. The researchers concluded that this possibly indicates the early stages of skeletal muscle catabolism.

Eight male Judokas (age 19.3±2.0 y, body height 178.1±6.3 cm, body weight 81.7±10.7 kg, professional experience 9.6±1.6 y) reduced total caloric intake by 35% for 6 days which was then followed by a total food restriction on the seventh day that was also weigh-in day (Drid et al., 2019). The Judokas dropped from 81.7±10.7 kg at baseline vs. 76.8±10.3 kg at follow-up from the intervention and serum creatinine levels were significantly increased at follow-up (mean difference 10.9 µmol/L; 95% CI 0.2 µmol/L to 22.0 µmol/L), while serum creatine and guanidinoacetic acid were not affected during the study. High levels of creatinine are a sign of impaired kidney function. Athletes must therefore be monitored closely to make sure RWL is not taken too far.

Reljic et al. (2016) divided twenty-eight well-trained male combat athletes (thirteen wrestlers, six Boxers, five Judokas, three Tae Kwon Do athletes, and one Karate fighter, into two groups, one control group (n=14) and one weight loss group (n=14). The control group maintained their current weight, while the weight loss group reduced their body mass by 5.5% according to their self-selected, accustomed regimen over a 1-week period (time point 2: 1-2 days prior to competition). The weight loss group haemoglobin mass decreased by 37±23 g (−4.1%) after rapid weight loss (time point 2) and remained at a lower level at time point three (post competition after a period of normal dieting and training) compared with the baseline (time point 1: when the athletes are weight stable) value taken at (−2.6%). In this study a decrease in haemoglobin mass was noted. There was however no noted impact on the measure aerobic performance capacity tests.

In their entirety, the above studies demonstrate that there are changes induced that may indicate an increase in health risk with RWL over a short duration (2-7 days). Whether differing methods of RWL cause similar health risks, and whether these changes are transient and inconsequential and remain after a proper recovery strategy is implemented are key questions that are largely unexplored in combat sports athletes who use RWL methods in order to make weight prior to competitions.

Hydration Status and Dehydration in Combat Sports

It is important to note the limitations of using hydration scores to measure hydration levels. According to a review by Cheuvront et al (2014) it is stated that "Without exception, urine concentration thresholds often used to denote a well-hydrated state are first morning measurements. First morning measurements are more uniform as they are generally immune to acute alterations in diet, fluid intake, or activity……… when first morning measurements are possible, most measures of urine concentration (Uosm, urine specific gravity, and urine colour) increase, and provide good diagnostic accuracy for diagnosing intracellular dehydration under controlled laboratory conditions…….. It has also been demonstrated that concentration of first morning urine reflects 24-h concentrations.”.

There is little doubt that weight class athletes are dehydrated at weigh-in (Brandt et al., 2018; Kasper et al., 2019; Matthews & Nicholas, 2017; Pettersson, et al., 2013). Depending on the recovery time, dehydration can be largely reversed (Slater et al, 2006). The extent to which dehydration impacts on readiness for competition is hard to know given that overall studies are heterogenous in terms of whether the study assessed the performance while dehydrated at what would be weigh-in time, or whether they allowed for rehydration and recovery after RWL, in a way that would be analogous to recovery after weigh-in. Regardless of that point, the effects of dehydration are explored below.

When it comes to the RWL process and potential methods applied, the largest magnitude of weight loss comes from manipulation of total body water through dehydration. When combat sports athletes reduce body mass from between 5% to 10%, the majority of this loss comes from dehydration. Methods used to promote dehydration such as saunas, hot baths, additional exercise, wearing “sweat suits”, restricting fluids or a combination of all of these are noted in the above data from questionnaires (Anyzewska et al., 2018; Artioli et al. 2010a; Malliaropoulos et al., 2018; Ng et al., 2017).

There is little doubt that most combat sport athletes making weight present at weigh-in in a hypohydrated state. For example, in one study sixty-three elite athletes that included Wrestlers, Judokas, Boxers, and Tae Kwon Do athletes were split between evening before weigh-ins (n=31) and morning of weigh-ins (n=32) (Pettersson & Berg, 2014). In the morning weigh-in group, as measured by urine specific gravity, 53% were severely hypohydrated and 43% were significantly hypohydrated. In the evening before weigh-in group, 41% were severely hypohydrated and 38% were significantly hypo-hydrated (Pettersson & Berg, 2014). This is consistent with typical methods of RWL resulting in 100% of MMA athletes being dehydrated to various degrees at the time of the official weigh-in (Jetton et al., 2013; Matthews & Nicholas, 2017). In preparation for a competitive bout, 57% and 43% of fighters were reported to be dehydrated (1033±19 mOsmol/kg) and severely dehydrated (1267±47 mOsmol/kg), respectively, at weigh-in (Matthews & Nicholas, 2017). Perhaps more importantly in a performance context, many of these athletes were still reported to be in a hypohydrated state after their recovery. For example, 14% (Matthews & Nicholas, 2017) and 39% (Jetton et al., 2013) of fighters remained hypohydrated when measured in the final 2 hours prior to a competitive fight.

This research indicates that a large proportion of athletes' rehydration protocols are therefore not adequate to fully hydrate them prior to competition.

In a meta-analysis of 15 studies (Goulet, 2013) involving 122 male subjects (25±3 years, 72±4 kg, 178±4 cm), exercise induced dehydration (as measured by a drop in body mass) of *less than 4%* does not impair endurance performance (mainly cycling) under real-world exercise conditions. Furthermore, the comparison of ecologically-valid time-trial exercises between exercise-induced dehydration (2.19±1.0% bodyweight) versus euhydration showed no statistically significant difference. It must be noted that this review was conducted on aerobic exercise and in MMA anaerobic capabilities largely distinguished higher- from lower-level athletes (James et al., 2016).

In a review on the effect of dehydration on anaerobic performance the conclusion of the authors (Kraft et al., 2011) is that several factors namely the performance-duration, the method of dehydration, dehydration level, whether the mode of exercise is anaerobic or intermittent, the work to rest ratio and the training status play a role in performance. Taking all of these factors into account, initial studies therefore suggest that anaerobic performance is not impaired once dehydration is less than 4%.

Other notable studies on the effect of anaerobic and/or intermittent performance indicate as follows., (2015) noted dehydration by 2.25% of body weight or in a euhydrated condition made no significant difference in peak power during high-intensity intermittent cycling, designed to replicate amateur boxing performance. Higher levels of dehydration (4.5% versus euhydrated) in five participants (wrestlers) performing a modified Wingate anaerobic arm crank decrements in mean power and peak power were observed (Hickner, et al. 1991). It can therefore be concluded from this research that levels of up to 4% hydration does not significantly impact on performance levels for athletes whether they were involved in areobic or anaerobic activity.

Another study conducted by Judelson et al (2007) indicated that dehydration seemed to have more of an impact on an athlete's muscular endurance rather than their explosive power. The study took a group of seven healthy resistance trained subjects (23±4 yr, 1.79±0.58 m, 87.8±6.8 kg) completing workouts in different states of hydration (euhydrated, hypohydrated by 2.5% of body mass and hypohydrated by 5% of body mass) (Judelson et al., 2007). There were no significant differences among trials in vertical jump height, peak lower-body power (assessed via jump squat), or peak lower-body force (assessed via isometric back squat).

Hypohydration however did decrease resistance exercise performance (six sets of 10 repetitions of the parallel back squat at 80% of subjects predetermined 1RM) during sets 2–3 and 2–5 for the 2.5% and 5% hypohydration condition, respectively (Judelson et al., 2007). The study concluded that explosive power was less affected compared to muscular endurance by varying levels of hydration.

Kurylas et al. (2019) conducted a study that better reflected what fighters undertake during RWL prior to weigh-in. Urine specific gravity (USG, normal range is 1.005 to 1.030) and urine osmolality was measured at four different time points (1 = four weeks before fight, 2 = two weeks before fight, 3 = one day before fight, 4 = the fight day). In order to measure the effect of dehydration a 30 second upper and lower body Wingate test on peak power (PP), mean power and total work performed by six well trained combat sports athletes (27.3±0.5 years, 79.4±1.1 kg) was conducted. At the time of weigh-in, when athletes would be at their most dehydrated, there was a significant drop mean (PP = 672±107 W) in all physical performance measurements as compared to 4 weeks out (mean PP = 976±147 W) and 2 weeks out (mean PP = 982±140 W). However performance was still impaired by "fight day" (mean PP = 923 ± 126 W). The participants were considered hydrated at the 4 (mOsmol/kg = 340) and 2 (mOsmol/kg = 380) week mark but severely dehydrated at weigh-in (mOsmol/kg = 1121) and on average were still dehydrated at "fight day" (mOsmol/kg = 780) despite 24 hours of rehydration (Kurylas et al., 2019). This study closely mimicked what fighters go through in a "real world" weight cuts, in that performance is not required at weigh-in time (when the fighters are at most dehydrated) but 24 hours after weigh-in.

Aside from the effects of RWL on hydration status, it is worth noting that RWL can also impact on fuel stores via the manipulation of muscle glycogen. This is important as if carbohydrates are removed and glycogen is depleted for too long, the enzymatic activity of pyruvate dehydrogenase (PDH), which regulates the glycogen metabolism as a contributor to energy provision, is impaired and even with carbohydrate restoration, athletes may still struggle to utilise these glycogen stores for high intensity exercise (Impey et al., 2018). The measure of the introduction and timing of these methods for RWL is important too. As an example, in one study, twelve highly trained male wrestlers achieved 5% body weight loss in a 3-day period with an energy restricted diet of 1,141 kcal/day, fluid restriction, exercise and dehydration methods that included sauna and exercise in a heated room (Tarnopolsky et al., 1996). These methods resulted in large decreases (54%) in muscle glycogen levels as measured by muscle biopsy from the dominant biceps brachii. However, like the effects of recovery on hydration status, the muscle glycogen levels were largely reinstated after a 17 hour repletion period. Half of the group (n=6) participated in a simulated wrestling tournament and there was no reduction in biceps brachii muscle glycogen levels while allowing ad libitum carbohydrates feeding between matches.

However, signs of glycogen depletion are evident after RWL (Reljic et al., 2015), and may have implication for performance as glycogen is critical for performance in MMA as it has been shown that the grappling aspects of the sport relies heavily on glycolytic metabolism (Abad et al., 2016; Andreato et al., 2016; Coswig et al., 2016).

Therefore, the overall evidence would suggest that the impact of dehydration on short duration, high intensity exercise that would be analogous to combat sports is equivocal, but cannot be discounted. A salient issue for weight class sports, in particular is that while dehydration is a key strategy in RWL, it is important to ensure that the period of recovery until competition allows for recovery of most, or all, of total body water deficits. Whether this overall process or any residual dehydration impacts performance has not been rigorously investigated. In that regard, an interesting study was recently reported by Barley et al. (2018). In that study, athletes were dehydrated by ~5% in through exercise in a heated room, with hand-grip strength, a repeat sled push test, medicine ball chest throw, and vertical jump tests completed 3 hours and 24 hours after the intervention. Vertical jump was unaffected by dehydration and recovery, hand-grip strength was weaker at 3 hours but not 24 hours, medicine ball chest throw was shorter at 24 hours, but not 3 hours, and repeated sled push performance was worse at both 3 and 24 hours after dehydration (Barley et al. 2018). Therefore, while the evidence is not yet conclusive, there is likely to be time course-specific effects on a given performance outcome in response to dehydration and recovery, and which may also be impacted by the method of inducing dehydration i.e. passive vs. active.

Effects of Weight Regain after RWL

Weight regain, often termed rapid weight gain (RWG), is defined as the period between the official weigh-in and the start of the competition. This section will look at the effects of weight regain on performance and some methods to achieve this.

Acute weight loss of 4% in a time trial resulted in a small but non-significant performance compromise in 17 nationally competitive rowers that consisted of 8 males (22.3±3.9 y, 183.2±1.8 cm, 74.2±1.3 kg), and 9 females (22.6±4.1 y, 171.7±5.0 cm, 63.2±2.6 kg), when there was only a 2% regain of body mass (Slater et al., 2006). When two other time trials were performed with a "complete" recovery of body mass, no compromise in performance was observed. The authors of this study recommend that athletes should be encouraged to maximise recovery in the 12-16 hours following racing when attempting to optimise subsequent performance. The recovery strategy employed was 2.3 g/kg carbohydrate, 34 mg/kg sodium, and 28.4 mL/kg fluid in the first 90 minutes of the two hours between weigh-in and performance trials.

In a related study in twelve nationally competitive male lightweight rowers (19.6±1.6 y, 182.1±4.2 cm, 74.0±1.8 kg) that dropped 5.2% body mass in 24 hours before testing, fluid intake had a greater influence on restoration of performance than sodium or carbohydrates (Slater et al., 2007). The rowers performed three 2,000 metre ergometer time trials separated by 48 hours. To simulate competition, weigh-ins were 2 hours before the time trials in which time different nutritional strategies were used for recovery. The athletes had to cut 5.2% body mass in the 24-hours before the first time trial and had to return to their baseline weight before the remaining two time trials. All participants presented as fully hydrated, bar 1 person, as measured by urine osmolality. Ad libitum water was allowed in addition to the specific recovery strategy. As part of the recovery strategy, athletes were provided with either a fluid drink (only water) (FLU), a carbohydrate/sodium supplement (CHO) and a combination of both (COM). The authors concluded that fluid either in water only (FLU) or in combination form (COM) was most important as in the time trials, performance was slower in CHO compared to both COM and FLU, but performance in FLU was not slower than COM.

A group of 16 trained wrestlers (age, 22.5±3.9 y; height, 179.4±7.2 cm; BM, 81.4±9.7 kg) simulated a typical weight cut for a competition by producing a drop in body mass of 5.4% ± 0.5% that was restored in a 16-hour recovery period before testing (Timpmann et al., 2017). The group (n=8) that supplemented with sodium citrate (3 x 200 mg/kg initial body mass) in the 16 hour recovery period had increased blood pH (7.32±0.14 versus 7.26±0.15), blood buffering capacity (18.85±8.55 versus 17.24±8.03 mmol·L–1), and plasma volume (18.4% ± 9.6% versus 9.1% ± 6.0%). The supplement group also had enhanced body mass regain (3.46±0.64 kg versus 2.70±0.28 kg). Hydration, as measured by USG normalised during the 16-hour recovery, and no between-group differences existed in USG at any stage of the study. Ultimately, there was no significant difference between groups on upper body intermittent sprint performance.

Eighteen male combat (Judo, Brazilian Jiu-jitsu, Wrestling, and Mixed Martial Arts) athletes dropped 5% body mass in 5 days (Mendes et al., 2013). There were two distinct groups: experienced weight cutters (n=10, 28±7 y, 77.7±12.3 kg, 1.75±0.06 cm) and non-experienced weigh cutters (n=8, 21±3 y, 73.8±9.5 kg, 1.77±0.06 cm). There was a 4 hour period in which participants ate and drank fluids ad libitum. The athletes' total energy intake and carbohydrate intake over the 4 hour recovery were higher (although no numerical data were provided) than daily intake over the weight loss period. Hydration scores were not given. However, in this study, RWL did not elicit measurable impairments in high-intensity upper-body intermittent performance, regardless of previous experience in rapid weight loss procedures.

Weight Making and RWL Effects on Performance

While the previous section focused on dehydration, this section will instead focus on the broader RWL process for effects on performance. With limited research on the effects of RWL on performance in MMA athletes specifically, data needs to be used from other sports that also use weight classes to have a clearer picture of the outcomes of such practices on performance.

Eleven international weightlifters reduced their weight from a reduction in energy intake (-40%) from restricting all macronutrients in a 6-day period resulting in a 4.34% drop in body mass (Durguerian et al., 2016). After a 2-hour recovery window (subjects were allowed to consume fluids and solids following the usual practice of weightlifters that included an average of 100 g of carbohydrate intake), athlete's performance in a simulated weightlifting competition was not reduced in comparison to baseline. Relatedly, a body mass loss of 1-3% due to diet (overnight fast) and sauna (-1-1.5% body mass) in seven collegiate Olympic weightlifting athletes does not change the ground reaction force, rate of force development, or vertical jump (Budd, & Jensen, 2015). The authors concluded that it could be beneficial for an athlete to lose 1-2% of body mass to get a competitive advantage in weightlifting. Even though the above two studies are from weightlifting, it shows that given the very short time from weigh-ins to weightlifting competitions (minimum of two-hours from weigh-ins to first lift) that strength, power and coordination can be fully restored from aggressive rehydration and refuelling strategies.

Returning the focus to combat sports, eleven well-trained wrestlers (20.45±2.69 y, 74.36±9.22 kg, and 177±5.71 cm) reduced their body mass by 5.03 ± 1.01% (from 79.3±9.7 to 75.3±9.2 kg) in a 3-day period (Cengiz, 2015). The wrestlers achieved RWL using methods they had used previously that included continuous reduction of food and fluid. The Wingate anaerobic test for legs and arms was performed three times: on day 1 before RWL (test 1) with the wrestlers at their 'natural' body weight, on day 4 after RWL (test 2), and after 12 hours of recovery (test 3). Peak power was significantly reduced from RWL in lower body (from 864.7±85.7 to 824.4±96.6 W) and upper body (from 601.1±104.7 to 508.9±115.9 W) but peak power had returned to baseline after 12 hours of recovery. There was a significant increase in fatigue index after RWL (from 55.6±4.4 to 60.6±5.0%) but after 12 hours of recovery it returned to baseline.

In thirteen male Turkish wrestlers, biomechanical segments and points studied had a sharp decline in both hemispheres of the wrestlers' body. Data showed linear velocity in shoulder, pelvis and knee strongly decreased due to a rapid weight loss of 3.5-4% of body mass through means of three 20-minute sauna exposures (Moghaddami, 2015). The biggest decline in performance was recorded immediately post-sauna, although such effects can be difficult to isolate as the effects may be due to dehydration or heat exposure. Tests performed however after 18 hours of recovery (including rehydration) showed an improvement, but not a return to pre-RWL levels.

Coordination can also be effected by RWL (Moghaddami, 2015) but may return to baseline if the necessary recovery time and strategies are used (Durguerian et al., 2016). Eight members of a college's men's boxing club performed tasks on a mechanical boxing ergometer after weight loss of 3-4% (Smith et al., 2000). The test consisted of the subjects completing three rounds, each 3 min long and consisting of 18 repetitions of a 9 item activity delivered by audio-tape. Overall data were equivocal with some subjects appearing able to resist the deleterious effects. When removing the outlier data, a mean decline in boxing performance of 26.8%. was recorded However, the authors noted the small sample affected the power of the analysis and the size of the effect (Smith et al., 2000).

During official competition preparations, 20 male Muay Thai fighters (age of 27.7±3.9 y) had their hand-grip strength tested at three different time points (2 wks before the official weighing for the fight, on the official weighing day, and 1 wk after the fight). The findings indicate that RWL techniques used by the fighters had a negative impact on their strength performance, and it is possible that they did not present their maximum physical potential in the competition (Ribas et al., 2019). There was a drop of 13% in hand-grip strength at the weigh-in, and there was still a drop of 6% in hand-grip strength two weeks post competition.

From the above results, if recovery is adequate or the physical qualities are more strength and power based, then RWL of up to 5% of body mass is not deleterious. If recovery is inadequate or occurs too close to the maximum body mass loss, performance is negatively affected. Coordination shows particular negative affects over other physical attributes.

Characteristics of MMA Performance

It is important when examining the potential effects of RWL on performance to establish the performance markers that are of most importance in the sport of MMA. By establishing the physiological demands and key performance indicators of MMA success, this will provide greater insight into determining the effects of RWL on performance.

MMA is a mixture of grappling and striking, the physical capabilities to succeed in these individual sports can however be varied . It is important to understand what the physical demands of the entire sport of MMA are and how these differ from the individual skills or disciplines of the sport such as striking, the clinch, and the ground game. Understanding the various aspects is important as physical attributes (such as power, strength, and endurance) can be affected varyingly by weight cutting. This will be explored further in later sections.

The sport of MMA has a very high work-to-rest ratio. In a trial of six male (age 26.17±5.04 y; stature 176.50±5.86 cm; mass 73.33±7.84 kg) MMA-trained participants in simulated bouts of three 5-minute rounds with 1 minute rest between rounds, the work to rest ratio ranged from 1:1.01 -1:1.27 (Kirk et al., 2016) with bout winners having more successful takedowns (2.5±3.21 per 15 minutes). MMA's W:R ratio sits in the middle of the striking and grappling spectrum. Mean lactate increased from 5.43±2.74 to 9.25±2.96 mmol/L after round 3. Competitive fights are more demanding on the body than sparring in MMA (Coswig et al., 2016). In a comparison between simulated sparring and official competitive matches in twenty-five male (26.5±5 y with 80±10 kg, 1.74±0.05) professional MMA fighters, glycolytic demand was higher in official matches (OFF) compared to simulated matches (SIM). This was measured by higher blood glucose concentrations (OFF= 6.1±1.2 mmol/L and SIM = 4.4±0.7 mmol/L) (Coswig et al., 2016). Notably, MMA matches produced higher lactate concentrations than Judo, BJJ, and Boxing (16 mmol/L (this is a median with a IQR of [(13.8 - 23.5), (all other measurements are mean and SD), 12.3±0.8 mmol/L, 11.6±1.1 mmol/L, and 13±2.0 mmol/L respectively). There were no differences found in biochemical markers (magnesium, lactate, glucose, total creatine kinase, aspartate aminotransferase, and alanine aminotransferase) between winners and losers in the official and simulated matches.

In a systemic review of 23 studies, albeit none of which were MMA, maximal strength was a greater predictor of successful outcome in grappling sports while in striking disciplines maximal force production is superior (James et al., 2016). Anaerobic or aerobic measures were reported in 19 articles and maximal strength or neuromuscular power variables were described in 16 investigations. The 5 combat sports represented in the studies were boxing, BJJ, wrestling, judo and karate. Across purely grappling combat sports anaerobic capabilities would largely distinguish higher from lower level athletes (James et al., 2016). Maximal strength was determined to be a greater predictor of performance outcomes in grappling sports as several studies showed a stratification of strength with different levels of competitors. 1RM bench press and 1RM smith machine squat was significantly greater in elite international wrestlers versus non-international wrestlers, in BJJ 1RM bench press was significantly higher in higher versus lower level athletes, and in Judo 1RM squat was significantly higher in international versus recreational athletes. Anaerobic capabilities showed a stratification of grappling sports across several studies. In wrestlers, lower-body mean power and upper-body relative mean power was significantly greater amongst higher-level competitors in an upper and lower body Wingate test. In an 8s abbreviated Wingate test in Judokas, mean power was significantly greater amongst higher-level competitors. Maximal force production was shown to predict performance in striking sports in karate as higher level competitors had significantly greater scores in counter movement jump and squat (James et al., 2016).

When the action is on the ground in MMA, BJJ is considered a large piece of the skill set. With regards to BJJ, there is a strong energy contribution from glycolytic metabolism (due to high peak blood lactate concentrations of 10.1±1.7 mmol/L) according to a study of twenty-one male BJJ athletes (27.8±8.0 y; 166±3.9 cm; 74.1±18.3 kg) (Abad et al., 2016). The high lactate concentrations are probably due to work to rest ratios of between 6:1 and 13:1 (Andreato et al., 2016).

Endurance, as defined in MMA by a high output of striking and takedowns, is a critical component in determining the outcome of a fight. James et al. (2017) obtained data from all male bouts in the UFC between July 2014 and December 2014 (234 total bouts) from the raw performance data supplied by the UFC's official statistics company, Fightmetrics. Analysis was performed by the authors on 13 key performance indicators and effect sizes. Their associated 95% confidence intervals were employed to determine the magnitude of the differences between Wins and Losses for each indicator in their rate-dependent and accuracy form. Results from the main effect size comparisons revealed differences between Wins and Losses for the majority of performance indicators. The actions that were the most influential in explaining the final outcomes in elite MMA bouts were total strikes landed per minute, total strikes attempted per minute, significant strikes landed per minute, significant strike accuracy, and significant ground strikes landed, while takedown accuracy also contributed (James et al., 2017).

As stated by the above research a high work to rest ratio is needed in MMA. Other factors that can impact on endurance are that competitions are more demanding than sparring (Amtmann et al., 2008), high lactate concentrations across all of MMAs sub disciplines (BJJ, Boxing and Judo) and higher work to rest ratios of ground work versus striking work. The effort/pause ratio during grappling matches was 6:1 to 13:1 (Andreato et al., 2016) while the activity-to-rest ratio was 1:1 in elite striking sports (Slimani et al., 2017). Since endurance is such a key component of successful outcomes in MMA, it is therefore very important that any study that measures the effects of RWL ensures that there is an endurance test as part of physical testing batteries.

Weight Making and RWL Specifically In MMA

RWL is a very common practice in MMA. In a sample of 179 Brazilian MMA athletes (Santos-Junior et al., 2020) 100% of athletes stated that they had engaged in weight loss procedures to make weight for competition. Crighton et al (2015) have raised concerns about some of the alarming practices in MMA such as consuming prescription and over-the-counter diuretics, extremely low energy diets, and consuming supplements without knowledge of what they were for. The following is an examination of research that has specifically looked at the effects of RWL in the sport of MMA.

Effect of RWL on Blood-based Biomarkers in MMA

In a comparison of 17 MMA athletes (age: 27.4 ±5.3 y; body mass: 76.2±12.4 kg; height: 1.71±0.05 m and training experience: 39.4±25 months) undergoing minimal weight loss versus RWL of up to 10% of body mass in official competitive matches, there were several significant differences noted between the groups (Coswig et al., 2015). There were 12 athletes in the non-RWL group and 5 athletes in the RWL group. The RWL group lost 7.4±1.1 kg in the 24 hours before their event and up to 10% of body mass the week leading up to the fight. Asparate Aminotransferase (AST) and Lactate Dehydrogenase (LDH) activity were all higher in the RWL group after the fights. LDH (median [interquartile range]; pre to post) (NWL= 211.5[183–236] to 231[203–258]U/L and RWL= 390[370.5–443.5] to 488[463.5–540.5]U/L) and AST (NWL= 30[22–37] to 32[22–41]U/L and 39[32.5–76.5] to 72[38.5–112.5] U/L). Creatinine was significantly lower in the RWL group before and after the matches (NWL= 101.6 ± 15–142.3 ± 22.9µmol/L and RWL= 68.9 ± 10.6–79.5 ± 15.9µmol/L). Lactate and cortisol showed no significant difference between the groups.

Over an 8-week period a reduction of 18.1% of body mass in a mixed martial artist severe changes in blood markers were noted (Kasper et al., 2019). The last phase of this weight cut (24 hours before the weigh-in until post weigh-in) led to a ~3-fold increase in plasma cortisol concentration to approximately 1500 nmol/L, sodium concentration to 148 mmol/L, and an increase to serum creatinine concentrations (53 µmol/L) consistent with acute kidney failure (>26 µmol/L).

Effect of RWL on Mood State in MMA

In addition to the physical effects of RWL, the psychological effects of extreme weight cutting cannot be ignored. For example, weight cutting negatively influences mood (Brandt et al., 2018). The mood states of anger, confusion, depression, fatigue, tension, vigour as well as total mood disturbance were assessed in professional male MMA athletes who used strategies to rapidly lose weight (n=9) and compared with MMA athletes who did not (n=3). The RWL group was associated with reporting higher confusion and greater total mood disturbance at each assessment point, than the group who did not practice RWL. RWL was also associated with high anger levels at the official weigh-in (Brandt et al., 2018). In another study on five male Brazilian MMA athletes no correlations were found between profile of mood and weight loss (Andreato et al., 2014). However, the athletes presented higher scores for vigour than for tension, depression and anger on the Brunel Mood Scale (Brums).

In a study of one hundred and forty-four Spanish national level judo athletes (66 females and 78 males, ages between 15 and 30 years) on a total of four psychological assessment tools, it noted that cadet and junior females are more likely to suffer from the psychological-related states associated to weight loss (Escobar-Molina et al., 2014). The Spanish versions of four psychological assessment tools were used: (i) State-Trait Anxiety Inventory, trait version (STAI-T), (ii) Food Craving Questionnaire-Trait [FCQ-T], (iii) Restraint Scale [RS], and (iv) Eating Attitude Test (EAT-40). STAI-T anxiety scores were higher for females (19.9±8.5) compared to males (17.5±6.8). EAT-40 scores indicated that females (16.4±13.1) had more eating disorders symptomatology than males (12.0±7.4).

Hydration Status during RWL in MMA

As described in an earlier section, a significant proportion of MMA fighters are not successfully rehydrating before competition and subsequently are competing in a dehydrated state (Jetton et al. 2013; Matthews & Nicholas, 2017).

In a study of 40 male MMA fighters where urinary measures of hydration status conducted approximately 24 hours before and then again approximately 2 hours before competition showed that fighters were in a severely dehydrated state at weigh-in, and were still considered dehydrated 2 hours before fighting, although the USG decreased from 1.028±0.001 to 1.020±0.001. Body mass increased by 3.4±2.2 kg or 4.4% in the approximately 22-hour period before competition.

Using urine osmolality as a means of measuring hydration status in seven male MMA athletes (mean ± SD, age 24.6±3.5 y, body mass 69.9±5.7 kg, competitive experience 3.1±2.2 y) at official weigh-ins (32 hours before competition), 57% of athletes were dehydrated (1033±19 mOsmol/kg) and the remaining 43% were severely dehydrated (1267±47 mOsmol/kg). When measured pre-competition 57% of athletes exhibited hyperhydration (108±38 mOsmol/kg), 29% were euhydrated, and 14% remained dehydrated (930 mOsmol/kg) (Matthews & Nicholas, 2017):

At the official weigh-in, and again one hour before the fight, saliva samples were taken to estimate the salivary osmolality in five Brazilian MMA athletes (age: 23±6 y, body mass: 76.9±7.7 kg, height: 1.79±0.03 m) (Andreato et al., 2014). The salivary osmolality did not show significant difference between weigh-in (55.6±30.7 mOsmol/kg H_2O) and pre-match moment (40.2±27.9 mOsmol/kg H_2O). This may have been accounted for as there was a high variation in the response for the different athletes, or because the time between weighing in and competition was long enough for recovery of this variable.

All twelve male amateur MMA athletes (age: 20.1±1.2 y, BM: 70.4±1.2 kg, height: 174.2±1.2 cm, and training experience: 4 yrs) at baseline were classified as well-hydrated (Alves et al., 2018). However, at the official weigh-in none of the athletes were classified as well-hydrated. The mean value of urine density at the official weigh-in was 1018.8±7.1 g/mL indicating dehydration. 9 subjects were classified as having minimal dehydration (75.0%), and 3 subjects had significant dehydration (25.0%). When tested at fight time, 2 subjects were well-hydrated (16.7%), 5 subjects had minimal dehydration (41.7%), and 5 subjects were significantly dehydrated (41.7%). At the time of the match the mean for urine density was 1019.2±9.3 g/mL.

The current evidence is that MMA athletes can be close to classification as being hypohydrated before they even start their weight cut, and are severely dehydrated at weigh-in time. After a recovery period most increase their hydration levels to a certain extent, but a large proportion are still in a hypohydrated state when competing.

How far weigh-in times are from competition, effects hydration levels too. Competitions that have weigh-ins the day before competitions have higher levels of hypohydration than competitions that have weigh-ins the morning of the competition. Combat sports that have weigh-in immediately before competition have the most hydrated athletes in comparison to combat sports with the above stated weigh-in times.

As regards to performance, as described in an earlier section, strength and power are more resilient to the effects of dehydration up to a 4% drop in body mass, and endurance performance can vary with drops of 2-4%. As described in previous sections, weight regain is important in combat sports, and in MMA specifically, weight regain has been shown to be a better indicator of winning outcomes in 15 MMA athletes than RWL (Coswig et al., 2018).

Hot Baths And Hot Salt Baths as an Approach to RWL

While the sections above describe the breadth of research on RWL across combat sports and MMA specifically, my next studies and therefore the remainder of this thesis ultimately focused on the use of hot bath and hot salt baths as a specific component of the RWL process. This was following the results of my survey on RWL practices outlined in Chapter 2.

Hot salt baths as a means of RWL has been observed among combat sports athletes (Brandt et al., 2018; Kasper et al., 2019; Matthews & Nicholas, 2017; Pettersson, et al., 2013). Exact protocols used to achieve RWL have not been reported. In my experience in the field there are no general or specific guidelines about hot bath protocols, the implementation of hot salt baths can therefore vary between different athletes.

Hot baths, as used by MMA fighters, do however broadly follow a general pattern. This pattern is as follows: 1. Sit in a hot bath, 2. Leave the bath (usually to "wrap" for a set period of time) 3. Repeat this 2 or 3 times or up to 10 times in the case of a severe weight cut (Kasper et al., 2019).

There are several factors involved in this process that need to be taken into consideration in the use of baths. These are the temperature of water, the duration of the time spent in the bath, the solution (or salt content) of the water, and how much of the body is immersed in the bath.

For total body immersion, water temperatures as low as 32ºC (Whitehouse et al., 1932) have been used. Fujishima (1986) used a 43º C temperature for full body immersion but the duration of the bath was only 8 minutes. 41º C has been the highest temperature for full body immersion used for an extended period of time (Brebner & Kerslake, 1964). The most common temperature range is however between 38-40º C. Higher temperatures for extended periods (60 min & 2 h respectively) have been used by Lee et al. (42º C) and Ogawa et al. (43ºC). Neither of these cases were however full body immersion, with one being submersion of both legs and calves and the other only arms submerged to the elbow respectively.

Fujishima (1986) bath of 8 minutes was the lowest duration of any hot bath study found in the literature with the longest being 5 hours (Whitehouse et al., 1932 and Brebner & Kerslake, 1964). Much variation exists about the duration of hot water immersion in the literature to date e.g. Alison & Rogers (1992) used 21 minutes, Zurawlew et al. (2016) used 40 minutes, Lee et al. (2011) used 60 minutes Kraft et al. (2011) did 2 hours.

The last component of the evidence is for salt as a means to augment body mass losses during hot water immersion. The mechanistic basis for why this effect has been observed is further explored in the Discussion sections of Chapters 4, 5, and 6.

Chapter 3 - Study 1

Published as:

Connor, J., & Egan, B. (2019). Prevalence, Magnitude and Methods of Rapid Weight Loss Reported by Male Mixed Martial Arts Athletes in Ireland. *Sports (Basel)* 7(9), 206. https://doi.org/10.3390/sports7090206

Abstract:

Rapid weight loss (RWL) is frequently practiced in weight category sports including Mixed Martial Arts (MMA). The aim of the present study was to evaluate self-reported methods of RWL in a sample of competitive MMA athletes comprising of both amateur and professional fighters. The previously-validated Rapid Weight Loss Questionnaire, with the addition of questions on water loading and hot salt baths, was completed anonymously online by athletes (n=30; all male, n=15/15 professional/amateur) from MMA clubs around Dublin, Ireland. All but one (97%) of athletes surveyed lost weight in order to compete, with the average weight loss being 7.9±3.1% of habitual body mass. The RWL score (mean±SD) for this sample was 37.9±9.6, and a tendency for higher [6.0 (95%CI; -1.1, 13.1)(P=0.093; d=0.64)] RWL scores for professional (40.8±8.9) c compared to amateur (34.8±9.6) athletes. Frequencies of "always" or "sometimes" were reported as 90% for water loading, 76% for hot salt baths and 55% for 24 h of fasting. Fellow fighters (41%) and coaches/mentors (38%) were "very influential" on RWL practices of these athletes, with doctors (67%), dieticians (41%), and physical trainers (37%) said to be "not influential". RWL is highly prevalent in MMA across both amateur and professional athletes, and RWL scores are higher than other combat sports. Water loading and hot salt baths are amongst the most commonly used methods of RWL despite little research on these methods for body mass reduction or effects on performance in weight category sports.

Introduction

Rapid weight loss (RWL) is frequently practiced in sports that have weight class restrictions (Barley, Chapman, & Abbiss, 2019; Franchini, Brito, & Artioli, 2012; Khodaee et al, 2015; Matthews et al, 2019; Reale, Slater, & Burke, 2017). Many of these sports include combat sports such as traditional Olympic sports, wrestling, judo, boxing and taekwondo, as well as other mainstream sports such as horse riding and rowing (Khodaee et al, 2015; Sundgot-Borgen, 2013). RWL generally refers to the methods employed by an athlete in reducing body mass in the range of 5% to 10% in the final one to two weeks before competition, and typically averages ~2% to 10% depending on the sport (Barley, Chapman, & Abbiss, 2019; Franchini, Brito, & Artioli, 2012; Khodaee et al, 2015; Matthews et al, 2019; Reale, Slater, & Burke, 2017). Subsequent to the weigh-in, combat sport athletes generally proceed to regain often the majority of this weight from within a few hours up to 36 hours before competing (Coswig et al, 2019; Matthews & Nicholas, 2017; Pettersson, Ekstrom, & Berg, 2013). RWL followed by rapid weight regain is employed, especially in combat sports, as a means of gaining a size and/or strength advantage over an opponent as the heavier fighter is generally seen to have an advantage (Coswig et al, 2019; Franchini, Brito, & Artioli, 2012; Matthews et al, 2019; Reale et al, 2016).

Mixed martial arts (MMA) is a combat sport comprised of styles of various martial arts and involves striking, grappling, wrestling and submission techniques (James et al, 2017). MMA athletes are required to compete under specific weight categories, namely: atomweight, 105 lbs (47.6 kg); strawweight 115 lbs (52.2 kg); flyweight, 125 lbs (56.7 kg); bantamweight, 135 lbs (61.2kg); featherweight, 145 lbs (65.8 kg); lightweight, 155 lbs (70.3 kg); welterweight, 170 lbs (77.1 kg);

middleweight, 185 lbs (83.9 kg); light-heavyweight, 205 lbs (93.0 kg); heavyweight, 205-265 lbs (93.0-120.2 kg); and super-heavyweight, no limit. In professional bouts for MMA, the timeline between weigh-ins and fight time can vary depending on the organisation sanctioning the fight. All professional organisations have weigh-ins on the day before the fight. For the majority of organisations, weigh-ins are at least 24 hours before the fight and up to 36 hours beforehand. The timeframe for amateur MMA fights again depends on the organisation sanctioning the bout. Many organisations will follow the same outline as the professional bouts on their card (24 to 36 hours before the fight), but under new rules set forth by the International Mixed Martial Arts Federation (IMMAF) weigh-ins for amateur fights are on the morning of competition.

MMA was established on the international stage as the Ultimate Fighting Championship (UFC) in 1993, but despite being one of the fastest growing international sports (Ko & Kim, 2010), only recently have reports begun to emerge on the weight-making practices of these athletes (Andreato et al, 2014; Coswig et al, 2019; Coswig, Fukuda, & Del Vecchio, 2015; Crighton, Close, & Morton, 2016; Jetton et al, 2013; Kasper et al, 2019; Matthews & Nicholas, 2017). One survey described MMA athletes losing 9±2% of body mass in the week before a fight, and a further 5±2% in the 24 h before weigh-in (Crighton, Close, & Morton, 2016). This is achieved due to employing one or all of the following methods: water loading, fluid restriction, prescription and over-the-counter diuretics, complete fasting or low carbohydrate diets in the final 3 to 5 days prior to weigh-in (Crighton, Close, & Morton, 2016). Such drastic methods for RWL result in 100% of athletes being dehydrated to various degrees at the official weigh-in (Jetton et al, 2013; Matthews & Nicholas, 2017) and 14% (Matthews & Nicholas, 2017) and 39% (Jetton et al, 2013) remaining dehydrated when measured in the final 2 h pre-fight.

Considering the increasing popularity of MMA, but documented adverse health outcomes and deaths attributed to RWL practices (Crighton, Close, & Morton, 2016; Kasper et al, 2019; Murugappan et al, 2019) the creation of bodies such as Safe MMA recognised that RWL practices may increase risk of injury and health consequences. Indeed, there have been calls to ban RWL in combat sports, partly because of the potential health risk to the athlete (Artioli et al, 2016). Conversely, the case has been made that a well-designed RWL strategy supported by appropriate recovery and weight regain strategies, when the time from weigh-in to competition allows, may confer a performance advantage (Reale, Slater, & Burke, 2017). While the data across weight category sports as a whole remain equivocal (Matthews et al, 2019; Reale, Slater, & Burke, 2017a), weight regain has been linked to a performance advantage in judo (Reale et al, 2016) and MMA (Coswig et al, 2019). Further studies are needed to characterise the prevalence and methods of RWL in MMA, with additional work then required to establish the safety, or otherwise of these methods. Therefore, the aim of the present study was to evaluate self-reported methods of RWL in a sample of competitive MMA athletes comprising of both amateur and professional fighters based in Dublin, Ireland.

Study design and participants

The study was approved by the Research Ethics Committee at the Dublin City University (DCU), Ireland (permit: DCUREC 2017_055) in accordance with the

Declaration of Helsinki. Participants, all of whom were male, were recruited from several MMA clubs around Dublin that are associated with Straight Blast Gym (SBG), the largest MMA gym franchise in Ireland. Participants were invited to participate in a survey of current and previous weight-making practices via the fighters' private page on Facebook. Participants clicked through via a link that gave them access to the anonymous online questionnaire. A participant information leaflet was presented on arrival, after which participants needed to consent via a tick box option in order to proceed to the questionnaire. Prior to commencing the questionnaire, RWL was defined to the participants as reducing body mass by 5 to 10% in seven days or less.

The private Facebook page, is for active fighters only (i.e. have previously competed and continuing to prepare for future fights), and has a membership of fifty athletes with an even split of amateur and professional fighters. Thirty athletes (60%) completed the online survey, with a final split (self-reported) of n=15 amateur fighters, and n=15 professional fighters. Professional and amateur fighters were categorised based on the rules set under which they fought at the time of the questionnaire being administered. The major distinctions between the respective groups are that amateur fights consist of 3 x 3 min rounds (compared to 3 or 5 x 5 min rounds in professional fights), and amateur fighters wear shin guards and a rash guard, and are not permitted to perform certain strikes and holds that are permitted under professional MMA rules. Even though there can be different rule sets in amateur and professional MMA with regards to regulations around the timing of weigh-in, all of the amateurs in this study competed under rules equivalent to professional MMA rules i.e. with a day before competition weigh-in rules.

Questionnaire

The questionnaire used in this study was a previously-validated Rapid Weight Loss Questionnaire (RWLQ) (Artioli et al, 2010b) with slight modifications. The questionnaire has demonstrated good stability, reliability and discriminant validity (Artioli et al, 2010a; Artioli et al, 2010b) having been conducted with a relatively large and heterogeneous sample, including competitors of both genders, a wide range of competitive levels and ages. This questionnaire was originally designed for the assessment of RWL in judo athletes, but was then modified and validated for other combat sports (Brito et al, 2012). Subsequently, the questionnaire has been modified and utilised for MMA athletes (Andreato et al, 2014; Barley, Chapman, & Abbiss, 2018; Coswig et al, 2019; Hillier et al, 2019; Matthews & Nicholas, 2017) and other combat sports (Da Silva Santos et al, 2016; Reale, Slater, & Burke, 2018). Similar to previous work (Reale, Slater, & Burke, 2018), our modifications were to change all instances of "judo" to the combat sport of interest to this study i.e. "MMA", and to add questions that better reflected current practices related to MMA such as water loading and hot salt baths (Barley, Chapman, & Abbiss, 2018; Brandt et al, 2018 Crighton, Close, & Morton, 2016; Hillier et al, 2019; Kasper et al, 2019; Matthews & Nicholas, 2017; Reale, Slater, & Burke, 2018). Specifically, we added the option to answer "hot salt baths" and "water loading" under the question "How often did you use each one of the following methods to lose weight before competition?" with same frequency options of always, sometimes, almost never, never used, and I don't use anymore. The questionnaire was recreated in Google Forms, and shared as a link to the aforementioned private Facebook page. The questionnaire was open for 8 weeks beginning April 1st 2017, with reminder requests for participation posted to the page once per fortnight.

Data analysis

The RWLQ was scored as described previously to produce a Rapid Weight Loss Score (RWLS) for each athlete and frequency analysis was performed where appropriate (Artioli et al, 2010b). Our additional questions on water loading and hot salt baths were not scored in the final calculation of RWLS. Therefore, the calculated RWLS remained directly comparable to other studies that employed the RWLQ. One amateur athlete indicated that he had never engaged in RWL and was excluded from the calculation of RWLS. Data were analysed and illustrated using PRISM v7 (GraphPad Software, USA). All data were assessed for normality using the Shapiro-Wilk test. For normal distributions, descriptive statistics are reported as mean±SD, and differences between groups were assessed using an independent samples t-test. For non-normal distributions, descriptive statistics are reported as median (interquartile range) (IQR), and differences between groups were assessed using a Mann-Whitney U test. The significance level was set at $\alpha=0.05$ for all tests. Differences between groups are reported as mean (lower 95% confidence interval, upper 95% confidence interval). Effect size was calculated using Cohen's d and interpreted using thresholds of <0.2, ≥0.2, ≥0.5 and ≥0.8 for trivial, small, moderate, and large, respectively.

Results

Of the n=30 athletes surveyed, respondents had, on average, 4.7±2.7 y of experience of formally competing in MMA (Table 3.1), and all but one athlete (97%) had previously engaged in RWL in preparation for competition. The percentage of habitual body mass usually lost in the overall weight cut preparation for a fight averaged 7.9±3.1%, and 100% of this weight loss was usually regained in the week after a fight (Table 3.1). In this cohort, the amateur fighters had a lower body mass index (23.6±1.8 vs. 25.0±2.2 kg m-2; P=0.030; d=0.70), and tended to have less years of competitive experience (3.8±2.6 vs. 5.6±2.6 y; P=0.067; d=0.69).

Table 3.1: Participant characteristics

	All (n= 30)	**Amateur (n=15)**	**Professional (n=15)**	**Amateur vs. Professional P value**
Age (y)	25.5±4.4	24.3±4.2	26.7±4.4	
Years of experience competing in MMA (y)	4.7±2.7	3.8±2.6	5.6±2.6	0.067
Weight category	AW, n=0: SW, n=0; FLW, n=2; BW, n=4; FEW, n=4; LW, n=10; WW, n=6; MW, n=3; LHW, n=0; HW, n=1	AW, n=0: SW, n=0; FLW, n=1; BW, n=2; FEW, n=1; LW, n=6; WW, n=5; MW, n=0; LHW, n=0; HW, n=0	AW, n=0: SW, n=0; FLW, n=1; BW, n=2; FEW, n=3; LW, n=4; WW, n=1; MW, n=3; LHW, n=0; HW, n=1	
Habitual body mass (kg)	78.3±9.7	76.3±7.3	80.3±11.5	0.257
Height (m)	1.79±0.07	1.80±0.07	1.79±0.08	0.743
Habitual body mass index (kg m-2)	24.3±2.1	23.6±1.8	25.0±2.2	0.030
Fights in previous 12 months	2.5 (1.0, 3.3)	2 (1, 4)	1 (1, 3)	0.853

Usual weight cut (% of current body mass)	7.9±3.1	7.2±3.4	8.6±2.8	0.397
Usual weight regain in week after fight (% of weight cut)	100 (85, 133)	100 (80, 131)	100 (91, 133)	0.612

The RWLS for this sample of MMA athletes was 37.9±9.6 (Figure 3.1). Comparison of RWLS across codes revealed a tendency for higher RWLS [6.0 (-1.1, 13.1); P=0.093] for professional (40.8±8.9) compared to amateur (34.8±9.6), with the magnitude of effect interpreted as 'moderate' (d=0.64) (Figure 3.1).

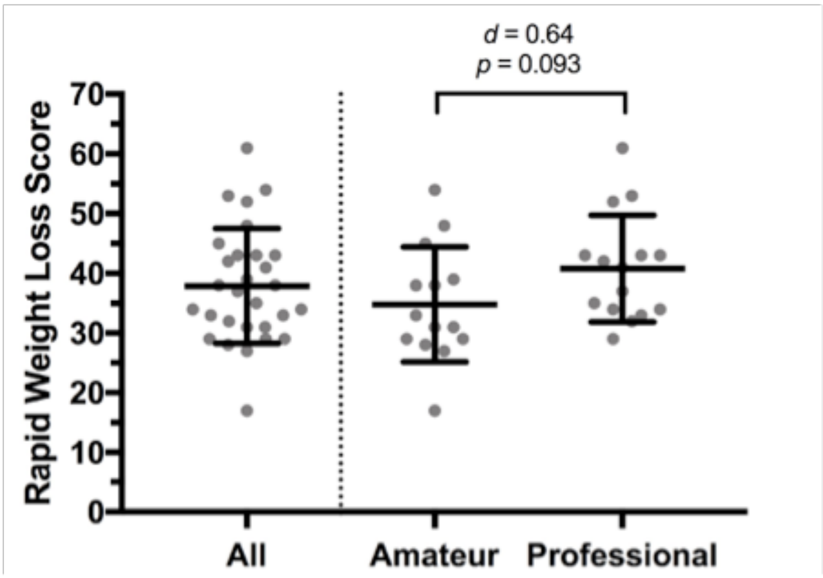

Figure 3.1. Rapid Weight Loss Score obtained by the RWLQ from the group as a whole (All, n=29), and based on self-reported status as Amateur (n=14) or Professional (n=15). Data bars are mean values with error bars representing standard deviation.

While energy restriction strategies (i.e. gradual dieting, fasting) are frequently used, methods that reduce body water stores (i.e. water loading, fluid restriction, and hot salt baths) are also commonly employed for RWL by this cohort (Table 3.2). Water loading was the most commonly used method for RWL with 90% of the athletes using water loading "sometimes" or "always". Of those that used water loading, 70% of the athletes start water loading between 5 and 8 days out from the weigh-in. When using water loading, 70% of the athletes consumed between 6 and 9 L of water for the high water intake days. Fluid restriction was used "sometimes" or "always" by 79% of the athletes, with 75% of this number employing the method at between 1 and 24 hours prior to weigh-ins. Hot salt baths are commonly used, with 76% of athletes using the method "always" or "sometimes", compared to 48% of the athletes "always" or "sometimes" using saunas to dehydrate. Gradual dieting was used "sometimes" or "always" by 76% of the athletes, in addition to fasting for 24 h being used "sometimes" or "always" by 55%. Using winter or plastic suits, spitting, laxatives, diuretics, diet pills, and vomiting were the RWL methods that were least commonly used in this cohort.

Table 3.2. Frequency analysis of the weight loss methods reported by the mixed martial arts athletes (N=29)

Method	Always (%)	Sometimes (%)	Almost Never (%)	Never (%)	Do Not Use Anymore (%)
Gradual dieting	62.1	24.1	6.9	0.0	6.9
Skipping one or two meals	20.7	27.6	24.1	17.2	10.3
Fasting	31.0	24.1	10.3	24.1	10.3
Restricting fluids	62.1	17.2	10.3	6.9	3.4
Increased exercise	34.5	31.0	13.8	17.2	3.4
Heated training rooms	13.8	34.5	3.4	48.3	0.0
Sauna	27.6	20.7	27.6	10.3	13.8
Hot salt baths	34.5	41.4	20.7	3.4	0.0
Training with rubber/plastic suits	31.0	13.8	20.7	24.1	10.3

Using winter or plastic suits	0.0	3.4	0.0	96.6	0.0
Spitting	10.3	17.2	6.9	65.5	0.0
Laxatives	3.4	17.2	3.4	72.4	3.4
Diuretics	3.4	3.4	0.0	89.7	3.4
Diet pills	0.0	3.4	0.0	86.2	10.3
Vomiting	0.0	0.0	0.0	100.0	0.0
Water loading	62.1	27.6	3.4	6.9	0.0

In the present cohort, athletes receive the majority of their advice about weight-making methods from fellow fighters and their coaches/mentors (Table 3.3). Fellow fighters were "very influential" to 41% of the athletes, and coaches/mentors were "very influential" to 38% of the athletes in their weight-making practices, with these sources being "somewhat influential" to another 31% and 24% of the athletes, respectively. Very little influence was provided health and fitness professionals. Doctors, dieticians and physical trainers were said to be "not influential" by 67% 41%, and 37% of the athletes, respectively.

Table 3.3. Frequency analysis of the individuals who are influential on the weight-making practices reported by the mixed martial arts athletes

Source	Very Influential (%)	Somewhat Influential (%)	Unsure (%)	A Little Influential (%)	Not Influential (%)
Online/written Material	18.5	14.8	14.8	14.8	37.0
Fellow fighter/ training colleague	41.4	31.0	17.2	3.4	6.9
Physician/ doctor	3.7	7.4	3.7	18.5	66.7
Physical trainer	14.8	11.1	29.6	7.4	37.0
Coach/mentor	37.9	24.1	20.7	6.9	10.3
Parents	0.0	3.7	3.7	11.1	81.5
Dietitian	14.3	14.3	10.7	14.3	46.4

Discussion

The present study establishes that a variety of methods for RWL are widely used by MMA athletes at amateur and professional levels. In addition to energy restriction by gradual dieting and short-term fasting, the methods most commonly being employed by this Irish cohort are those that reduce body water stores i.e. water loading, fluid restriction, and hot salt baths. Even discounting water loading and hot salt baths, RWL scores were higher in these athletes than those reported in other combat sports, and a tendency existed for higher RWL scores in professional compared to amateur fighters. Fellow fighters and coaches are the dominant sources of information on methods of RWL in this cohort of athletes.

Despite the increasing popularity of MMA (Ko & Kim, 2010), and the concerns expressed around the safety of weight-making practices in the sport (Artioli et al, 2016; Crighton, Close, & Morton, 2016), there has been a scarcity of studies describing the prevalence and magnitude of RWL by these athletes, or indications of the personnel who are influencing these practices. During the execution of the present study, two other reports emerged describing weight-making practices in MMA in athlete cohorts of n=70 (Barley, Chapman, & Abbiss, 2018) and n=314 (Hillier et al, 2019). The findings of these studies are largely confirmed in our study, but in addition we report an estimate of prevalence of the use of hot salt baths by MMA athletes.

Hot baths generally describe the practice of hot water immersion (e.g. >38°C), and supported by “wrapping” in warm towels or bedclothes for period of time prior to further exposures to hot water immersion (Kasper et al, 2019). As part of the hot bath protocol, fighters will often add Epsom salt (magnesium sulfate) with the prevailing wisdom that this addition elicits greater loss of body mass through sweating-induced dehydration. Indeed, the addition of a salt to a hot water immersion to produce greater body mass loss does have some empirical evidence to support its practice (Hope, Aanderud, & Aakvaag, 2001). Hot baths/hot salt baths have been briefly mentioned as part of weight-making practices in a number of case and small cohort studies (Brandt et al, 2018; Kasper et al, 2019; Matthews & Nicholas, 2017; Pettersson, Ekstrom, & Berg, 2013), but to date their prevalence in a larger cohort has not been documented. In the present cohort, 76% of the athletes reporting using hot salt baths "always" or "sometimes", with one only athlete reporting to have "never" used them. Clearly, there is a need for future work to explore the detailed protocols, and outcomes of this method for RWL given this prevalence.

Like other work (Barley, Chapman, & Abbiss, 2018; Hillier et al, 2019) methods that reduce body water stores (i.e. water loading, fluid restriction, and hot salt baths) are the most commonly employed methods for RWL by this cohort. All but one (97%) of the n=30 of those surveyed lost weight in order to compete, with water loading being the most prevalent method employed at a frequency of "always" or "sometimes" in 90% of respondents. The high prevalence of RWL is consistent with other reports in MMA athletes (Barley, Chapman, & Abbiss, 2018; Hillier et al, 2019), and is greater than that reported, on average, in other combat and weight category sports (Barley, Chapman, & Abbiss, 2018; Brito et al, 2012; Reale, Slater, & Burke, 2018). The prevalence of RWL varies considerably between the various combat and weight category sports, with a number of reviews summarising the prevalence as between 50% and 80% (Barley, Chapman, & Abbiss, 2019; Franchini, Brito, & Artioli, 2012; Matthews et al, 2019). Combat sports tend to report a higher prevalence of RWL compared to other weight category sports (Barley, Chapman, & Abbiss, 2019; Franchini, Brito, & Artioli, 2012; Matthews et al, 2019), and the prevalence of RWL in MMA is generally >95% of athletes (Barley, Chapman, & Abbiss, 2018; Hillier et al, 2019). Similarly, the prevalence of water loading observed in MMA athletes in the present study, and by others (Barley, Chapman, & Abbiss, 2018; Hillier et al, 2019), appears to be higher than the prevalence of water loading reported in other combat sports (Barley, Chapman, & Abbiss, 2018; Reale, Slater, & Burke, 2018). Differences in methods of RWL between sports is not solely limited to methods to reduce body water stores; for example, the use of fasting "always" or "sometimes" was only reported by 24% of boxers compared to 70% of wrestlers. The specific reasons for differences in methods of RWL between other combat and weight category sports remains to be explored. Several factors are likely to be at play including the culture of the sport itself, the number of weight categories, and the duration of the time period between weigh-in and competition (Barley, Chapman, & Abbiss, 2019; Franchini, Brito, & Artioli, 2012; Matthews et al, 2019; Reale, Slater, & Burke, 2017a).

The level of competition, calibre of athlete and/or professional status have been observed to varying degrees to be influencing factors in the prevalence and/or magnitude of RWL in several studies (Artioli et al, 2010a; Barley, Chapman, & Abbiss, 2018; Reale, Slater, & Burke, 2018), i.e. higher prevalence of RWL, greater % body mass lost, and/or higher RWL scores were associated with more elite performers. A similar tendency was noted in the present study, with a moderate effect size observed for higher RWL score in the professional fighters. The RWL score is an outcome based on scoring of the RWLQ as described by the original validation papers (Artioli et al, 2010a; Artioli et al, 2010b) which allow for direct comparison between studies. The RWL score for this sample of MMA athletes was 37.9±9.6, which is higher than scores of ~31 reported in boxing, judo, taekwondo, and wrestling (Reale, Slater, & Burke, 2018).

This scoring system and calculated RWL scores do not include a weighting attributed to water loading or hot salt baths, which are common practices by MMA athletes. Whether these methods are commonly used in other combat sports, or whether the prevalence of hot baths reported herein is similar in other MMA cohorts, remains to be confirmed. Nevertheless, separate to the RWL scoring system, it is generally accepted that the %body mass lost as part of the RWL process in greater in MMA than other sports (Barley, Chapman, & Abbiss, 2019; Matthews et al, 2019). In other combat sports, the %body mass lost during averages ~2% to 6% (Barley, Chapman, & Abbiss, 2018; Brito et al, 2012; Reale, Slater, & Burke, 2018), whereas the average is ~5% to 10% in MMA (Barley, Chapman, & Abbiss, 2018; Coswig et al, 2019; Coswig, Fukuda, & Del Vecchio, 2015; Crighton, Close, & Morton, 2016; Hillier et al, 2019; Matthews & Nicholas, 2017). The 7.9±3.1% reported by our cohort is, therefore, consistent with the magnitudes in the latter studies cited.

Fellow fighters and coaches/mentors were the most influential sources of information for weight-making practices in this cohort of MMA athletes, whereas health and fitness professionals such as doctors, dieticians and physical trainers are generally reported to have limited influence. This finding is not exclusive to MMA, and in fact, is widely reported across a range of combat and weight category sports (Artioli et al, 2010a; Barley, Chapman, & Abbiss, 2018; Hillier et al, 2019; Reale, Slater, & Burke, 2018). Whether it is possible to overcome ingrained practices in a sport such as MMA remains to be seen, but support staff should be aware of these key influencers of the practices of their athletes. Governing bodies should consider formal education modules for their coaches and athletes on the potential health, safety and performance consequences of methods for RWL.

Aside from the limitations generally associated with self-reported data, another limitation that must be acknowledged in the present study is that the cohort of athletes surveyed were part of the same larger MMA franchise, SBG. Although the athletes trained in several different MMA gyms, the convenience sampling approach using the internal social media pages likely resulted in the recruitment of athletes with largely similar coaching and support staff. While circulation of nutrition and weight-making advice is not a feature of the social media page, given the described influence of coaches and fellow fighters, on methods of RWL, this sampling approach may have introduced a bias to the results. Specifically, the finding of a high prevalence of hot salt bath use will need to be confirmed in other MMA cohorts. However, the overall results in terms of prevalence, magnitude and methods of RWL are largely similar to that of surveys of larger MMA cohorts

(Barley, Chapman, & Abbiss, 2018; Hillier et al, 2019).

Therefore, we conclude that manipulation of body water stores through water loading, fluid restriction and hot salts baths, and in addition to gradual dieting and short-term fasting, are the most common methods of RWL employed by MMA athletes. Given the greater degree of RWL in MMA compared to other sports, whether measured by prevalence, % body mass loss or RWL score, there is a need for research on the physiological responses to these methods of RWL in addition to understanding the safety and performance characteristics of athletes who have undertaken aggressive weight regain strategies subsequent to these weight-making practices. Such research will benefit fighters, coaches and administrators alike in developing evidence-based practices, recommendations and policies for the sport.

Chapter 4 - Study 2

Published as:

Connor, J., Shelley, A., & Egan, B. (2020). Comparison of hot water immersion at 37.8°C with or without salt for rapid weight loss in mixed martial arts athletes. *Journal of Sports Sciences* 38(6), 607–611. https://doi.org/10.1080/02640414.2020.1721231

Abstract

Objectives: Hot water immersion, known as a hot bath, is common practice in MMA athletes to produce rapid weight loss (RWL) by means of passive fluid loss. This study investigated the magnitude of body mass losses using a standardised hot bath protocol with or without the addition of salt.

Methods: In a crossover design, eleven male MMA athletes (26.3±4.0 y; 1.77±0.08 m; 74.5±5.3 kg) performed a 20 min immersion at 37.8°C followed by a 40 min wrap in clothing in a warm room. This bath and wrap was performed twice per visit. During one visit, only fresh water was used (FWB), and in the other visit, Epsom salt (magnesium sulfate; 2.5% wt/vol) was added to the bath (SWB). Prior to each visit, 24 h of carbohydrate, fibre and fluid restriction were undertaken as part of the RWL protocol.

Results: Body mass losses induced by the hot bath protocols were 1.63±0.75 kg and 1.60±0.80 kg for FWB and SWB, respectively (P=0.825 between trials), and equivalent to ~2.1% body mass. Total body mass losses induced by the entire RWL protocol were 3.92±1.22 kg and 3.84±1.35 kg for FWB and SWB, respectively (P=0.756 between trials). All but one participant reported immersion at 37.8°C to be colder than the bathing that they usually employ.

Conclusions: Under the conditions employed, the magnitude of body mass lost in SWB was similar to FWB. However, further research should explore bathing in a

temperature that is consistent with that habitually used by fighters, and/or higher concentrations of salt.

Introduction

Rapid weight loss (RWL) is frequently practiced in sports that have weight class restrictions (Khodaee et al, 2015; Reale, Slater, & Burke, 2017a), including combat sports such as mixed martial arts (MMA) (Barley, Chapman, & Abbiss, 2019; Matthews et al, 2019). The weight-making practices of MMA athletes has recently been a subject of much interest (Andreato et al., 2014; Barley, Chapman, & Abbiss, 2018; Connor & Egan, 2019; Coswig, Fukuda, & Del Vecchio, 2015; Coswig et al., 2019; Crighton, Close, & Morton, 2016; Hillier et al., 2019; Jetton et al., 2013; Kasper et al., 2019; Matthews & Nicholas, 2017). Notably, the prevalence and magnitude of the RWL process is greater in MMA than other combat and weight category sports (Barley et al., 2019; Matthews et al., 2019), with the %body mass loss usually ~5% to 10% in the week prior to competition (Barley, Chapman, & Abbiss, 2018; Coswig et al., 2015, Crighton, Close, & Morton, 2016; Hillier et al., 2019; Matthews & Nicholas, 2017). At both professional and amateur levels, these athletes are using strategies that reduce body water stores (e.g. water loading, fluid restriction, and increasing sweat losses through heat exposure) as the predominant methods of RWL (Barley, Chapman, & Abbiss, 2018; Connor & Egan, 2019; Hillier et al., 2019).

A means of passive fluid loss known as hot baths has been briefly mentioned as part of weight-making practices in a number of case and small cohort studies (Brandt et al, 2018; Kasper et al, 2019; Matthews & Nicholas, 2017; Pettersson, Ekstrom, & Berg, 2013). We recently identified hot baths as a highly prevalent method of RWL in MMA athletes with 76% of a cohort of n=29 male fighters reporting using hot baths "always" or "sometimes" (Connor & Egan, 2019). Hot baths generally describe the practice of hot water immersion followed by wrapping in warm clothing for period of time prior to further exposures to hot water immersion (Kasper et al, 2019). As part of the hot bath protocol, fighters will often add Epsom salt with the prevailing wisdom that this addition elicits greater loss of body mass through sweating-induced dehydration. The addition of salt to a hot water immersion to produce greater body mass loss does have some empirical evidence to support its practice (Hertig, Riedesel, & Belding, 1961; Hope, Aanderud, & Aakvaag, 2001). For example, 4 h of immersion up to the neck in 38ºC water produced ~0.6 kg more body mass loss in seawater (~2.5 kg/4 h) compared to fresh water (~1.9 kg/4 h) (Hope, Aanderud, & Aakvaag, 2001). While the loss of body mass by hot water immersion is primarily through sweating-induced dehydration, the addition of salt increases the osmotic pressure difference between the immersion medium and body fluids, which likely contributes to the greater fluid loss compared to fresh water (Hertig, Riedesel, & Belding, 1961; Hope, Aanderud, & Aakvaag, 2001; Whitehouse, Hancock, & Haldane, 1932). However, a comparison of fresh versus salt water immersion has not been investigated in an athletic population as part of RWL practice.

Therefore, the aim of the present study was to determine the magnitude of body mass losses in MMA athletes using a standardised hot bath protocol, with or without the addition of Epsom salt.

Methods

Participants

Eleven male professional MMA athletes (age, 26.3±4.0 y; height, 1.77±0.08 m; body mass, 74.5±5.3 kg) with previous experience of RWL provided written informed to participate. The study was approved by the Human Research Ethics Committee of Dublin City University (permit number: DCUREC/2019/021).

Design

A repeated measures, crossover design was employed to compare the effects on passive fluid loss of hot water immersion under conditions of fresh water bathing (FWB) compared to salt water bathing (SWB). Participants performed two main experimental trials separated by at least seven days, with the trials being identical with the exception of the water condition in which they were immersed. The bathing protocol comprised of 20 min of hot water immersion ("bath") followed by 40 min wrapped in heavy clothing and blankets in a warm room ("wrap"). This 60 min bath and wrap protocol was repeated twice per main experimental trial i.e. 2 h total. On the day prior to bathing, participants were prescribed to eliminate carbohydrate and fibre-rich foods from their diet and consume 22 kcal/kg body mass. Fluid intake was prescribed to be restricted to 15 mL/kg for the 24 hours before bathing.

Change in body mass, measured to the nearest 0.05 kg (model #63667; Soehnle, Germany), was the primary outcome measure. Body mass was measured in minimal clothing at several time-points: (i) upon waking on the day prior to bathing (Morning Day -1), (ii) upon waking on the day of bathing (Morning Day 0), (iii) immediately prior to the first bath, (iv) immediately before the second bath, (v) immediately after the second wrap, and finally, (v) upon waking on the day after bathing (Morning Day +1).

Urine osmolality was measured (Osmocheck Portable Osmometer; Vitech Scientific, UK) at the same time points except immediately before the second bath and wrap. Participants were defined as dehydrated using a criteria of urine osmolality of >700 mOsmol/kg (Sawka et al, 2007).

Methodology

For each bath, participants were submerged up to the neck for 20 min bath at 37.8ºC. A floating thermometer (Avent Bath & Room Thermometer; Philips, UK) was checked frequently and the bath was topped up with hot water as needed to maintain the target temperature.

After 20 min of bathing, participants dried off in the bathroom and as quickly as possible put on a beanie, cotton t-shirt, hoodie, tracksuit bottoms and socks. Participants were then covered in blankets on a bed in an adjacent room with only their face exposed. This wrap was performed for 40 min. This 60 min bath and wrap protocol is considered one round and was repeated twice per main experimental trial.

Upon completion of the second round, participants began the weight regain process and were prescribed to consume fluids (in L) to the equivalent to 150% of total body mass lost (in kg) (Sawka et al, 2007) from Morning Day -1, and to consume 6 g/kg body mass of carbohydrate throughout the rest of the day.

For the FWB trial, only fresh tap water was used in the bath. For the SWB trial, Epsom salt (magnesium sulfate) were added to the bath at a concentration of 2 kg in 80 L of water (i.e. ~2.5% wt/vol).

Upon completion of the second trial, each participant completed a questionnaire examining their experiences of the study and their habitual practices of hot baths for RWL.

Statistical Analysis

Statistical analysis and graphical representation were performed using GraphPad Prism v8.1 (GraphPad Software, Inc., USA). Normality of data was assessed with the Shapiro-Wilk normality test, for which all data passed. All data are presented as mean±SD. A two way (condition x time) repeated measures analysis of variance (ANOVA) was used to assess responses to the interventions. When a main or interaction effect was observed, pairwise comparisons were performed with Bonferroni's correction for which multiplicity-adjusted p-values are reported. The level of significance for all tests was set at $P<0.05$. Standardised differences in the mean were used to assess magnitudes of effects between conditions. These were calculated using Cohen's d effect size (ES) and interpreted using thresholds of <0.2, ≥0.2, ≥0.5 and ≥0.8 for trivial, small, moderate, and large, respectively.

Results

For change in body mass in absolute (kg) (Table 4.1) and relative (%initial body mass) (Figure 4.1) terms, a main effect of time (P<0.001), but neither a main effect of condition, nor a condition*time interaction effect, was observed. Similarly, there was no difference between conditions for changes in urine osmolality at the various time points (Table 4.1).

Table 4.1. Body mass (kg) and hydration status assessed by urine osmolality (mOsmol/kg) at time points during a rapid weight loss protocol featuring a hot bath protocol in fresh (FWB) or salt water (SWB).

	Morning Day -1	**Morning Day 0**	**Before 1st bath**	**After 1st bath & wrap**	**After 2nd bath & wrap**	**Morning Day +1**	P value
Body mass (kg)							Time, P = 0.001***
FWB	82.49±9.14	80.69±8.55	80.20±8.66	79.36±8.75	78.57±8.73	81.55±8.31	Condition, P = 0.271
SWB	82.04±9.12	80.28±9.00	79.79±8.75	79.05±8.92	78.20±8.98	81.34±8.82	Interaction, P = 0.817
Urine osmolality (mOsmol/kg)							Time, P = 0.004**
FWB	693±235	894±137	845±115		928±78	796±219	Condition, P = 0.468
SWB	637±204	871±163	852±157		925±214	750±314	Interaction, P = 0.737

Data are presented as mean±SD, n = 11. **P < 0.01; ***P < 0.001.

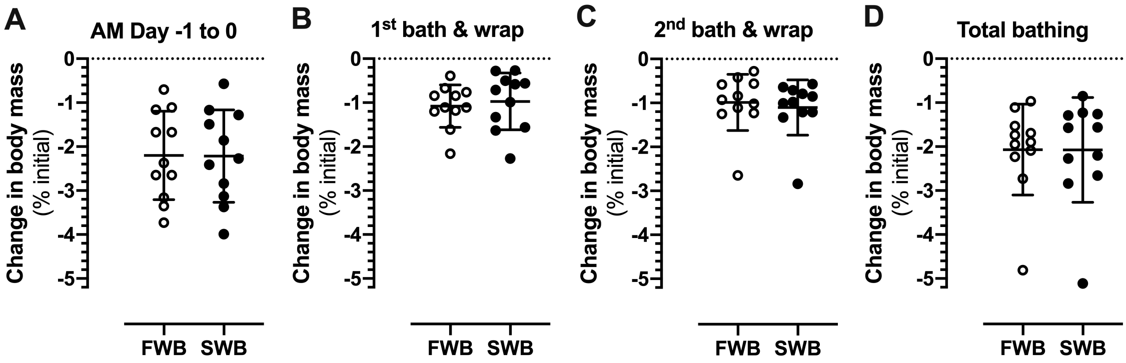

Figure 4.1. – Percentage changes in body mass (relative to baseline) induced by diet and fluid restriction, and a hot bath protocol in fresh (FWB) or salt water (SWB). Data are mean±SD.

Body mass losses induced by carbohydrate and fluid restriction were 2.29±0.82 kg (P<0.001; d=0.26) and 2.25±0.86 kg (P<0.001; d=0.25) in preparation for the FWB and SWB trials (P=0.881 between trials), respectively. Body mass losses induced by the hot bath protocols were 1.63±0.75 kg (P<0.001; d=0.20) and 1.60±0.80 kg (P<0.001; d=0.20) for the FWB and SWB protocols (P=0.825 between trials), respectively.

Total body mass losses induced by the entire RWL protocol were 3.92±1.22 kg (P<0.001; d=0.44) and 3.84±1.35 kg (P<0.001; d=0.42) for the FWB and SWB protocols (P=0.756 between trials), respectively. These values represented losses of initial body mass of 4.27±1.50% and 4.29±1.84% for the FWB and SWB protocols, respectively (Figure 4.2A).

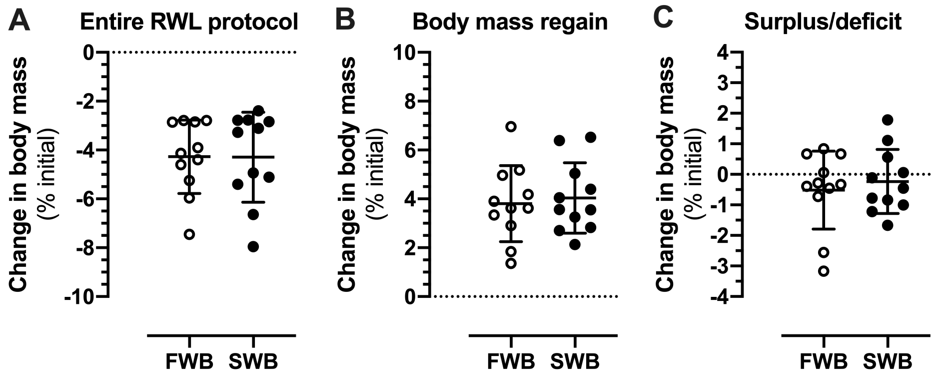

Figure 4.2. – Percentage changes in body mass (relative to baseline) during (A) the entire rapid weight loss protocol featuring a hot bath protocol in fresh (FWB) or salt water (SWB), (B) the period of weight regain prior to weigh-in, and (C) as a measure of total body fluid deficit or surplus at weigh-in. Data are mean±SD.

Weight regain was 2.97±1.15 kg (P<0.001; d=0.35) and 3.14±1.04 kg (P<0.001; d=0.35) during recovery from the FWB and SWB protocols (P=0.508 between trials), respectively, resulting in a body fluid deficit of 0.95±1.06 kg and 0.70±1.03 kg (P=0.503 between trials), respectively. At Morning Day +1, 10 (FWB trial) and 8 (SWB trial) participants were in a body fluid deficit compared to Morning Day -1, and 9 (FWB trial) and 6 (SWB trial) participants were defined as dehydrated (urine osmolality >700 mOsmol/kg).

Exit questionnaires were completed by the 10 of the 11 participants. Eight out of 10 participants used hot baths or hot salt baths “always” or “sometimes” as part of the RWL process, with 6 out of these 8 participants usually spending 11 to 20 min immersed in hot water, 6 out of 8 participants usually spending 11 to 30 min wrapped in warm towels/bed clothes, and 6 out of 8 participants usually repeating the bath and wrap twice.

Body mass loss during the bath and wrap process was reported as usually being 1.1 to 1.5 kg. One participant reported a usual weight loss of 5.1 to 5.5 kg, with this individual reporting using two 60 min hot water immersions separated by a 15 min wrap. Another participant reported a usual weight loss of 3.6 to 4.0 kg, with this individual reporting using a 15 min hot water immersions followed by a 60 min wrap repeated for two rounds.

All but one participant found our bathing protocol at 37.8ºC to be colder than the hot water immersion that they usually employ, but only two participants reported using a thermometer to measure the water temperature as part of their usual practice. All participants reported increasing the water temperature throughout each immersion, either using hot tap water or boiled kettle water.

All but one participant reported adding salt to their hot baths, each of whom used Epsom salt, with the average quantity being 1 to 2 kg of salt. One participant reported using the salts for “muscle relaxation”, whereas the remaining participants reported adding salts because they were led to believe that it enhanced the weight cutting effect of a hot bath.

Discussion

This is the first study to describe a standardised hot bath protocol in MMA athletes, and investigate if adding salt to hot water immersion at 37.8ºC increases body mass loss during a RWL protocol. The main finding is that the body mass loss when bathing in a hot bath of fresh water (FWB) is similar to bathing in a hot bath with ~1.6% Epsom salt added (SWB).

The absence of difference between body mass loss during FWB compared to SWB is in contrast to previous work demonstrating ~32% greater body mass loss over 4 h of immersion at 38ºC in seawater compared to fresh water (Hope, Aanderud, & Aakvaag, 2001). The differences between the study protocols are most obviously the duration (4 h continuous immersion versus the present 2x 20 min bath/40 min wrap protocol), the salt concentration (seawater being ~3.5% salt versus ~1.6% in our protocol), and the type of salt (seawater versus added Epsom salt). Whether the latter would make any difference to the outcome remains to be explored, but is unlikely. The contention is that in salt water immersion, the osmotic pressure difference between the immersion medium and body fluids results in greater fluid loss compared to fresh water (Hertig, Riedesel, & Belding, 1961; Hope, Aanderud, & Aakvaag, 2001; Whitehouse, Hancock, & Haldane, 1932). Such a difference was not observed in the present study, wherein body mass loss in both FWB and SWB trials averaged ~1.6 kg, or 2.1% body mass. However, the concentration of salt in the hot bath is an important factor to consider in this context. The present protocol employed a salt concentration of ~1.6% wt/vol magnesium sulfate. This quantity and type of salt was chosen based on our personal experience of working with combat sport athletes during weight-making efforts, and was confirmed during exit interviews to be the usual quantity and type of salt per bath used by this cohort of fighters. Early work established that even in thermoneutral water i.e. in the absence of sweating, immersion in a strong salt solution (either 11.5% or 20.0% salt as sodium chloride) produces passive fluid loss (Whitehouse, Hancock, & Haldane, 1932). In water heated to 36/37ºC, addition of 5% sodium chloride allowed for higher sweat rates during 3 h of immersion when compared to fresh water (Hertig, Riedesel, & Belding, 1961). This effect was more pronounced at salt concentrations of 10% and 15%, with the authors suggesting that the salt did not serve as a stimulus for sweating, but rather served to remove an inhibitory influence on the decline in sweat rate that usually occurs with prolonged immersion in fresh water (Hertig, Riedesel, & Belding, 1961). Therefore, it may be that the concentration of salt in a hot bath should at least 3.5% (Hope, Aanderud, & Aakvaag, 2001), or possibly greater (Hertig, Riedesel, & Belding, 1961), if the aim is to augment the rate of passive fluid loss that would otherwise occur in fresh water.

Notably, there was a greater loss of body mass by the 24 h of restriction of carbohydrate, fibre and fluid (~2.2 kg), than from either bathing protocol (~1.6 kg). This magnitude of body mass loss is consistent with the suggestion of a ~3% reduction in body mass to be expected by short duration glycogen depletion and emptying of the intestinal contents (Reale, Slater, & Burke, 2017b).

All participants were classified as dehydrated when measured after the second wrap, a time point selected to be representative of weigh-in time for these fighters. This is consistent with typical methods of RWL resulting in 100% of MMA athletes being dehydrated to various degrees at an official weigh-in (Jetton et al., 2013; Matthews & Nicholas, 2017). For example, in preparation for a competitive bout, 57% and 43% of fighters were reported to be dehydrated (1033±19 mOsmol/kg) and severely dehydrated (1267±47 mOsmol/kg), respectively, at weigh-in

(Matthews & Nicholas, 2017). Moreover, 14% (Matthews & Nicholas, 2017) and 39% (Jetton et al., 2013) of fighters remained dehydrated when measured in the final 2 h prior to a competitive fight. In the present study, after a 20 hour recovery period, 9 (FWB trial) and 6 (SWB trial) participants remained dehydrated.

Although mentioned briefly in a number of case and small cohort studies (Brandt et al, 2018; Kasper et al, 2019; Matthews & Nicholas, 2017; Petterson, Ekstrom, & Berg, 2013) our recent survey reported the use of hot baths to be prevalent (76%) in MMA (Connor & Egan, 2019), but this present study suggests that the exact protocol varies considerably between individual fighters. Within the current cohort, duration of immersions varied from 11 to 60 min, and duration of wraps varied from 6 to 60 min. Most fighters reported that the number of combined baths with wraps is two round for a 'normal' weight cut. In contrast, one case study reported nine hot baths being used in the 20 h prior to weight-in as part of one fighter's weight cut (Kasper et al, 2019). All but one participant found our bathing protocol at 37.8ºC to be colder than the hot water immersion that they usually employ. All participants reported increasing the water temperature throughout each immersion, either using hot tap water or boiled kettle water. Clearly there are large variations in methods employed for hot baths, but the present study may act as a reference point for further research. For example, whether a higher water temperature and/or differences in salt type and/or concentration would reveal differences between FWB and SWB protocols.

In summary, hot baths are commonly used by MMA athletes and are an effective method of RWL, but there are large variations in protocols used by fighters in practice. Under the standardised conditions employed in the present study, the total amount of body mass loss during a hot bath in water supplemented with ~1.6% Epsom salt was similar to a hot bath performed in fresh water (~2.1% over 2 h of bathing and wrapping). However, further research should explore hotter bathing temperatures that are consistent with those habitually used by fighters, and higher concentrations of salt in order to produce a large osmotic gradient between the bath water and body fluids. Carbohydrate, fibre, and fluid restriction for 24 h prior to commencing the bathing protocol resulted in ~2.8% loss of body mass, suggesting that dietary manipulation should be considered as a method of RWL prior to employing aggressive dehydration strategies, particularly if the desired weight loss is less than ~3% of body mass.

Chapter 5 - Study 3

Published as:

Connor, J., Egan, B. (2021). Comparison of hot water immersion at self-adjusted maximum tolerable temperature, with or without the addition of salt, for rapid weight loss in mixed martial arts athletes. *Biology of Sport* 38(1), 89-96. https://doi.org/10.5114/biolsport.2020.96947

Abstract

Hot water immersion is used by athletes in weight category sports to produce rapid weight loss (RWL) by means of passive fluid loss, and often is performed with the addition of Epsom salt (magnesium sulphate). This study investigated the magnitude of body mass losses during hot water immersion with or without the addition of salt, with the temperature commencing at 37.8°C and being self-adjusted by participants to their maximum tolerable temperature. In a crossover design, eight male MMA athletes (29.4±5.3 y; 1.83±0.05 m; 85.0±4.9 kg) performed a 20 min whole-body immersion followed by a 40 min wrap in a warm room, twice in sequence per visit. During one visit, only fresh water was used (FWB), and in the other visit, magnesium sulphate (1.6% wt/vol) was added to the bath (SWB). Prior to each visit, 24 h of carbohydrate, fibre and fluid restriction was undertaken. Water temperatures at the end of the first and second baths were ~39.0°C and ~39.5°C, respectively. Body mass losses induced by the hot bath protocols were 1.71±0.70 kg and 1.66±0.78 kg for FWB and SWB, respectively ($P = 0.867$ between trials, $d = 0.07$), and equivalent to ~2.0% body mass. Body mass lost during the entire RWL protocol was 4.5±0.7%. Under the conditions employed, the magnitude of body mass lost in SWB was similar to FWB.

Augmenting passive fluid loss during hot water immersion with the addition of salt may require a higher salt concentration than that presently utilised.

Introduction

Rapid weight loss (RWL) is frequently practiced in sports that have weight class restrictions [Khodaee et al, 2015; Reale, Slater, & Burke, 2017]. For example, in mixed martial arts (MMA), the percentage of body mass lost by these athletes is usually ~5% to 10% in the week prior to competition (Barley, Chapman, & Abbiss, 2018; Connor & Egan, 2019; Coswig et al, 2019; Coswig, Fukuda, & Del Vecchio, 2015; Crighton, Close, & Morton, 2016; Hillier et al, 2019; Matthews & Nicholas, 2017). To achieve losses of this magnitude, RWL strategies that reduce body water stores (e.g. water loading, fluid restriction, and increasing sweat losses through heat exposure) are the predominant methods of RWL (Barley, Chapman, & Abbiss, 2018; Connor & Egan, 2019; Hillier et al, 2019).

A means of passive fluid loss known as hot baths is often employed as part of weight-making practices in combat sports (Brandt et al, 2018; Connor & Egan, 2019; Kasper et al, 2019; Matthews & Nicholas, 2017; Park et al, 2019; Pettersson, Ekstrom, & Berg, 2013). A recent survey of RWL practices by MMA athletes reported the use of hot baths to be highly prevalent, with 76% of fighters reporting their use either "always" or "sometimes" (Connor & Egan, 2019). Hot baths generally describe the practice of hot water immersion followed by wrapping in warm clothing for a period of time prior to further exposures to hot water immersion and wrapping. However, there are large variations in how athletes perform a hot bath protocol (Connor, Shelley, & Egan, 2020). For instance, in a cohort of 11 fighters, duration of immersions varied from 11 to 60 min and duration of wraps varied from 6 to 60 min, and the number of combined immersions with wraps is typically two rounds for a "normal" weight cut (Connor, Shelley, & Egan, 2020). In contrast, one case study reported nine hot baths being used in the 20 h prior to weigh-in as part of one fighter's weight cut (Kasper et al, 2019).

As part of their personal hot bath protocol, many of the fighters described the addition of 1 to 2 kg of Epsom salt to the water with the aim of augmenting the loss of body mass compared to that achieved by immersion in fresh water (Connor, Shelley, & Egan, 2020). The addition of salt to this end does have some empirical evidence to support its practice (Hertig, Riedesel, & Belding, 1961; Hope, Aanderud, & Aakvaag, 2001; Whitehouse, Hancock, & Haldane, 1932), with the suggestion that the addition of salt increases the osmotic pressure difference between the immersion medium and body fluids, and/or removes the inhibitory effect on sweating, and thereby contributes to the greater fluid loss compared to fresh water (Brebner & Kerslake, 1964; Buettner, 1953; Hope, Aanderud, & Aakvaag, 2001; Peiss, Randall, & Hertzman, 1956; Whitehouse, Hancock, & Haldane, 1932). We recently tested the addition of Epsom salt to produce a 1.6% salt solution but found no difference in body mass losses comparing fresh water and salt water immersion when the water temperature was maintained at 37.8ºC. In the absence of previous studies in athletes, we used this fixed temperature in order to increase the internal validity of the experimental design. However, in an exit questionnaire, all but one participant found our bathing protocol at 37.8ºC to be colder than the hot water immersion that they usually employ, and all participants reported that they usually increase the water temperature throughout each immersion, either using hot tap water or boiled kettle water. Therefore, in practice in MMA, a hot bath protocol is completed by starting at a warm water temperature and increasing temperature to the fighter's maximum tolerable temperature. This difference in protocol compared to our recent experiment is salient because there is a suggestion from previous work that water temperature and salt concentration may interact such that the effect of the addition of salt, if any, is greater at higher water temperatures (Buettner, 1953; Whitehouse, Hancock, & Haldane, 1932).

Therefore, the aim of the present study was to determine the magnitude of body mass losses in MMA athletes using a hot bath protocol with immersion in hot water with or without the addition of Epsom salt, and wherein participants were encouraged to increase bathing temperatures to that which they would use during their typical hot bath protocol during a weight cut.

Materials And Methods

Participants

Eight male MMA athletes (age, 29.4±5.3 y; height, 1.83±0.05 m; body mass, 85.0±4.9 kg) provided written informed consent to participate. Participants comprised both amateur and professional fighters, including two former Ultimate Fighting Championship (UFC) fighters. All participants were competing under professional weigh-in rules at the time of the study i.e. weigh-in 24 h before competition. Each participant had previous experience of RWL and the use of hot baths as part of that process, and each had made weight for competition on at least ten occasions prior to participation in the study. The study was approved by the Human Research Ethics Committee of DCUREC/2019/115 . This study protocol was based on our previous work (Connor, Shelley, & Egan, 2020), but was performed independent of that work, separated by 4 to 6 calendar months, and under a different ethics committee permit. However, n=6 participants were common to both studies.

Protocol

A crossover-repeated measures design was employed to compare the effects on passive fluid loss of hot water immersion under conditions of fresh water bathing (FWB) compared to salt water bathing (SWB). Participants performed two main experimental trials separated by at least seven days, with the order of the FWB and SWB trials being assigned in a counterbalanced manner. The FWB and SWB trials were identical with the exception of the water condition in which they were immersed (Figure 5.1). On the day prior to bathing, participants were prescribed to eliminate carbohydrate- and fibre-rich foods from their diet and consume an energy intake of 22 kcal/kg body mass. Fluid intake was prescribed to be restricted to 15 mL/kg for the 24 hours before bathing. These dietary and fluid restriction protocols were typical of what was practiced by the participants in their previous RWL experiences, and compliance with the prescribed protocol was confirmed verbally on Morning Day 0. The bathing protocol was as previously described (Connor, Shelley, & Egan, 2020) and comprised of 20 min of hot water immersion (“bath”) followed by 40 min wrapped in heavy clothing and blankets in a warm room (“wrap”). This 60 min bath and wrap protocol was repeated twice per main experimental trial i.e. 2 h total (Figure 5.1). All experiments took place in the same bath, bathroom, and adjacent bedroom of a private residential dwelling.

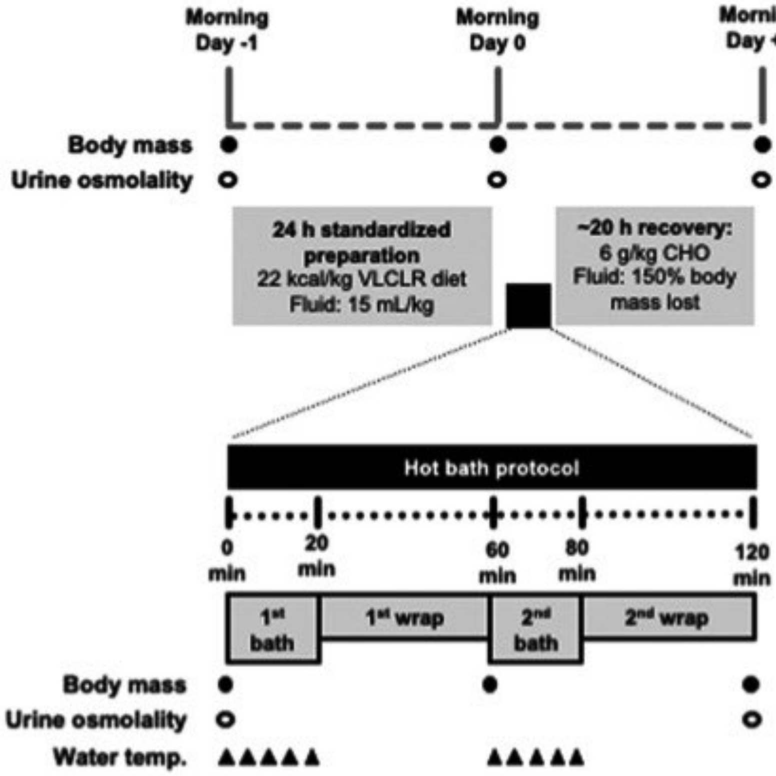

Figure 5.1. Study design schematic. Experimental trials were identical with the exception of the water condition in which they were immersed being with fresh water bathing or salt water bathing on separate days. CHO, carbohydrate; VLCLR, very low carbohydrate, low residue.

For each bath, participants were submerged up to the neck for 20 min. The initial water temperature of the bath was prepared to 37.8ºC, but participants were encouraged to bath in a water temperature that was typical for a normal weight cut bath protocol. In practice, this process usually involves bringing the water temperature up to a fighter’s maximum tolerable level, but this temperature will vary from fighter to fighter. To achieve this aim, participants requested from the researchers for the addition of boiling water from an electric kettle (1.5 L) to the bath ad libitum. The volume of additional boiling water per bath was noted. A floating thermometer (Avent Bath & Room Thermometer; Philips, UK) was checked at 4 min intervals for measurement of water temperature (Figure 5.1), but participants were not informed of the temperature during either bath or trial.

After 20 min of bathing, participants dried off in the bathroom and as quickly as possible put on a knitted wool hat, cotton t-shirt, hooded cotton sweatshirt, cotton tracksuit bottoms/sweatpants, and socks. Participants were then covered in blankets on a bed in an adjacent bedroom with only their face exposed. This wrap was performed for 40 min. Room temperature ranged from 24ºC to 29ºC during the trials. This 60 min bath and wrap protocol is considered one round and was repeated twice per main experimental trial (Figure 5.1).

Upon completion of the second round, participants began the weight regain process and were prescribed to consume fluids (in L) to the equivalent to 150% of total body mass lost (in kg) (Sawka et al, 2007) from Morning Day -1, and to consume 6 g/kg body mass of carbohydrate throughout the rest of the day.

For the FWB trial, only fresh tap water was used in the bath. For the SWB trial, Epsom salt (magnesium sulfate) were added to the bath with 160 L capacity at a concentration of 2 kg in 125 L of water (i.e. ~1.6% wt/vol). This quantity and type of salt was used in our previous work and was chosen based on our personal experiences of the practices of fighters making weight in combat sports, and was subsequently confirmed as approximating general practices of that participant cohort in exit questionnaires completed by the study participants (Connor, Shelley, & Egan, 2020).

Change in body mass, measured to the nearest 0.05 kg (model #63667; Soehnle, Germany), was the primary outcome measure. Body mass was measured in minimal clothing, i.e. lower body short underwear in the form of briefs or boxer briefs, at several time-points (Figure 5.1): (i) upon waking on the day prior to bathing (Morning Day -1), (ii) upon waking on the day of bathing (Morning Day 0), (iii) immediately prior to the first bath, (iv) immediately before the second bath, (v) immediately after the second wrap, and finally, (vi) upon waking on the day after bathing (Morning Day +1).

Urine osmolality was measured (Osmocheck Portable Osmometer; Vitech Scientific, UK) at the same time points except immediately before the second bath and wrap. Participants were defined as dehydrated using a criteria of urine osmolality of >700 mOsmol/kg (Sawka et al, 2007).

Sample size calculation

The primary outcome was change in body mass as a consequence of the 2 h bath and wrap protocol. Therefore, a sample size calculation was performed (G*Power v.3.1) based on previous research demonstrating an effect of salt water to augment the magnitude of body mass lost during hot water immersion when compared to fresh water (Hope, Aanderud, & Aakvaag, 2001). Using the body mass lost after 2 h of that 4 h immersion protocol, a time point analogous to the present work, and that being 0.98±0.44 kg and 1.24±0.80 kg for fresh water and salt water respectively, and an assumed correlation between conditions of 0.90, the required sample size to detect a difference between FWB and SWB at a Type I error rate (a) of 0.05 and a power (1-b) of 0.8 was n=26. However, because these data are based on a higher salt concentration of ~3.5%, and given the absence of effect in our previous research using a salt concentration of 1.6% (Connor, Shelley, & Egan, 2020) a priori we planned an interim data analysis for the assessment of futility, and therefore discontinuation, after completion of one-third (n~8) of the required sample size. In the absence of any difference between FWB and SWB for change in body mass with n=8 ($P = 0.867$ between trials, $d = 0.07$; data reported below), we discontinued recruitment at that time.

Statistical analysis

Statistical analysis and graphical representation were performed using GraphPad Prism v8.3 (GraphPad Software, Inc., USA). Normality of data was assessed with the Shapiro-Wilk normality test, for which all data passed. All data are presented as mean ± SD. A two way (condition x time) repeated measures analysis of variance (ANOVA) was used to assess responses to the interventions. When a main or interaction effect was observed, pairwise comparisons were performed with Bonferroni's correction for which multiplicity-adjusted P-values are reported. Paired t-tests were used to assess differences between trials for the quantity of boiling water added, and differences in body mass lost during bathing between this study and our previous study for the n=6 participants common to both studies. The level of significance for all tests was set at $P < 0.05$. Standardised differences in the mean were used to assess magnitudes of effects between conditions. These were calculated using Cohen's d effect size and are interpreted using thresholds of < 0.2, ≥ 0.2, ≥ 0.5 and ≥ 0.8 for trivial, small, moderate, and large, respectively.

Results

After starting each bath temperature at 37.8ºC, the participant's self-adjustment of bathing temperature resulted in progressive increases in water temperature in both the 1st and 2nd baths (main effect of time, $P < 0.001$) (Figure 5.2A & 5.2C). Average water temperature in the 1st bath of each trial was 38.41±0.31ºC and 38.16±0.31ºC for FWB and SWB, respectively ($P = 0.135$), and in the 2nd bath of each trial was 38.48±0.36ºC and 38.64±0.22ºC for FWB and SWB, respectively ($P = 0.341$). Final water temperature in the 1st bath of each trial was 38.94±0.70ºC and 38.93±0.63ºC for FWB and SWB, respectively ($P = 0.972$), and in the 2nd bath of each trial was 39.14±0.70ºC and 39.59±0.45ºC for FWB and SWB, respectively ($P = 0.154$). No condition or interaction effects were observed for the effect of salt (Figure 5.2A & 5.2C). The volume of boiling kettle water added to each bath was 4.50±1.96 L for FWB and 5.44 ± 1.12 L for SWB during the 1st bath of each trial ($P = 0.305$), and 4.69±2.03 L for FWB and 5.81±0.96 L for SWB during the 2nd bath of each trial ($P = 0.080$) (Figure 5.2B & 5.2D).

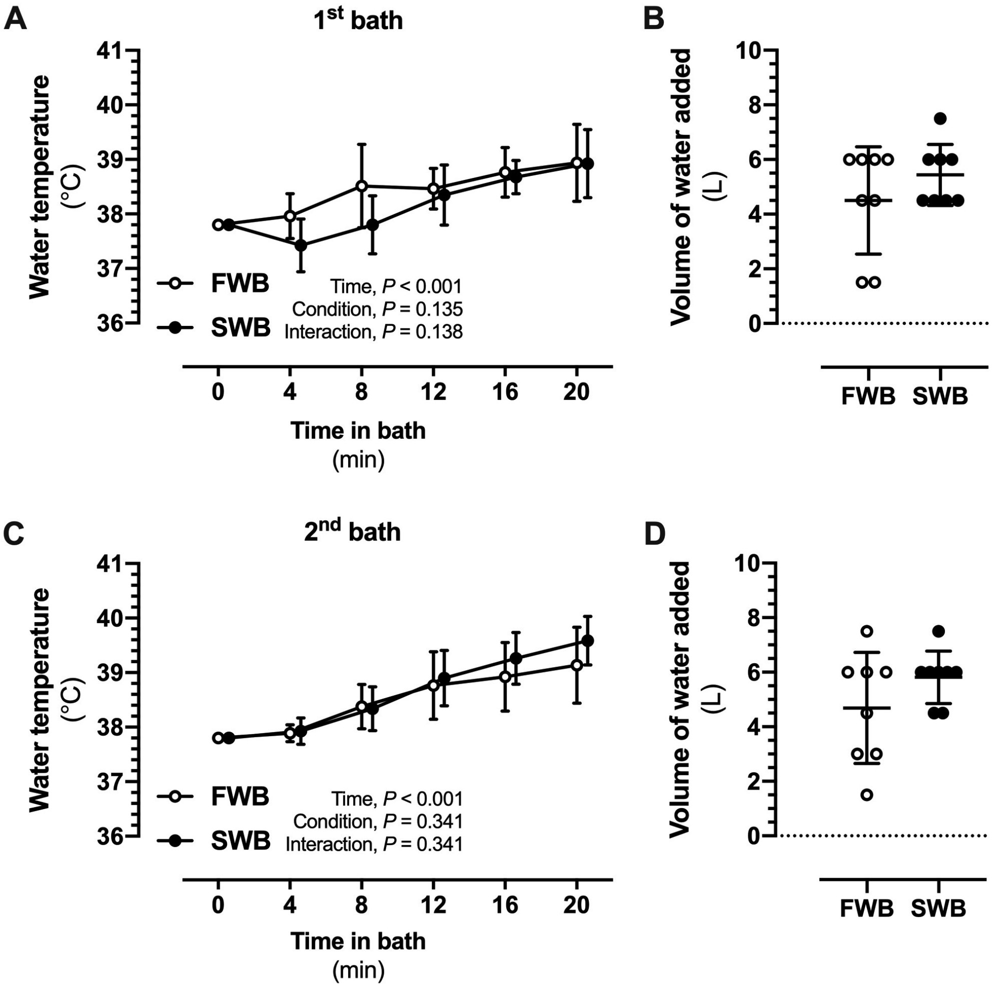

Figure 5.2. Water temperatures measured at 4 min intervals during each bath (A, 1st bath; C, 2nd bath) during experimental trials of fresh (FWB) or salt water (SWB); and quantity of boiling kettle water added per bath (B, 1st bath; D, 2nd bath). White (FWB) and black (SWB) circles in panels B and D represent individual data points. Otherwise data are mean values with vertical bars representing SD.

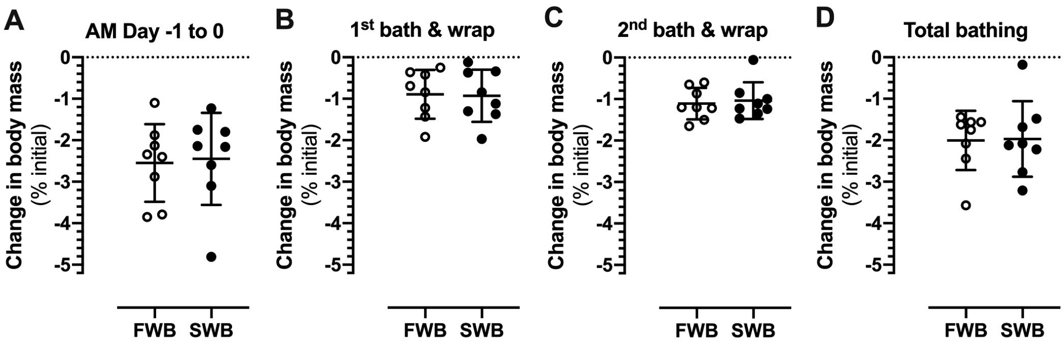

Figure 5.3. Percentage changes in body mass (relative to baseline recorded on Morning Day -1) induced by diet and fluid restriction, and a hot bath protocol in fresh (FWB) or salt water (SWB) for (A) the period from Morning Day -1 to Morning Day 0, (B) the 60 min period comprising the first bath and wrap, (C) the 60 min period comprising the second bath and wrap, and (D) the 120 min period comprising both baths and wraps. White (FWB) and black (SWB) circles in each panel represent individual data points. Mean values are represented by the horizontal solid line with vertical bars representing SD for changes observed within each time period that is defined above each panel.

Table 5.1. Body mass (kg) and hydration status assessed by urine osmolality (mOsmol/kg) at time points during a rapid weight loss intervention featuring a hot bath protocol in fresh (FWB) or salt water (SWB).

	Morning Day -1	**Morning Day 0**	**Before 1st bath**	**After 1st bath & wrap**	**After 2nd bath & wrap**	**Morning Day +1**	P value
Body mass (kg)							Time, P = 0.001***
FWB	85.03±4.87	83.31±4.86	82.89±4.83	82.13±4.61	81.18±4.40	84.75±4.72	Condition, P = 0.919
SWB	84.94±5.45	83.34±4.98	82.86±4.85	82.09±5.01	81.21±4.87	84.60±5.04	Interaction, P = 0.953
Urine osmolality (mOsmol/kg)							Time, P = 0.001***
FWB	718±137	880±137	856±117		989±126	909±134	Condition, P = 0.333
SWB	709±234	939±121	897±152		943±90	954±133	Interaction, P = 0.615
Data are presented as ± SD, n = 8. P < 0.001***							

For change in body mass in absolute (kg) (Table 5.1) and relative (%initial body mass) (Figure 5.3) terms, a main effect of time ($P < 0.001$), but neither a main effect of condition, nor a condition*time interaction effect, was observed. Similarly, there was no difference between conditions for changes in urine osmolality at the various time points (Table 5.1).

Body mass losses induced by carbohydrate and fluid restriction were 2.14±0.78 kg ($P<0.001$;$d=0.44$) and 2.08±0.96 kg ($P < 0.001$; $d = 0.40$) in preparation for the FWB and SWB trials, respectively. Body mass losses induced by the hot bath protocols were 1.71±0.70 kg ($P<0.001$;$d=0.37$) and 1.66±0.78 kg ($P < 0.001$; $d=0.34$) for the FWB and SWB protocols, respectively. FWB resulted in body mass loss of 0.76±0.53k g ($P=0.005$; $d=0.16$) during the 1st bath and wrap, and 0.94±0.35 kg ($P=0.001$; $d=0.21$) during the 2nd bath and wrap. SWB resulted in body mass loss of 0.77±0.52 kg ($P=0.004$; $d=0.16$) during the 1st bath and wrap, and 0.88±0.40 kg ($P < 0.001$; $d = 0.18$) during the 2nd bath and wrap.

Total body mass losses induced by the entire RWL protocol were 3.84±0.74 kg ($P<0.001$;$d=0.83$) and 3.74±0.70 kg ($P < 0.001$; $d=0.72$) for the FWB and SWB protocols, respectively. These values represented losses of relative to initial body mass on Morning Day -1 of 4.55±0.77% and 4.44±0.66% for the FWB and SWB protocols, respectively (Figure 5.4A).

Weight regain was 3.57±0.86 kg ($P < 0.001$; d=0.78) and 3.39±0.87 kg ($P < 0.001$; d=0.89) during recovery from the FWB and SWB protocols, respectively (Figure 5.4B), resulting in a body mass deficit compared to Morning Day -1 of 0.28±0.44 kg and 0.34±0.89 kg, respectively (Figure 5.4C). At Morning Day +1, 6 (FWB trial) and 5 (SWB trial) participants were in a body mass deficit compared to Morning Day -1, and all participants, regardless of trial, were defined as dehydrated by having a urine osmolality >700 mOsmol/kg (Sawka et al, 2007) both immediately after the 2nd bath and wrap, and at Morning Day +1.

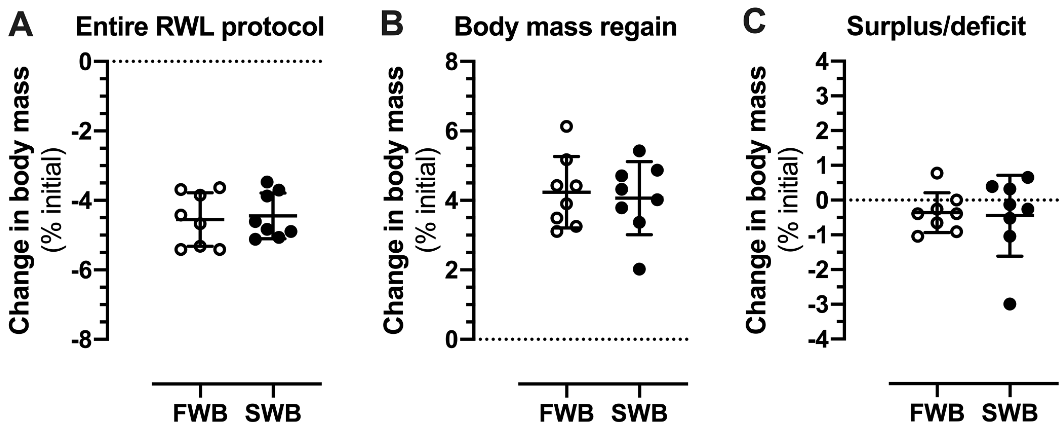

Figure 5.4. Percentage changes in body mass (relative to baseline recorded on Morning Day -1) during (A) the entire rapid weight loss intervention featuring a hot bath protocol in FWB or SWB, (B) the period of weight regain prior to weigh-in on Morning Day +1, and (C) as a measure of total body mass deficit or surplus on Morning Day +1 compared to Morning Day -1. White (FWB) and black (SWB) circles in each panel represent individual data points. Mean values are represented by the horizontal solid line with vertical bars representing SD for changes observed within each time period that is defined above each panel.

Comparing the n=6 participants common to the present study and our previous work (Connor, Shelley, & Egan, 2020) body mass lost during the bathing protocol using SWB was 1.57±0.46 kg for bathing at 37.8ºC, and 1.98±0.47 kg for the present study of self-adjusted maximum tolerable temperature of ~39.0ºC ($P=0.152$; $d=0.89$). Expressed as percentage of body mass prior to the 1st bath of each trial, this is equivalent to 2.07±0.61% and 2.62±0.62% for bathing at 37.8ºC and ~39.0ºC, respectively.

Discussion

The present study demonstrates that the body mass lost when bathing in a hot bath of fresh water (FWB) is similar to bathing in a hot bath with ~1.6% Epsom salt added (SWB). This finding is consistent with our previous work using the same bathing protocol but performed at a fixed water temperature of 37.8ºC (Connor, Shelley, & Egan, 2020). The present study extends that work by investigating body mass lost when the water temperature is self-adjusted to a fighter's own maximum tolerable temperature. While there was greater body mass lost in hotter water temperatures in those participants common to both studies, there was again no effect of adding salt on the magnitude of body mass lost compared to fresh water.

Investigating body mass loss when the water temperature is self-adjusted to a fighter's maximum tolerable temperature was undertaken as a means to extend the ecological validity of our previous hot bath study (Connor, Shelley, & Egan, 2020). An exit questionnaire performed during that study revealed that the most obvious difference between that study design and protocols that fighters were currently practicing was the temperature of the water i.e. all but one participant found our bathing protocol at 37.8ºC to be colder than the hot water immersion that they usually employ, and all participants reported that in practice they increase the water temperature throughout each immersion. However, even at the increased water temperature of ~39.0ºC, there was still no difference observed on body mass lost between the FWB and SWB trials. This finding, combined with our previous work, suggests that an interaction effect between water temperature and salt concentration, i.e. that addition of salt produces greater loss of body mass or body water at higher water temperatures, does not exist in the hot bath protocol employed. This is unsurprising given that of the work that previously suggested an interaction effect between water temperature and salt concentration, one study was performed with an n-size of one participant (Whitehouse, Hancock, & Haldane, 1932) and the other employed a forearm model of water exposure under rubber or neoprene sleeves (Buettner, 1953).

That said, the addition of salt during hot baths is common practice in MMA athletes (Connor, Shelley, & Egan, 2020), and there is some empirical evidence of the effect of adding salt to increase body mass lost during immersion in water (Hertig BA, Riedesel ML, Belding, 1961; Hope, Aanderud, Aakvaag, 2001; Whitehouse, Hancock, & Haldane, 1932). Early work established that even in thermo-neutral water, i.e. in the absence of sweating, immersion in a strong salt solution (either 11.5% or 20.0% salt as sodium chloride) produces passive fluid loss (Whitehouse, Hancock, & Haldane, 1932). In water heated to 36/37ºC, addition of 5% sodium chloride allowed for higher sweat rates during 3 h of immersion when compared to fresh water, with the effect more pronounced at salt concentrations of 10% and 15% (Hertig, Riedesel, Belding, 1961). Lastly, during immersion in seawater compared to fresh water, ~32% greater body mass was lost in the former during 4 h of immersion at ~38ºC (Hope, Aanderud, Aakvaag, 2001). Given that seawater is ~3.5% salt, it may be that the concentration of salt in a hot bath should at least 3.5% (Hope, Aanderud, Aakvaag, 2001), or possibly greater (Hertig, Riedesel, Belding, 1961; Buettner, 1953), if the aim is to augment the rate of passive fluid loss that would otherwise occur in fresh water. Despite these indications, we employed a salt concentration of only ~1.6% wt/vol magnesium sulfate, but this quantity and type of salt was chosen for its ecological validity based on data from exit questionnaires in our previous work (Connor, Shelley, & Egan, 2020).

Future work should certainly investigate higher concentrations of salt in order to produce a larger osmotic gradient between the bath water and body fluids. The suggested mechanisms for how the addition of salt influences the loss of body mass during immersion are (i) that salt water serves to remove an inhibitory influence on the decline in sweat rate that usually occurs with prolonged immersion in fresh water, and/or (ii) that during immersion in salt water, the osmotic pressure difference between the immersion medium and body fluids results in greater fluid loss compared to fresh water (Brebner & Kerslake, 1964; Buettner, 1953; Hertig BA, Riedesel ML, Belding, 1961; Hope, Aanderud, Aakvaag, 2001; Peiss, Randall, & Hertzman, 1956; Whitehouse, Hancock, & Haldane, 1932). However, in studies where an additive effect of salt has been observed, these have been 3 to 4 h immersions (Hertig BA, Riedesel ML, Belding, 1961; Hope, Aanderud, Aakvaag, 2001), in contrast to the only 40 min of immersion time across the 2 h bath and wrap protocol that we employed. Moreover, whether the type of salt (i.e. seawater versus added Epsom salt) would make any difference to the outcome remains to be explored, but is unlikely. In previous work, when the osmotic gradient was produced by either sodium chloride, potassium chloride or cane sugar, the diffusion of water through the skin was similar in all conditions (Buettner, 1953).

Absent an effect of the addition of salt under the conditions employed in our two studies, because there were six participants common to both studies, it was possible to explore the effect of self-adjusting the water temperature on body mass loss. Expressed as percentage of body mass prior to the respective 1st bath, the magnitude of loss was 2.07±0.61% for the previous study at a water temperature of 37.8ºC, and 2.62±0.62% for the present study at ~39.0ºC. While this difference was not statistically significant (P=0.152), perhaps given the small n-size, the magnitude of the effect size was 'large' (d=0.89), and in practical terms translates to an extra ~410 g of body mass lost. As part of the process of making weight in weight category sports, this is a practically-meaningful amount of weight loss and speaks to the importance of water temperature in the hot bath process, but should be kept within safe limits, which remain to be defined. For illustration, water temperatures rarely exceeded 40ºC across all participants and baths, and previous immersion studies have typically used temperatures of ~38/39ºC (Connor, Shelley, & Egan, 2020; Heathcote et al, 2019; Hertig, Riedesel, Belding, 1961; Hoekstra et al, 2018; Hope, Aanderud, Aakvaag, 2001; Kraft et al, 2011; Leicht et al, 2019), but water temperatures of ~41ºC acutely (Brebner & Kerslake, 1964) and ~40ºC repeated daily for six days (Zurawlew et al, 2016), have also been employed without adverse effects being reported.

Despite the greater body mass loss with the higher water temperature in the present study, consistent with our previous work, there was a greater loss of body mass by the 24 h of restriction of carbohydrate, fibre, and fluid intake (FWB, -2.54±0.93%; SWB -2.45±1.11%), than from either bathing protocol (FWB, -2.00±0.71%; SWB, -1.97±0.91%). The loss of body mass with 24 h of such restriction is attributed to dehydration, short-duration glycogen depletion, and emptying of the intestinal contents (Reale, Slater, & Burke, 2017), and like the present study is typically ~2–3% of body mass (Connor, Shelley, & Egan, 2020; Reale et al, 2018; Reale, Slater, & Burke, 2017). Therefore, while gradual weight loss using an appropriate energy deficit is central to a weight loss strategy lasting several weeks or months (Reale, Slater, & Burke, 2017), for the RWL period prior to weigh-in, acute (< 48 h) dietary manipulation (carbohydrate, fibre, and fluid intake) should be considered prior to employing aggressive heat-stimulated dehydration strategies, particularly if the desired weight loss is less than ~3% of body mass.

After the second wrap, a time point chosen to be typical of a weigh- in time for MMA athletes, total body mass lost including the 24 h restriction and 2 h hot bath protocol was ~4.5%. At this timepoint, all participants were classified as dehydrated based on a urine osmolality of >700 mOsmol/kg (Sawka et al, 2007). This finding is consistent with typical methods of RWL resulting in 100% of MMA athletes being dehydrated to various degrees at an official weigh-in (Jetton et al, 2013; Matthews & Nicholas, 2017). Body mass and hydration assessment performed on Morning Day +1 represents an ~20 hour recovery period after completing the second bath and wrap, and a body mass deficit and dehydration were observed at this timepoint. However, in practice the time from weigh-in until official competition in professional MMA is usually longer i.e. approximately 30 to 36 h. Even with a long time period for rehydration, the majority of MMA athletes remain dehydrated up to 2 h before competition (Jetton et al, 2013; Matthews & Nicholas, 2017). Therefore, regain of body mass alone is potentially not a good indicator of returning to euhydration, and indeed there remains some debate about the assessment of hydration status by spot analysis with urine measures (Cheuvront, Kenefick, & Zambrask, 2015).

The small sample size (n=8) employed may be considered a limitation of the present study. However, this sample size was finalised based on a pre-planned interim data analysis for the primary outcome of change in body mass during the 2 h bath and wrap protocol. The small sample size may result in assessment of the secondary outcomes by ANOVA being underpowered, and thereby increase the likelihood of a type II error (i.e. false negative) for these outcomes. Another limitation of this study may be the heterogeneity in the experience of the participants with RWL practices. All participants had prior experience with making weight for competition and the use of hot baths in that process, but during either our recruitment or analysis, we did not account for the number of lifetime exposures to these practices. While speculative, it may be that the response to such practices changes over time, but with participants acting as their own control in this crossover design, we do not anticipate that this aspect had a meaningful impact on the results. Lastly, the magnitude of body mass lost during the entire RWL process averaged ~4.5% of body mass, whereas in practice losses of ~5% to 10% are typical in these athletes in the week prior to competition (Barley, Chapman, & Abbiss, 2018; Connor & Egan, 2019; Coswig et al, 2019; Coswig, Fukuda, & Del Vecchio, 2015; Crighton, Close, & Morton, 2016; Hillier et al, 2019; Matthews & Nicholas, 2017). Therefore, whether there would be a differential effect of salt water when bathing has been preceded by RWL of greater magnitude cannot be excluded as a possibility.

Conclusions

In summary, hot baths are an effective method of RWL to produce a loss of ~2.0% body mass during 2 h of bathing and wrapping. When fighters self-adjust the water temperature in the bath, temperatures were ~39.0°C. However, using this protocol, the total amount of body mass lost during a hot bath in water supplemented with ~1.6% Epsom salt was similar to a hot bath performed in fresh water. Future research should explore bathing in higher concentrations of salt, which likely need to be >3.5% in order to produce a sufficient osmotic gradient between the bath water and body fluids.

Chapter 6 - Study 4

Abstract

Connor, J., Germaine, M., Gibson, C., Clarke, P., & Egan, B. (2022). Effect of rapid weight loss incorporating hot salt water immersion on changes in body mass, blood markers, and indices of performance in male mixed martial arts athletes. *European Journal of Applied Physiology, 10.1007/s00421-022-05000-7.* https://doi.org/10.1007/s00421-022-05000-7

Purpose

To investigate the effects of rapid weight loss (RWL), incorporating comparison of hot water immersion (HWI) in fresh or salt water, on changes in body mass, blood markers, and indices of performance in mixed martial arts athletes.

Methods

In a crossover design comparing fresh water (FWB) to salt water (SWB; 5.0%wt/vol Epsom salt) bathing, thirteen males performed 20 min of HWI (~40.3°C) followed by 40 min wrapped in a heated blanket, twice in sequence (2 h total). Before bathing, ~26 h to ~28 h of fluid and dietary restriction was undertaken, and ~24 h to ~26 h of a high carbohydrate diet and rehydration was undertaken as recovery.

Results

During the entire RWL process, participants lost ~5.3% body mass. Body mass lost during the 2 h hot bath protocol was 2.17±0.81 kg (~2.7% body mass) and 2.24±0.64 kg (~2.8% body mass) for FWB and SWB, respectively ($P=0.647$ between trials). Blood urea nitrogen, creatinine, sodium, chloride, hemoglobin and hematocrit were increased (all $P<0.05$), and plasma volume was decreased (~14%; $P<0.01$), but did not differ between FWB and SWB, and were similar to baseline values after recovery. No indices of performance (e.g. countermovement jump, isometric strength, and functional threshold power) were impacted when RWL was followed by the recovery process.

Conclusion

Under the conditions of this hot bath protocol, fluid loss was not augmented by the addition of ~5.0%wt/vol of Epsom salt during HWI, and RWL of ~5.3% body mass followed by >24 h of recovery did not impact indices of performance.

Introduction

In sports that have weight class restrictions, athletes attempting to 'make weight' frequently practice short-term weight loss, termed acute or rapid weight loss (RWL), in the ~72 h before weigh-in (Reale et al. 2017; Burke et al. 2021). Methods of RWL focus on the acute reduction of the contents of the gastrointestinal tract, and of total body water, through methods such as a low carbohydrate and low residue diet, fluid restriction, and increasing sweat losses through exercise and/or heat exposure (Barley et al. 2018a; Hillier et al. 2019; Connor and Egan 2019). In mixed martial arts (MMA) athletes, combinations of these methods typically induce losses of ~5% to ~10% of body mass in the week before weigh-in (Coswig et al. 2015; Matthews and Nicholas 2017; Barley et al. 2018a; Hillier et al. 2019; Coswig et al. 2019; Connor and Egan 2019; Brechney et al. 2019).

One method of heat exposure used to induce passive fluid loss is hot water immersion (HWI), which is often employed as part of weight-making strategies in combat sports (Pettersson et al. 2013; Matthews and Nicholas 2017; Brandt et al. 2018; Kasper et al. 2019; Connor and Egan 2019; Park et al. 2019; Gordon et al. 2021). Colloquially known as hot baths, this method typically involves short duration HWI followed by 'wrapping' in warm clothing for a period of time before further exposures to HWI and wrapping (Kasper et al. 2019; Connor et al. 2020). The HWI is often performed in salt water, usually by the addition of Epsom salt, with the aim of augmenting the loss of body mass compared to that achieved by immersion in fresh water (Connor et al. 2020). Indeed, there is some empirical evidence for salt water immersion to augment fluid loss when compared to fresh water immersion (Whitehouse et al. 1932; Hertig et al. 1961; Hope et al. 2001), albeit these studies involve prolonged (3 h) HWI. The mechanistic basis for this effect is proposed as the addition of salt increasing the osmotic pressure difference between the immersion medium and body fluids, and/or attenuating the inhibitory effect on sweating that occurs during prolonged HWI, and thereby resulting in the greater fluid loss compared to fresh water (Whitehouse et al. 1932; Buettner 1953; Peiss et al. 1956; Buettner 1959; Hertig et al. 1961; Brebner and Kerslake 1964; Hope et al. 2001).

Our recent work has investigated a hot bath protocol incorporating short duration (2x20 min) HWI with an Epsom salt concentration of ~1.6%wt/vol, but found no difference in body mass losses comparing fresh water and salt water immersion either at a fixed water temperature of 37.8ºC (Connor et al. 2020), or when athletes self-adjusted the water temperature to the maximum temperature each could tolerate (~39.0ºC) (Connor and Egan 2021). Our choice of a ~1.6% salt solution using Epsom salt was chosen for its ecological validity based on our knowledge of applied practice, and responses to a questionnaire in which fighters described typically the addition of 1 to 2 kg of Epsom salt to a standard sized bath (Connor et al. 2020). However, higher concentrations of salt, which would induce a larger osmotic gradient between the bath water and body fluids, may be required to augment fluid loss when compared to fresh water. For example, even in thermoneutral water, i.e. in the absence of sweating, immersion in a strong salt solution (either 11.5% or 20.0% salt as sodium chloride) induced passive fluid loss (Whitehouse et al. 1932). In water heated to 36/37ºC, addition of

5% sodium chloride (~1709 mOsmol/kg) allowed for higher sweat rates during 3 h of immersion when compared to fresh water, with the effect more pronounced at salt concentrations of 10% and 15% (Hertig et al. 1961). Lastly, immersion in seawater (~3.5% salt; ~1000 mOsmol/kg to ~1200 mOsmol/kg) resulted in ~32% greater loss of body mass compared to fresh water during 4 h of immersion at ~38ºC (Hope et al. 2001). Given these observations, it may be that the concentration of salt in a hot bath should be at least 3.5% (Hope et al. 2001), or possibly greater (Buettner 1953; Buettner 1959; Hertig et al. 1961), if the aim is to augment the rate of passive fluid loss that would otherwise occur during HWI in fresh water.

Therefore, the present study investigated the magnitude of body mass losses in MMA athletes using a hot bath protocol with immersion in fresh water or salt water at a concentration of ~5%wt/vol of Epsom salt. Extending our previous work (Connor et al. 2020; Connor and Egan 2021), we also investigated the effects of ~28 h to ~30 h of RWL on blood markers (plasma volume, kidney function and electrolytes) and indices of performance. Hypohydration is well-established as negatively impacting indices of performance (Savoie et al. 2015; Deshayes et al. 2020), and importantly, recent evidence suggests this to be the case even when major confounders such as expectancy effects because of a lack of blinding, and inadequate familiarization with methods of dehydration, are addressed (James et al. 2019). While RWL inherently involves dehydration processes, in professional combat sports such as MMA and boxing the competitive bout typically takes place ~30 h to ~36 h after weigh-in (Burke et al. 2021). This time-period may allow for rehydration and recovery of muscle glycogen (Burke et al. 2021), but several studies have observed a residual negative impact on indices of performance even after ~16 h to ~24 h of recovery (Oöpik et al. 1996;

Moghaddami et al. 2016; Alves et al. 2018; Yang et al. 2018; Barley et al. 2018b; Kurylas et al. 2019). Therefore, we also investigated the effect of RWL followed by ~24 h to ~26 h of recovery in the form of a high carbohydrate diet and rehydration on body mass, hydration status, blood markers, and indices of performance.

Methods

Participants

Thirteen male MMA athletes (29.5±6.7 y; 1.81±0.07 m; 83.0±8.8 kg) with previous experience of RWL provided written informed consent to participate. The study was approved by the Human Research Ethics Committee of Dublin City University (permit number: DCUREC/2020/186). Participants comprised both amateur and professional fighters, but all participants were competing under professional weigh-in rules at the time of the study i.e. weigh-in 24 h before competition. Each participant had previous experience of RWL, and the use of hot baths as part of that process, and each participant had made weight for competition on at least five occasions before participating in the study.

Study design

A crossover-repeated measures design was employed to compare the effects on passive fluid loss of HWI using fresh water bathing (FWB) compared to salt water bathing (SWB). Participants performed two main experimental trials separated by at least seven days, with the order of the FWB and SWB trials assigned in a counterbalanced manner, and participants randomised to which trial they performed first. The FWB and SWB trials were identical with the exception of the water condition in which they were immersed during the bathing periods (Figure 6.1).

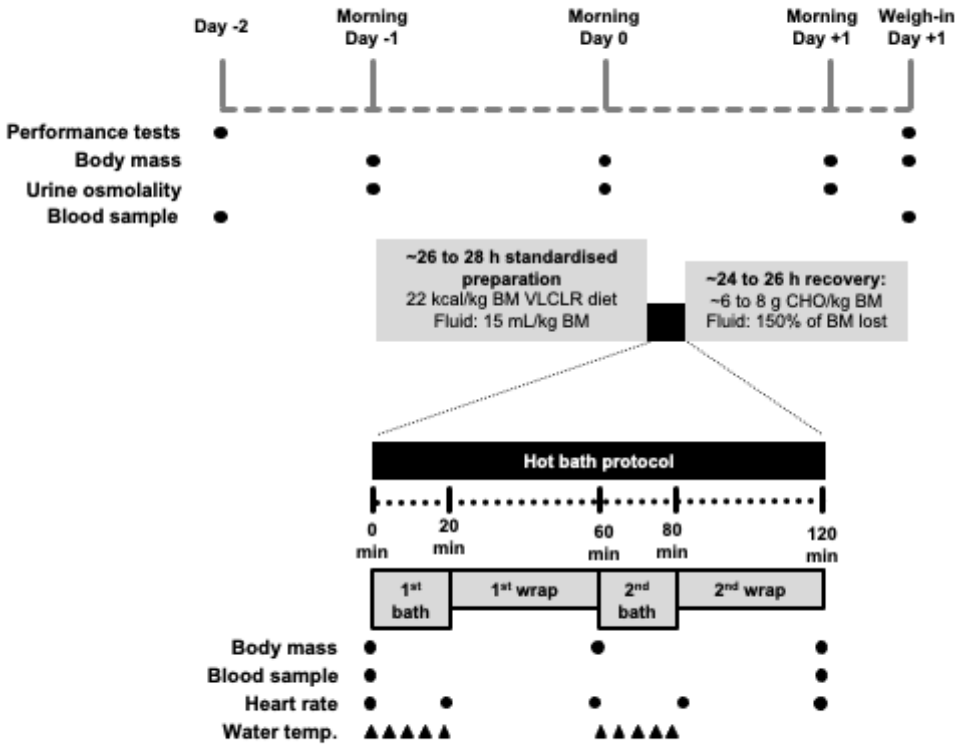

Figure 6.1 Study design schematic. Experimental trials were identical with the exception of the water condition in which they were immersed being with fresh water bathing or salt water bathing on separate days. CHO, carbohydrate; VLCLR, very low carbohydrate, low residue.

On Day -2, a performance test battery was performed consisting of tests of leg power (countermovement jumps; CMJ), maximal strength (isometric hand-grip strength and isometric mid-thigh pull; IMTP), and a 3 min all-out exercise test on a cycle ergometer to estimate functional threshold power (FTP). At least 72 h before the first trial commencing, a familiarisation session for the performance test battery was performed, which involved the participants undertaking the test battery in its entirety, and in an identical manner to that undertaken during the main trials.

On Day -1 (the day before bathing), participants were prescribed to restrict fluid intake to 15 mL/kg of body mass, eliminate carbohydrate- and fiber-rich foods from their diet, and consume an energy intake of 22 kcal/kg of body mass, which was tracked using the MyFitnessPal mobile phone application (UnderArmour, USA). These practices are similar to what these participants routinely undertake to make weight for competition, and compliance with the prescribed protocol was confirmed verbally on Day 0.

On Day 0, participants arrived ~2 h to ~4 h after waking to perform the bathing protocol. During this ~2 h to ~4 h period, participants remained fasted and did not consume any fluids. Upon completion of the bathing protocol, the total body mass lost from Morning Day -1 was calculated, and participants began the weight regain process by following the prescription to consume fluids (in L) to the equivalent to 150% of body mass lost (in kg) during the next 6 h (Sawka et al. 2007), and to consume 6 to 8 g/kg of body mass of carbohydrate during the rest of the day (Burke et al. 2021).

On Day +1, participants were advised to follow their habitual fight day nutrition practices, and returned to undertake the performance test battery ~24 h to ~26 h after completing the bathing protocol (Figure 6.1). For their first trial, participants were asked to keep a record of what food and fluid they consumed from waking to before testing on both Day -2 and Day +1. For their second trial, participants were asked to repeat the timing and quantity of this intake for the respective days. Compliance with this approach was confirmed verbally upon arrival for testing on each day. To minimise the potential influence of circadian rhythms on indices of performance, the testing on Day +1 was performed at the same time of day ±1 h as performed on Day -2. Participants completed their habitual training in the period between the main trials, but for the day before Day -2, only low intensity training was allowed, and like dietary standardisation was asked to be maintained consistent before each trial.

Bathing protocol

The bathing protocol comprised of 20 min of HWI ("bath") followed by 40 min wrapped in a rubberised sauna blanket ("wrap"). This 60 min bath and wrap protocol was repeated twice per main experimental trial i.e. 2 h total, as described in our previous work (Connor et al. 2020; Connor and Egan 2021) (Figure 6.1). One difference to these previous studies was that a sauna blanket (MiHIGH Infrared Sauna Blanket; MiHIGH Pty Ltd, Queensland, Australia) was used for the wrap periods, rather than a knitted wool hat, cotton t-shirt, hooded cotton sweatshirt, cotton tracksuit bottoms/sweatpants, and socks worn underneath several blankets in a heated bedroom. According to the manufacturer, the sauna blanket uses the same heating technology as an infrared sauna, emitting far infrared wavelengths.

For each bath, participants were submerged up to the neck for 20 min i.e. head-out HWI. For the FWB trial, only fresh tap water was used in the bath. For the SWB trial, Epsom salt was added to the bath with 160 L capacity at a concentration of 6.25 kg in 125 L of water (i.e. ~5.0%wt/vol). Based on the chemical composition of Epsom salt (magnesium sulfate heptahydrate; MgSO47H2O), 5.0%wt/vol of Epsom salt would result in the osmolality of the salt water being ~406 mOsmol/kg.

Another difference to our previous work in the present study was that the initial water temperature of the bath was prepared to ~40.3ºC rather than 37.8ºC (Connor et al. 2020; Connor and Egan 2021), and participants maintained the water temperature at their maximum tolerable level, rather than self-adjusting the water temperature upwards as previously (Connor and Egan 2021). To maintain the water temperature, participants requested from the researchers the addition of boiling water from an electric kettle (1.5 L) to the bath ad libitum. The volume of additional boiling water per bath was noted. Additional salt was not added to adjust for the additional boiling water, and therefore, during the bathing process the %wt/vol was estimated to decrease from 5.0% to ~4.7%, whereas the osmolality of the salt water was estimated to concomitantly decrease from ~406 mOsmol/kg to ~381 mOsmol/kg.

A floating thermometer (Avent Bath & Room Thermometer; Philips, UK) was checked at 4 min intervals for measurement of water temperature, but participants were not informed of the temperature during either bath or trial. At the same 4 min intervals, forehead temperature was measured as the mean of two measures using a digital infrared thermometer (Model HTD8813; LPOW, USA), whose range of precision according to the manufacturers' instructions is ±0.2ºC. After 20 min of bathing, the participants exited the bath, briefly dried themselves with a towel before entering the sauna blanket for the next 40 min. Heart rate was measured using an automated heart rate and blood pressure monitor (UA-611; A&D Company Limited, Japan) immediately before and after the 1st bath, immediately before and after the 2nd bath, and immediately after the 2nd wrap.

Body mass, urine and blood sampling

Change in body mass, measured to the nearest 0.05 kg (model #63667; Soehnle, Germany), was the primary outcome measure. Body mass was measured in minimal clothing, i.e. lower body short underwear in the form of briefs or boxer briefs, at several time-points: (i) upon waking on the day before bathing (Morning Day -1), (ii) upon waking on the day of bathing (Morning Day 0), (iii) immediately before the 1st bath, (iv) immediately before the 2nd bath, (v) immediately after the 2nd wrap, (vi) upon waking on the day after bathing (Morning Day +1), and finally (vii) on the day after bathing at "weigh-in" immediately to the performance test battery (Weigh-in Day +1). Change in body mass induced by the entire RWL process, and whether a body mass deficit was present after recovery, were both calculated compared to body mass at Morning Day -1.

Urine samples for the measurement of urine osmolality (Osmocheck Portable Osmometer; Vitech Scientific, UK) were taken upon waking on Day -1, Day 0, and Day +1. Participants were classified as hypohydrated using the criterion of urine osmolality of >700 mOsmol/kg (Sawka et al. 2007).

Capillary blood was sampled at four timepoints: (i) before undertaking the performance test battery on Day -2, (ii) immediately before the 1st bath, (iii) immediately after the 2nd wrap, and finally (iv) before re-testing of performance on Day +1. Participants were seated upright and stationary for ~3 min before a fingertip capillary blood sample (95 L) was collected and analyzed for blood chemistry (glucose, blood urea nitrogen [BUN], creatinine, hematocrit, hemoglobin, the Anion Gap, sodium, potassium, chloride, ionized calcium and total CO2) using the i-STAT 1 point-of-care handheld blood analyzer and CHEM8+ cartridges (Abbott

Laboratories, USA) according to the manufacturer's instructions. The CHEM8+ cartridges were the best available tool for point-of-care blood analysis, and our specific interest from this list of analytes were BUN and creatinine as indicators of acute kidney injury; sodium, potassium and chloride as indicators of change in circulating electrolytes sensitive to sweat losses and dehydration; whereas the data for hemoglobin and hematocrit were used to calculate percentage change in plasma volume using the method of calculation described by Dill and Costill (1974). Due to technical issues resulting in missing data points, data for blood analysis are reported as n=10 or n=11 where appropriate.

Performance test battery

The performance test battery was identical on Day -2 and Day +1. After arrival and having a capillary blood sample taken, participants performed a standardized general warm-up. First, 10 min of cycle ergometry (Wattbike Pro; Wattbike Ltd., Nottingham, England) at a cadence of >70 rpm and a self-selected moderate intensity (rating of perceived exertion of 12 to 15). Next, bodyweight exercises consisting of five squats, five split-squats each side, five push-ups, and five CMJs were performed, after which lastly, another 5 min of cycle ergometry and the same bodyweight exercises were performed.

Leg power was measured by CMJ for which five jumps in total were performed with ten seconds of rest taken between each jump. The participants were instructed to jump with maximal effort on each jump, and were required to keep the hands firmly placed on the hips throughout the jump. Jumps were performed on a dual force plate system sampling at 500 Hz (Pasco PS-2141; Pasco Scientific Corp, USA) and CMJ height was calculated as previously described (Jordan et al. 2018). Data are reported as jump height (in cm) calculated as the average of three jumps after the worst and best jumps of the five attempts were excluded. The coefficient of variation (%CV) for this parameter was 5.7% in this cohort of athletes.

Isometric hand-grip strength test was measured using a hand-grip dynamometer (TKK 5401 Grip-D; Takei Scientific Instruments Co, Japan). The dynamometer was held at shoulder height to start and the participants were instructed to apply maximum force while lowering their arm to their side while in full elbow extension (Savva et al. 2013). Two maximum efforts per hand were performed by alternating each side, with the best score for each hand being recorded and averaged as a composite score. The %CV for this parameter was 5.0% in this cohort of athletes.

IMTP was performed in a customised power rack (Grip Ltd.; Ireland) standing on a dual force plate system using a standardised protocol as previously described (Halperin et al. 2016). Participants were positioned in a body position similar to completing the second pull of a power clean with a flat trunk position and their shoulders in line with the bar. This position allowed participants to maintain a knee angle of ~120 to ~130°. Two 3 sec IMTP efforts were performed applying 50% and 80% of perceived maximum effort. After these priming efforts, 30 sec of rest was taken before completing the three 3 sec maximal efforts separated by seated rest for 150 sec. Data are reported as peak force given that this measure is the most reliable measure from the data output (Brady et al. 2020). The %CV for this parameter was 7.2% in this cohort of athletes.

FTP was estimated using the 3 min all-out test (Burnley et al. 2006) performed on an electromagnetically and air-braked cycle ergometer (Wattbike Pro; Woodway Inc., USA)

(Wainwright et al. 2017), using a previously-validated protocol (Hanson et al. 2019). Handlebar and saddle position/height were recorded during the familiarisation visit and replicated for each subsequent testing day. The warm-up was standardised as 5 min of cycling at cadence of >70 rpm and the same self-selected moderate intensity as above. The goal of this test is then to maintain the highest power output possible for the 3 min of effort. Cadence was kept between 90 and 110 rpm for the duration of the test. On conclusion of the test, maximum heart rate (via telemetry; Polar, Finland) and FTP were extracted for analysis. The %CV for maximum heart rate and FTP were 3.5% and 3.0%, respectively, in this cohort of athletes.

The smallest worthwhile difference (SWD) for each of the performance tests was set at 0.2 between-subject SD, which is suggested to represent a practically-relevant change in performance in athletes (Hopkins et al. 2009). Thus, in this study the SWD corresponded to 0.6 cm for CMJ height, 1.5 kg for hand-grip strength, 56 N for IMTP peak force, 2.3 bpm for maximum heart rate, and 5.1 W for FTP.

Sample size calculation and early termination

The primary outcome was change in body mass as a consequence of the 2 h bath and wrap protocol. Therefore, a sample size calculation was performed (G*Power v.3.1) based on previous research demonstrating an effect of salt water to augment the magnitude of body mass lost during HWI when compared to fresh water (Hope et al. 2001). Using the body mass lost after 2 h of that 4 h immersion protocol, a time point analogous to the present work, and that being 0.98±0.44 kg and 1.24±0.80 kg for fresh water and salt water respectively, and an assumed correlation between conditions of 0.90, the required sample size to detect a difference between FWB and SWB at a Type I error rate () of 0.05 and a power (1-) of 0.8 was n=26. However, given the absence of effect in our previous research using a salt concentration of ~1.6% (Connor et al. 2020; Connor and Egan 2021), a priori we planned an interim data analysis for the assessment of futility, and therefore discontinuation, after completion of 50% of the required sample size i.e. n=13. In the absence of any difference between FWB and SWB for change in body mass with n=13 (P=0.647 between trials, d=0.09; data reported below), we discontinued recruitment at that time.

Statistical Analysis

Statistical analysis and graphical representation were performed using GraphPad Prism v9.1 (GraphPad Software, Inc., USA). Normality of data was assessed with the Shapiro-Wilk normality test for which all data passed. All data are presented as mean±SD. A two-way (condition*time) repeated measures analysis of variance (ANOVA) was used to assess responses to the interventions for variables with serial measurements. A one-way repeated measures ANOVA was used to assess whether an order effect was present in the indices of performance from Trial 1 to Trial 2 regardless of salt condition. When a main or interaction effect was observed, pairwise comparisons were performed with Bonferroni's correction for which multiplicity-adjusted P-values are reported. Paired t-tests were used to assess differences between conditions for variables with two measurements, including to assess whether an order effect was present when comparing Trial 1 and Trial 2. The level of statistical significance for all tests was set at $P<0.05$. Standardised differences in the mean were used to assess magnitudes of effects between conditions. These were calculated using Cohen's d effect size and are interpreted using thresholds of <0.2, ≥0.2, ≥0.5 and ≥0.8 for trivial, small, moderate, and large, respectively.

Results

Water temperature

The starting water temperature did not differ between trials (1st bath, P=0.374; 2nd bath, P=0.133). The starting water temperature was 40.31±0.32ºC and 40.62±0.37ºC for the 1st and 2nd baths, respectively, in FWB (P=0.240), and 40.46±0.44ºC and 40.42±0.31ºC for the 1st and 2nd baths, respectively, in SWB (P=0.744). No interaction effect was observed for the effect of salt (1st bath, P=0.343; 2nd bath, P=0.297), and average water temperature remained above 40ºC throughout the bathing periods (Figure 6.2A & 6.2B). The volume of boiling kettle water added to each bath was 4.39±1.14 L for FWB and 3.46±1.42 L for SWB during the 1st bath of each trial (P=0.055), and 2.31±1.80 L for FWB and 2.65±1.64 L for SWB during the 2nd bath of each trial (P=0.513).

Forehead temperature and heart rate response to the bathing protocols

Forehead temperature increased in response to the hot bath protocol in both the 1st and 2nd bath periods (main effect of time, P<0.001 for both) (Figure 6.2C & 2D). Resting heart rate was similar for each trial before the 1st bath (FWB, 67±18 bpm; SWB, 65±11 bpm). Heart rate increased in response to the hot bath protocol (main effect of time, P<0.001) and reached a measured peak of 128±19 bpm and 127±21 bpm after the 2nd bath period during FWB and SWB respectively, but no main effect of condition (P=0.166) or interaction effect (P=0.762) were observed (Figure 6.3).

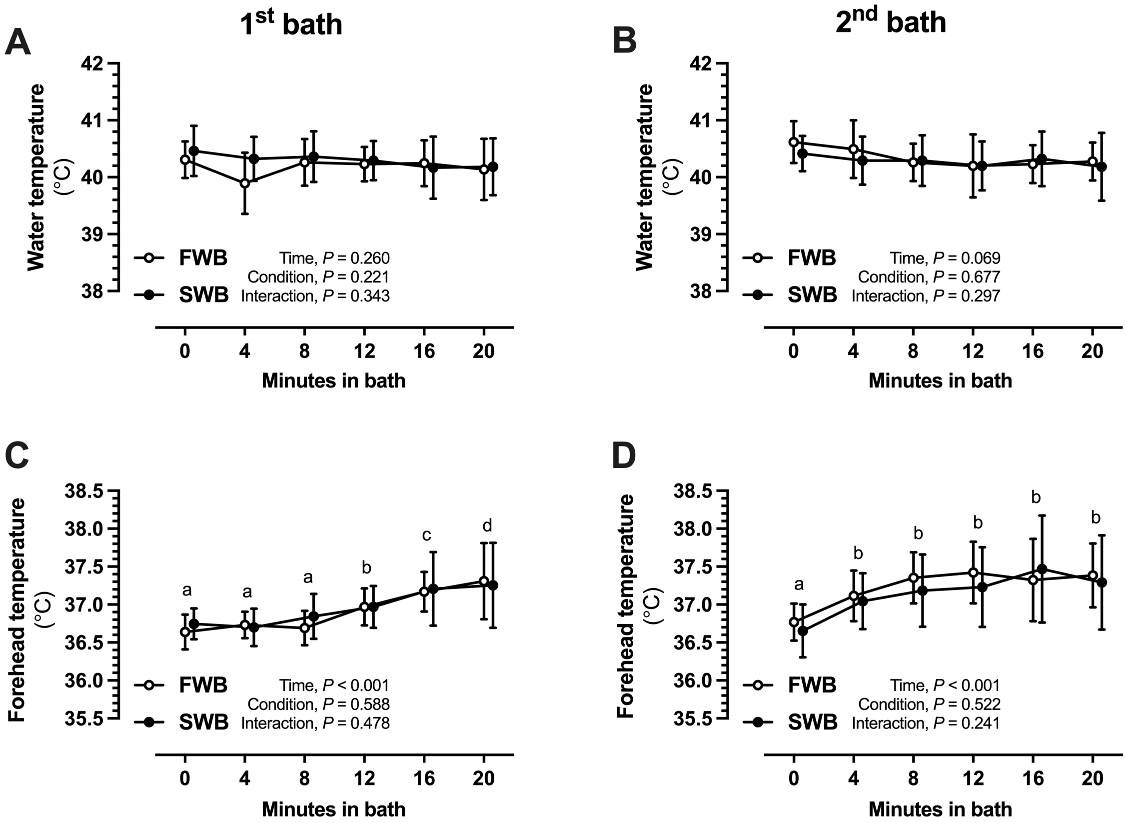

Figure 6.2 Water temperatures measured at 4 min intervals during each bath during experimental trials of fresh water or salt water; and quantity of boiling kettle water added per bath

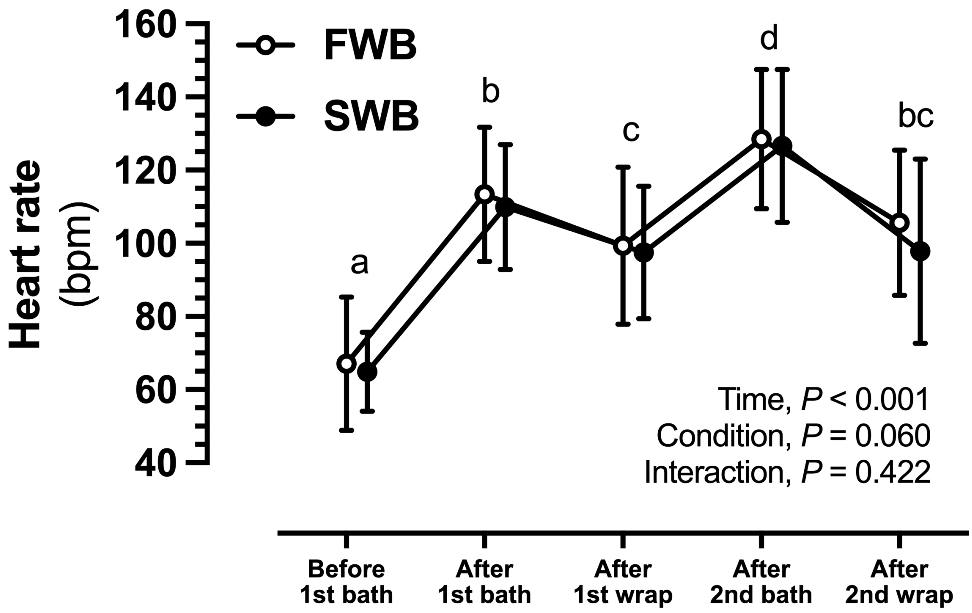

Figure 6.3. Heart rate responses to hot water immersion during experimental trials of fresh (FWB) or salt water (SWB). Data points are mean values (n=13, all male) with vertical bars representing SD. Differences within conditions are noted by different letters representing significant differences (P<0.05) between respective timepoints, whereas timepoints with the same letter are not different to each other.

Changes in body mass

For change in body mass in absolute (kg) (Table 6.1) and relative (%initial body mass) (Figure 6.4) terms, a main effect of time ($P<0.001$), but neither a main effect of condition, nor a condition*time interaction effect, was observed. Similarly, there was no difference between conditions for changes in urine osmolality at the various time points (Table 6.1).

Body mass losses induced by carbohydrate and fluid restriction were 2.18±1.18 kg ($P<0.001$; d=0.26) and 1.98±0.95 kg ($P<0.001$; d=0.24) in preparation for the FWB and SWB trials, respectively. These values represented losses of relative to initial body mass on Morning Day -1 of 2.72±1.47% and 2.47±1.18% for the FWB and SWB protocols, respectively.

Body mass losses induced by the hot bath protocols were 2.17±0.81 kg ($P<0.001$; d=0.28) and 2.24±0.64 kg ($P<0.001$; d=0.28) for the FWB and SWB protocols, respectively, which corresponded to 2.70±1.01% of initial body mass for FWB, and 2.78±0.79% of initial body mass for SWB (Figure 6.4A). Analysis for the presence of an order effect demonstrated no difference ($P=0.704$) in body mass losses induced by the hot bath protocols when analyzed as Trial 1 (2.27±0.71 kg) versus Trial 2 (2.14±0.75 kg). FWB resulted in body mass loss of 1.15±0.63 kg ($P=0.001$; d=0.15) during the 1st bath and wrap, and 1.02±0.31 kg ($P<0.001$; d=0.13) during the 2nd bath and wrap. SWB resulted in body mass loss of 1.18±0.25 kg ($P<0.001$; d=0.15) during the 1st bath and wrap, and 1.06±0.45 kg ($P<0.001$; d=0.13) during the 2nd bath and wrap.

Total body mass losses induced by the entire RWL protocol were 4.35±1.60 kg ($P<0.001$; d=0.53) and 4.22±1.17 kg ($P<0.001$; d=0.51) for the FWB and SWB protocols, respectively. These values represented losses of relative to initial body mass on Morning Day

-1 of 5.42±1.99% and 5.25±1.46% for the FWB and SWB protocols, respectively (Figure 6.4B).

On Morning Day -1, 9 (FWB trial) and 7 (SWB trial) were classified as hypohydrated with a urine osmolality of >700 mOsmol/kg (Sawka et al. 2007). On Morning Day +1, 7 (FWB trial) and 9 (SWB trial) were classified as hypohydrated, and 8 (FWB trial) and 6 (SWB trial) participants were in a body mass deficit compared to Morning Day -1. However, at Weigh-in Day +1 i.e. before the performance test battery, only 4 (FWB trial) and 2 (SWB trial) participants were in a body mass deficit compared to Morning Day -1. Overall, weight regain from the end of the 2nd wrap period to Weigh-in Day +1 was 4.83±1.41 kg ($P<0.001$; d=0.62) and 4.92±1.27 kg ($P<0.001$; d=0.59) during recovery from the FWB and SWB protocols, respectively (shown as % of initial body mass in Figure 6.4C), resulting in a body mass surplus compared to Morning Day -1 of 0.47±1.48 kg and 0.69±0.83 kg, respectively (shown as % of initial body mass in Figure 6.4D).

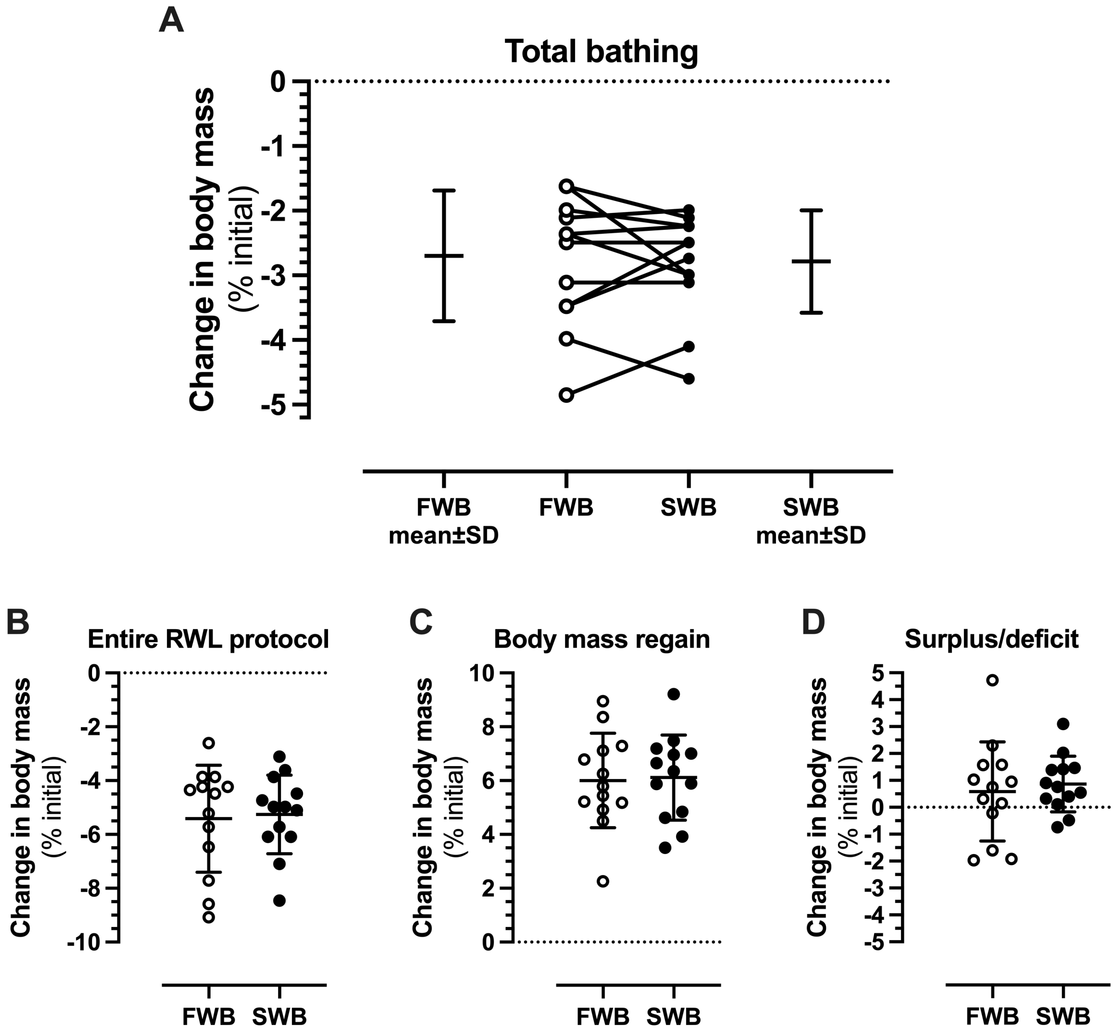

Figure 6.4 Percentage changes in body mass (relative to baseline recorded on Morning Day -1) induced during (A) a hot bath protocol in fresh (FWB) or salt water (SWB) for a 2 h period comprising both baths and wraps, (B) the entire rapid weight loss (RWL) intervention, (C) the period of weight regain before weigh-in on Day +1, and (D) as a measure of total body mass deficit or surplus at weigh-in on Day +1 compared to Morning Day -1. White (FWB) and black (SWB) circles in each panel represent individual data points. Mean values (n=13, all male) are represented by the horizontal solid line with vertical bars representing SD for changes observed within each time period that is defined above each panel.

Indices of performance

Across the five timepoints measured (FAM, Trial 1 Day -2, Trial 1 Day +1, Trial 2 Day -2, Trial 2 Day +1), there was no order effect observed for any of indices of performance, i.e. CMJ height (P=0.907), hand-grip strength (P=0.722), IMTP peak force (P=0.537), maximum heart rate (P=0.284), and FTP (P=0.874). Comparing between FWB and SWB trials, in the 3 min all-out test the absence of interaction effects or main effects of time are indicative of there being no significant differences in FTP or maximum heart rate either between or within conditions (Table 6.2). Similarly, no differences in CMJ height, hand-grip strength, or IMTP peak force either between or within conditions were observed (Table 6.2).

Blood markers

For blood markers, the absence of interaction effects or main effects of condition are indicative of there being no significant differences between conditions on these markers (Table 6.3). A main effect of time (all P0.01) was observed for several markers (BUN, chloride, creatinine, hemoglobin, hematocrit, and sodium) with each being increased in blood samples taken immediately after the 2nd wrap period, but returning to values similar to Day -2 when measured after the period of recovery up to sampling at Weigh-in Day +1 (Table 6.3). Declines in plasma volume induced by the entire RWL protocol were estimated as being -14.1±12.1% for FWB (P=0.003; d=1.64) and -13.0±11.4% for SWB (P=0.004; d=1.62).

Discussion

Given that our previous work using ~1.6% salt solutions did not reveal an effect of salt to augment body mass loss during a hot bath protocol (Connor et al. 2020; Connor and Egan 2021), the present study investigated body mass losses when the salt concentration is increased to ~5.0%wt/vol This higher concentration is more similar to immersion studies where an effect of salt to augment the loss of fluid and/or body mass has been observed (Whitehouse et al. 1932; Hertig et al. 1961; Hope et al. 2001). However, the present study demonstrates that the body mass lost during a hot bath protocol using fresh water (FWB) is similar to a protocol using ~5.0%wt/vol of Epsom salt (SWB).

Body mass losses induced by ~26 h to ~28 h of restriction of fluid intake combined with a low residue, low carbohydrate diet were ~2.6% of body mass. This is similar in magnitude to the suggestion of a ~3% reduction to be expected by short-duration restriction of carbohydrate and fluid, and emptying of the gastrointestinal contents using a low residue diet (Reale et al. 2017; Burke et al. 2021), and is also similar to our previous work (Connor et al. 2020; Connor and Egan 2021). However, the percentage of body mass lost during the entire RWL process was greater in the present study at ~5.3% compared to those previous studies where ~4.3% (Connor et al. 2020) and ~4.5% (Connor and Egan 2021) was observed. The larger magnitude is explained by differences in percentage of body mass lost in the bathing protocol, which was ~2.7% loss of body mass in this study compared to ~2.1% in the other studies. This difference may simply reflect inter-individual differences in RWL between studies. Alternatively, commencing bath in water of higher temperature (e.g. ~40.3ºC versus 37.8ºC), and using a sauna blanket for the wrap periods rather than cotton clothing in a warm room, may result in more efficient loss of body mass per unit of time invested in such a protocol.

Together these findings across three studies suggest that the addition of salt to HWI does not augment the loss of body mass compared to fresh water, at least in the hot bath protocol employed. The caveat that this conclusion only applies to the hot bath protocol employed is important because several prior studies do indeed demonstrate an effect of salt to augment immersion-induced loss of fluid and/or body mass in various experimental models including whole-body immersion, and localised immersion of an arm/hand or leg/foot (Whitehouse et al. 1932; Buettner 1953; Peiss et al. 1956; Buettner 1959; Hertig et al. 1961; Brebner and Kerslake 1964; Hope et al. 2001). There are two suggested mechanisms for this phenomenon. Firstly, that during immersion in salt water, the osmotic pressure difference between the immersion medium and body fluids results in greater fluid loss compared to fresh water, and/or secondly, that salt water serves to attenuate an inhibitory influence on the decline in sweat rate that usually occurs with prolonged immersion in hot fresh water (Whitehouse et al. 1932; Buettner 1953; Peiss et al. 1956; Buettner 1959; Hertig et al. 1961; Brebner and Kerslake 1964; Hope et al. 2001).

The absence of an effect of salt in our work may be explained by duration of immersion being much shorter than those previous studies observing an effect. For example, those studies have used immersion times of 3 h (Hertig et al. 1961), 4 h (Hope et al. 2001) and 5 h (Whitehouse et al. 1932; Brebner and Kerslake 1964). Despite our protocol comprising of 2 h of passive heating, HWI only accounts for 2x20 min of this time period. Hope et al. (2001) observed a difference of ~600 g of body mass lost in 4 h when comparing immersion in fresh water to salt water (sea water) at 38ºC (Hope et al. 2001). This difference between conditions would be the equivalent of ~2.5 g per minute assuming linearity in the response. Translating this rate into our 40 min of total time spent immersed in water would result in an expected difference of just 100 g between FWB and SWB. Therefore, for the addition of salt to have the desired impact of augmenting loss of body mass through passive fluid loss, much longer immersion times than the 2x20 min employed in this study may be required.

Another consideration, however, is the osmolality of the salt water given the proposed mechanism around the osmotic pressure difference between the immersion medium and body fluids. While the %wt/vol of salt is most commonly used as the descriptor of the salt water condition, the osmolality will be a function of both the concentration and type of salt. Our previous work using 1.6%wt/vol of Epsom salt (Connor et al. 2020; Connor and Egan 2021), and the present study using 5.0%wt/vol of Epsom salt, would result in an osmolality of ~130 mOsmol/kg and ~406 mOsmol/kg, respectively, which would decline somewhat with the addition of boiling water to maintain or increase the water temperature while bathing. Thus, these salt water baths were, respectively, hypotonic and only mildly hypertonic relative to the osmolality of body fluids (i.e. ~280 mOsmol/kg to ~295 mOsmol/kg). In contrast, when augmented body mass losses have been previously observed, these salt water baths were markedly hypertonic i.e. 5%wt/vol of sodium chloride (Hertig et al. 1961) being ~1709 mOsmol/kg, and seawater (Hope et al. 2001) being ~3.5% salt and ~1000 mOsmol/kg to ~1200 mOsmol/kg. Therefore, while Epsom salt was used for its ecological validity, a salt such as sodium chloride may be more effective on a %wt/vol basis. Alternatively, Epsom salt would need to be used at >12.3%wt/vol to produce an osmolality of >1000 mOsmol/kg. These points assume that the osmotic gradient is an important mechanism by which salt water augments loss on body mass during HWI, and tentatively that >1000 mOsmol/kg is a valid threshold above which these effects would be observed.

The present study extends our previous work by measuring heart rate during the hot bath protocol, and measuring changes in blood markers during the RWL process, in addition to investigating of effects the RWL followed by ~24 h to ~26 h of recovery on indices of performance. The heart rate data during the hot bath protocol demonstrates a moderate degree of cardiovascular stress was induced as indicated by heart rate averaging ~110 bpm throughout the 2 h period and a measured peak at 128±19 bpm and 127±21 bpm during FWB and SWB, respectively. These values are equivalent to ~68% of the participants' age-predicted maximum heart rate.

The concentrations of several analytes in blood were increased during the hot bath protocol. Specifically, in blood samples taken immediately after the 2nd wrap period, concentrations of

BUN, chloride, creatinine, hemoglobin, and sodium were each increased, as was the hematocrit value, but each returned to values similar to baseline by weigh-in on Day +1. Calculation of plasma volume from hemoglobin and hematocrit revealed an average decrease in plasma volume induced by RWL of ~14% when measured upon completion of the 2nd wrap. This value is somewhat greater than that observed by Hope et al. (2001) of ~7 to ~12%, but perhaps unsurprising given that the overall loss of body mass during the RWL protocol in the present study was approximately double of that previous work. In contrast, when elite amateur boxers undertook RWL in which a similar quantity of body mass was lost (5.6% ±1.7%), the reduction in plasma volume was smaller at 8.6±3.9% (Reljic et al. 2013). In that study, the RWL process was over a five day period, which potentially suggests that a shorter time frame of RWL and/or exposure to HWI may lead to greater loss of plasma volume or differential effects on different compartments of body water. There are two caveats that apply to the interpretation of these data for plasma volume. Firstly, the i-STAT blood analyser derives the value for hemoglobin using a proportionality constant after the measurement of hematocrit by a conductometric method, so the plasma volume data are based on an estimation of hemoglobin rather than direct measurement. Alternatively, by using hematocrit only and thereby calculating loss of blood volume (Dill and Costill 1974), RWL resulted in a decrease in blood volume by ~8% in both conditions upon completion of the 2nd wrap. Secondly, postural changes are known to acutely influence measures of plasma volume (Pivarnik et al. 1986; Lippi et al. 2015), and a reduction in plasma volume of ~4.8% was previously observed within the initial 5 min after moving from a supine to seated (Pivarnik et al. 1986). Although the seated posture and rest period was consistent before blood sampling on Day -2, before the 1st bath on Day 0, and on Day +1, the sample taken upon completion of the 2nd wrap was preceded by 40 min in a supine position and only ~3 min of equilibration in a seated position. Therefore, the change in posture from a supine to seated position may have also contributed to decrease in plasma volume observed in response to the hot bath protocol.

Also of note is the observation of increased BUN and creatinine concentrations as these are often used as biomarkers of acute kidney injury (AKI) (Edelstein 2008; Kellum and Lameire 2013; Ostermann et al. 2020). RWL of >4% of body mass consistently results in an increase in BUN and creatinine, which has been suggested as an indication of AKI being caused by RWL (Lakicevic et al. 2021). AKI has been previously defined as an increase in serum creatinine concentration by ≥0.3 mg/dL within 48 h (Kellum and Lameire 2013), a threshold which just two of our participants exceeded and which occurred within the 2 h bathing period. Additionally, the utility of circulating BUN and creatinine concentrations as sensitive and specific markers of AKI has been questioned (Edelstein 2008; Ostermann et al. 2020), whereas traditional measures of AKI are limited in their ability to classify AKI during heat stress, especially when combined with dehydration and/or exercise (Chapman et al. 2021). BUN and creatinine concentrations are indirect measures of AKI rather than direct measures of tissue injury such as with creatine kinase and cardiac troponin from skeletal muscle and heart, respectively. Direct measures of AKI in the circulation remain to be firmly established, especially those that can differentiate between 'pre-renal' and 'intrinsic' causes of change in circulating markers (Edelstein 2008; Ostermann et al. 2020; Chapman et al. 2021). Moreover, changes in BUN and creatinine concentrations are generally delayed in their response to AKI rather than acutely responsive (Edelstein 2008; Ostermann et al. 2020). Our data indicate an acute response in creatinine concentration to increase over the 2 h bathing period, whereas BUN concentration was already increased after the diet and fluid restriction, and increased further during bathing. Hence, it remains unclear whether these increases can indeed be considered to be evidence of AKI, or whether these simply reflect the well-established hemoconcentration effect of an acute decrease in plasma volume (Harrison 1985). In favour of the former is that it well-established that heat stress, especially when combined with physical exertion, can result in AKI (Chapman et al. 2021). Especially relevant to the present study is that heat stress-associated AKI is also influenced by hydrostatic pressure of water when HWI is used to apply the heat stress in experimental contexts (Chapman et al. 2021). Therefore, changes in markers of AKI during RWL and comprehensive assessment of kidney function should continue to be investigated by future research in order to better understand this phenomenon given its implications for the welfare of athletes who repeatedly undertake RWL.

Immediately before the performance testing on Day +1 represented a ~24 to ~26 h recovery period at which point only 4 (FWB trial) and 2 (SWB trial) participants remained in a body mass deficit compared to Morning Day -1. On average, there was a body mass surplus of 0.47±1.48 kg and 0.69±0.83 kg compared to Morning Day -1 after recovery from FWB and SWB, respectively. This surplus is in contrast to the deficit observed on average in our previous work (Connor et al. 2020; Connor and Egan 2021), but is explained by the ~2 h to ~4 h longer recovery time in the present study due to the inclusion of the performance tests. Therefore, despite the loss of ~5.3% of body mass in ~28 h to ~30 h, blood markers had returned to values similar to baseline after ~24 h to ~26 h of recovery. In practice, the time from weigh-in until official competition in professional MMA is usually longer i.e. ~30 h to ~36 h, but even with a longer time period for rehydration, the majority of MMA athletes have been observed to be hypohydrated up to 2 h before competition (Jetton et al. 2013; Matthews and Nicholas 2017). Based on these observations, regain of body mass alone was suggested as potentially not being a good indicator of returning to a euhydrated state, but there is some debate about the validity of the classification of hypohydration through assessment of hydration status by spot analysis with urine measures (Cheuvront et al. 2015; Barley et al. 2020). For example, an alternative to the criterion of urine osmolality of >700 mOsmol/kg being classified as hypohydration (Sawka et al. 2007) has been proposed as 925 mOsmol/kg (Armstrong et al. 2010). Using the Armstrong et al. threshold, only 3 (FWB trial) and 3 (SWB trial) participants were classified at hypohydrated at Morning Day +1 compared to 7 (FWB trial) and 9 (SWB trial) using the Sawka et al. threshold.

No indices of performance were impacted by the RWL and recovery process when compared to pre-RWL values in either the FWB and SWB conditions. These results are in contrast to studies that have demonstrated a residual negative impact on indices of performance after ~24 h of recovery (Alves et al. 2018; Barley et al. 2018b; Kurylas et al. 2019). In one study, athletes were dehydrated by ~5% of body mass through exercise in a heated room, and performance tests were completed 3 h and 24 h after the intervention (Barley et al. 2018b). Vertical jump was unaffected by dehydration and recovery; hand-grip strength was weaker at 3 h but not 24 h; medicine ball chest throw distance was shorter at 24 h, but not 3 h; and repeated sled push performance was worse at both 3 h and 24 h after dehydration (Barley et al. 2018b). Therefore, there are likely to be time course-specific effects on performance in response to RWL and recovery, and which may also be impacted by whether passive or active methods of dehydration are employed, and the choice of performance test. Active methods of dehydration, i.e. involving exercise, may lead to residual fatigue and depletion of energy stores (Savoie et al. 2015), and can produce divergent responses in relation to changes in plasma volume, serum and urine osmolality, and performance, compared to passive dehydration (Nielson et al. 1981; Caldwell et al. 1984; Muñoz et al. 2013). Moreover, if a chosen performance test is not sensitive enough to detect physiological and performance changes, if any, that may be happening in response to RWL, the conclusion that there are no negative performance consequences of RWL when followed by adequate recovery and rehydration may be a type II error i.e. false negative finding.

Relatedly, this study was powered using the primary outcome of change in body mass as a consequence of the 2 h bath and wrap protocol. Given the absence of effect in our previous research using a salt concentration of ~1.6% (Connor et al. 2020; Connor and Egan 2021), like our previous approach (Connor and Egan 2021) a priori we planned an interim data analysis for the assessment of futility, and therefore discontinuation. However, the sample size was based on data derived from pre-to-post differences in a crossover design, and therefore, it is likely that the sample size is underpowered for the analysis of serial time point data such as those analysed by ANOVA. In this scenario, again a type II error for observing the lack of differences between FWB and SWB cannot be fully discounted. Additionally, there are several methodological limitations that could be addressed in future work including the measurement of body temperature with a valid measure of core temperature (either esophageal or rectal), and the inclusion of a body mass measurement immediately after each period of HWI in order to isolate the effects of salt versus fresh water during HWI specifically rather than the entire hot bath protocol including wrapping periods.

In summary, short duration HWI combined with periods under an infrared sauna blanket is an effective method of RWL to induce a loss of ~2.7% of body mass during 2 h of bathing (2x20 min) and wrapping (2x40 min). Using this protocol, the total amount of body mass lost when the water was supplemented with ~5.0%wt/vol of Epsom salt was similar to fresh water. When an appropriate refuelling and rehydration strategy was followed, the ~5.3% loss of body mass during the overall ~28 h to ~30 h RWL period was not detrimental in terms of blood markers or indices of performance measured after the ~24 h to ~26 h recovery period.

Table 6.1. Body mass (kg) and hydration status assessed by urine osmolality (mOsmol/kg) at time points during a rapid weight loss protocol featuring a hot bath protocol in fresh (FWB) or salt water (SWB).

	Morning Day -1	**Morning Day 0**	**Before 1st bath**	**After 1st bath & wrap**	**After 2nd bath & wrap**	**Mornin g Day +1**	**Weigh-in Day +1**	***P* value**
Body mass (kg)								T, *P*<0.00 1***
FW B	82.95±8. 78 [a]	81.09±7. 89	80.76± 7.79	79.62± 7.70	78.59± 7.64	82.45± 7.83 [a]	83.42± 7.84 [a]	C, *P*= 0.754
SW B	82.86± 8.69 [a]	81.16± 8.24	80.88± 8.02	79.70± 8.00	78.64± 7.99	85.14± 7.52 [a]	83.56± 8.63 [a]	I, *P*= 0.655
Urine osmolality (mOsmol/kg)								T, P= 0.002**
FW B	762±217 [a]	955±145			1102± 70	673± 318 [a]		C, P= 0.570
SW B	695±252 [a]	872±185			992±98	794± 274 [a]		I, P= 0.067

Data are presented as mean±SD, n=13. **P<0.01 and ***P<0.001 for main and interaction effects from the two-way (condition*time) ANOVA analyses. Differences within conditions are noted by superscripted letters where different letters represent significant differences (P<0.01) between respective timepoints, whereas timepoints with the same letter are not different to each other.

Table 6.2. Countermovement jump (CMJ) height, hand-grip strength, isometric mid-thigh pull (IMTP) peak force, functional threshold power (FTP) and maximum heart rate (HR) measured before (Day -2) and ~28 h after (Day +1) a rapid weight loss intervention featuring a hot bath protocol in fresh (FWB) or salt water (SWB).

	FAM	FWB		SWB		*P* Values
		Day -2	Day +1	Day -2	Day +1	
Hand-grip strength (kg)	46.7±6.5	48.0±6.8	48.9±7.9	48.8±8.0	47.5±7.6	T, $P = 0.503$; C, $P = 0.739$; I, $P = 0.066$
CMJ height (cm)	31.11± 4.36	32.93± 2.71	32.86 ± 3.30	31.81 ± 3.86	32.29 ± 3.06	T, $P = 0.572$; C, $P = 0.080$; I, $P = 0.435$
IMTP Peak Force (N)	1766 ± 280	1690 ± 267	1757 ± 276	1739 ± 288	1729 ± 287	T, $P = 0.435$; C, $P = 0.785$; I, $P = 0.152$
3 min all-out test FTP (w)	213.8 ± 21.0	219.6 ± 22.9	220.6 ± 26.4	222.2 ± 25.4	223.7 ± 27.8	T, $P = 0.642$; C, $P < 0.068$; I, $P = 0.854$
3 min all-out test Max HR (bpm)	182.5 ± 9.4	183.5 ± 10.1	185.2 ± 11.4	185.2 ± 12.4	186.1 ± 11.4	T, $P = 0.374$; C, $P = 0.228$; I, $P = 0.790$

Data are presented as mean±SD, n=13. FAM, familiarization trial. Data for FAM are included for descriptive purposes. P values are obtained from two-way (condition*time) ANOVA analyses on the FWB and SWB data.

Table 6.3. Blood markers measured at time points during a rapid weight loss intervention featuring a hot bath protocol in fresh (FWB) or salt water (SWB).

		Day -2	Before 1st Bath	After 2nd Wrap	Day + 1	P Value
Glucose mg/dL	FWB	94.3±9.2	88.1±9.1	91.8±14.8	100.2±13.1	T, *P* = 0.041; C, *P* = 0.290; I, *P* = 0.024*
	SWB	94.8±8.7	90.7±6.1	104.9±16.1	94.9±11.5	
BUN mg/dL	FWB	22.2±4.4	26.9±6.9 [a]	27.8±7.3 [a]	20.9±6.6	T, *P* = 0.001**; C, *P* = 0.030; I, *P* = 0.481
	SWB	18.8±5.2	25.5±7.4 [a]	27.3±6.7 [aa]	20.0±5.8	
Creatinine mg/dL	FWB	0.99±0.16	1.03±0.14	1.19±0.23 [a]	1.13±0.18	T, *P* = 0.001**; C, *P* = 0.125; I, *P* = 0.071
	SWB	0.96±0.11	1.00±0.11	1.20±0.12 [aaa]	0.94±0.10	
Hematocrit %	FWB	44.2±3.7	45.5±3.1	48.2±4.0 [a]	45.1±3.4	T, *P* = 0.001*; C, *P* = 0.517; I, *P* = 0.644
	SWB	44.3±3.2	46.2±2.1	47.9±2.3 [a]	46.1±2.7	
Hemoglobin g/dL	FWB	15.0±1.3	15.5±1.1	16.4±1.4 [a]	15.3±1.2	T, *P* = 0.001**; C, *P* = 0.536; I, *P* = 0.640
	SWB	15.1±1.1	15.7±0.7	16.3±0.8 [a]	15.7±0.9	
Change in Plasma Volume (%)	FWB		-5.1±7.4	-14.1±12.1 [a]	2.7±13.6	T, *P* = 0.001**; C, *P* = 0.630; I, *P* = 0.657
	SWB		-7.0±10.0	-13.0±11.4 [a]	-6.3±12.8	
AnGap mmol/L	FWB	16.4±1.8	16.4±2.7	16.0±2.4	15.2±2.8	T, *P* = 0.805; C, *P* = 0.851; I, *P* = 0.173
	SWB	15.7±1.6	15.7±2.1	16.3±2.6	16.6±1.9	
Sodium mmol/L	FWB	139.9±2.2	141.3±1.6	143.5±2.8	139.7±1.7	T, *P* < 0.001***; C, *P* = 0.361; I, *P* = 0.814
	SWB	140.5±1.5	141.3±1.3	144.1±2.5	140.1±1.8	
Potassium mmol/L	FWB	4.63±0.43	4.87±0.37	4.75±0.39	4.67±0.41	T, *P* = 0.642; C, *P* = 0.227; I, *P* = 0.272
	SWB	4.82±0.36	4.80±0.27	4.80±0.32	4.90±0.24	
Chloride mmol/L	FWB	103.6±2.7	105.5±2.5	108.3±3.1	103.6±1.6	T, *P* = 0.001**; C, *P* = 0.547; I, *P* = 0.507
	SWB	103.6±1.6	105.5±1.5	107.8±2.9	102.7±1.7	

		Day -2	Before 1st Bath	After 2nd Wrap	Day + 1	P Value
iCalcium mmol/L	FWB	1.25±0.07	1.29±0.08	1.29±0.07	1.35±0.23	T, *P* = 0.311; C, *P* = 0.983; I, *P* = 0.583
	SWB	1.29±0.07	1.26±0.08	1.32±0.10	1.31±0.07	
TCO2 mmol/L	FWB	25.5±1.6	25.7±2.3	25.3±1.6	26.5±2.2	T, *P* = 0.125; C, *P* = 0.283; I, *P* = 0.334
	SWB	27.1±1.6	25.8±1.2	25.6±1.4	26.6±1.6	

Data are presented as mean±SD, n=10 or 11. BUN, blood urea nitrogen; CO2, carbon dioxide. *P<0.05; **P<0.01; ***P<0.001 for main and interaction effects from the two way (condition*time) ANOVA. Where a main effect of time was indicated, differences within conditions are noted by aP<0.05, aaP<0.01, and aaaP<0.001 compared to Pre-testing Day -2, and bP<0.05, bbP<0.01, and bbbP<0.001 compared to Before 1st bath.

Chapter 7 - Conclusion

Main research findings

When starting this body of work in winter 2017, a study demonstrating the efficacy of water loading had just been released, but there were no intervention studies on the other effects of the methods used on RWL in combat sport athletes (Reale et al., 2018). The study of MMA fighters and the sport have grown massively since then. For example, a PubMed search for "mixed martial arts" produces 147 articles up to the end of 2016, and 195 articles since the start of 2017 to October 2021. Yet there remains a need for many more studies, especially in the interventions used for RWL, their efficacy, safety and implications for performance. In that regard, this PhD thesis has made several original contributions that have advanced knowledge in the field:

Study 1

The aim of Study 1 was to evaluate self-reported methods of RWL, using a reliable and validated questionnaire (the RWLQ), in a sample of competitive MMA athletes comprised of both amateur and professional athletes. The results from this questionnaire showed higher scores in the RWLQ (40.8±8.9) and in the magnitude of RWL in the professional MMA athletes then in other studies of similar design with combat sports and other weight class sports. Of particular note was the finding that hot (salt) baths were among the most commonly used methods for RWL in this cohort.

Study 2

Having identified hot baths as a highly prevalent method of RWL in MMA athletes in the previous study, the aim of Study 2 was to determine the magnitude of body mass losses in MMA athletes using a standardised hot bath protocol with or without the addition of Epsom salt. The protocol that we devised and the addition of Epsom salt were based on my personal experience of what combat sport athletes were using in the field, a fact that was later confirmed by exit interviews in this study. The main finding was that the body mass loss when bathing in a hot bath of fresh water (FWB) is similar to bathing in a hot bath with ~1.6% Epsom salt added (SWB). A secondary finding was the demonstration of reproducible effects of RWL on body mass lost under controlled conditions, including a 2-3% reduction in body mass induced by a low residue diet combined with carbohydrate and fluid restriction for ~24 hours.

Study 3

Exit questionnaires from Study 2 revealed that fighters found the standardised water temperature (37.8°C) used in Study 2 to be too cold relative to their habitual practices, so this follow-up study investigated the magnitude of body mass losses during hot water immersion with or without the addition of ~1.6% Epsom salt, with the temperature commencing at 37.8°C and being self-adjusted by participants to their maximum tolerable temperature. Like Study 2. under the conditions employed, the magnitude of body mass lost in SWB was similar to FWB. As a sub-analysis, we compared the n=6 participants that were common to Studies 2 and 3 and in the hotter bath of Study 3, there was a loss of 2.62±0.62% as compared to 2.07±0.61% in Study 2. Increased water temperature made a difference to the magnitude of body mass lost, but the addition of salt was still having no discernible effect on this outcome.

Study 4

Studies 2 and 3 showed no difference in body mass losses using hot water immersion with the salt concentration at ~1.6%, regardless of water temperature. The next logical step was to increase the concentration of the salt given that previous studies that observed an effect of salt did so at concentrations >3.5%. The primary aim of Study 4 was to determine the magnitude of body mass losses during hot water immersion with and without a 5% solution of Epsom salt. In addition, to extend the methodology of our previous work we also investigated the effects of RWL using hot baths on blood markers (plasma volume, kidney function and electrolytes), and exercise performance after rehydration. Again, there were no differences in body mass losses between FWB and SWB, nor were there differences in the blood markers between conditions. Performance tests were not negatively impacted by the RWL process followed by ~20 hours of recovery.

Consistency in the RWL and RWG processes

Although the central focus of this PhD was the question of whether the addition of salt augments body mass losses during hot water immersion, an important finding for both research and practice was the consistency in responses to the RWL and RWG processes across the studies. A general finding across Studies 2, 3, and 4 is that restricting fluid intake to 15 ml/kg the day before a weigh-in and following a low energy, low residue diet consisting of predominantly fat and protein, consistently leads to a reduction of approximately 2 to 3% body mass. Specifically:

- Study 2: Body mass losses induced by carbohydrate and fluid restriction were 2.29±0.82 kg and 2.25±0.86 kg in preparation for the FWB and SWB trials, respectively. These values represented losses of relative to initial body mass on Morning Day -1 of 2.0±1.01% and 2.22±1.05% for the FWB and SWB protocols, respectively.
- Study 3: Body mass losses induced by carbohydrate and fluid restriction were 2.14±0.78 kg and 2.08±0.96 kg in preparation for the FWB and SWB trials, respectively. These values represented losses of relative to initial body mass on

Morning Day -1 of 2.55±0.93% and 2.45±1.11% for the FWB and SWB protocols, respectively

- Study 4: Body mass losses induced by carbohydrate and fluid restriction were 2.27±1.18 kg and 2.12±0.72 kg in preparation for the FWB and SWB trials, respectively. These values represented losses of relative to initial body mass on Morning Day -1 of 2.82±1.47% and 2.64±0.90% for the FWB and SWB protocols, respectively

This 24 hour strategy with low residue foods and carbohydrate and fluid restriction could be translated easily into practice as in my experience it is easy to plan for and replicate. Such a strategy could act as a critical tool for use in any weight class sport, especially those with morning of weigh-ins where reductions of body mass tend to be lower as a percentage of body mass. Future research would need to explore the effects on performance in various weight class sports depending on their physiological demands and the length of the recovery window from weigh-in to competition.

In a similar manner, the magnitude of body mass lost in the hot bath protocol of the RWL process was largely consistent across studies with Studies 2 and 3 showing approximately the same amount of body mass loss of 2.1% and 2.0%, respectively, with Study 4 showing a further increase to ~2.8% body mass lost in the 2 hour protocol. The larger magnitude of loss in Study 4 is notable because of slight differences in the protocol employed. For that study, the temperature for the bath started between 40ºC and 41ºC and remained above 40ºC throughout each of the 20-minute rounds of bathing, which differed from Studies 2 and 3 where the temperature started and 37.8ºC and was either maintained (Study 2) and self-adjusted upwards (Study 3). Another change for Study 4 was that the “wrap protocol” was changed from wrapping up in clothes and staying under the blankets in a heated bedroom to using a sauna blanket i.e. effectively a heated sleeping bag lined with plastic. Whether the higher starting water temperature or the wrap protocol, or both, resulted in the greater % of body mass lost is unknown, and it is also not possible to exclude the possibility that the participants in Study 4 were simply heavier sweaters than Studies 2 and 3. Ultimately, the RWL process in Study 4 produced losses of relative to initial body mass on Morning Day -1 of 5.33±2.20% and 5.13±1.52% for the FWB and SWB protocols, respectively.

Therefore, the combination of the above and any of the hot bath protocols used led to an ~5% body mass reduction in 24 hours, that was almost completely reversed in the following 24 hours but there were still signs of dehydration in many of the participants. Despite the differing, though similar, amounts of total body mass loss between the studies, the RWG process was again consistent in that body mass returned to a similar level on all three studies after aggressive refuelling and rehydration protocols. Specifically:

- Study 2: Weight regain was 2.97±1.15 kg and 3.14±1.04 kg during recovery from the FWB and SWB protocols, respectively, resulting in a body mass deficit compared to Morning Day −1 of 0.95±1.06 kg and 0.70±1.03 kg, respectively. At Morning Day +1, 10 (FWB trial) and 8 (SWB trial) participants were in a body mass deficit compared to Morning Day −1.
- Study 3: Weight regain was 3.57±0.86 kg and 3.39±0.87 kg during recovery from the FWB and SWB protocols, respectively, resulting in a body mass deficit compared to Morning Day -1 of 0.28±0.44 kg and 0.34±0.89 kg, respectively. At Morning Day +1, 6 (FWB trial) and 5 (SWB trial) participants were in a body mass deficit compared to Morning Day -1.

- Study 4: Weight regain was 4.00±1.13 kg and 4.34±1.10 kg during recovery from the FWB and SWB protocols, respectively, resulting in a body mass deficit compared to Morning Day -1 of 0.52±1.55 kg and 0.06±0.64 kg, respectively. At Morning Day +1, 4 (FWB trial) and 4 (SWB trial) participants were in a body mass deficit compared to Morning Day -1,

In Study 4, assessment of effects of RWG using the blood markers and performance tests showed that all parameters had returned to baseline (Day -2) values, which could be interpreted as a lack of negative effect of the RWL process if RWG is adequate. However, as noted in Chapter 6, this observation could be explained by two contrasting views: first that there are no negative effects of RWL due to the deleterious effects of RWL being reversed by adequate RWG, or alternatively that the tests employed were not being sensitive enough to detect negative effects of RWL. However, in my experience athletes are going to make weight whatever the weight and whatever the means, and this fact is evidenced in several case studies of combat sport athletes (Kasper et al., 2019) and therefore, I would have no hesitation to recommend a weight cut of up to 5% if there was ~24 hours or more for recovery.

Emerging issues and future directions for research

In my opinion, these bath studies are only a starting point for research in the field of RWL methods and consequences, and even within the specifics of hot water immersion, there remains several unanswered questions. Specifically there are two main questions that need to be studied: duration of hot water immersion, and type of salt used.

In relation to the type of salt used, we started using Epsom salt as that was the type of salt that I had observed being used by fighters in practice, and was also reported to us by the fighters in Studies 1 and 2. Of those studies that have observed the effects of salt to augment the sweating response or body mass losses, sodium chloride or sea water has been the salt used (Hertig, et al., 1961; Hope et al., 1994; Whitehouse, et al., 1932). While the physiological mechanism of augmentation by salt may be due to the osmotic effect and in which case, the type of salt should not matter, I cannot exclude that possibility at this time. However, I do believe that the duration of immersion is likely to be the most important consideration as outlined in the Discussion section of Chapter 6. Briefly again, work by Hertig et al. (1961) used 5% sodium chloride in water heated to 36-37ºC and this allowed for higher sweat rates during 3 hours of immersion when compared to fresh water; Whitehouse et al., (1932) also used sodium chloride in hot water immersion for 5-hours to elicit a greater body mass loss in comparison to fresh water. Therefore, the durations of studies that show effects of salt water on body mass reduction have been done with immersions of 3 to 5 hours, so the total of 40 minutes in our protocol may be inadequate to elicit a greater weight loss effect.

Other questions arising from this work are around inter-individual variation. As evidenced by the individual data plots in each study, there were wide variations in body mass reductions between individuals in the hot water immersion, and it would be valuable to understand why this is the case. Is there a mechanism involved with this that can be trained to elicit more weight loss without incurring any health hazards? Possible mechanisms for this could be sweat gland number, sweat gland hypertrophy and sweat gland fatigue based on the work of Michael J. Buono of San Diego University (Buono et al., 2018). Barley et al. (2019) concluded that after heat acclimation participants were able to lose the weight significantly faster (possibly due to increased sweat rate).

We induced 5% body mass loss in many of our subjects but as noted in the literature review and beyond there are many cases above this level and even as much 10%. I would like to see research conducted that looks at higher magnitudes of body mass reduction in the lead-up to a hot bath protocol and whether this impacts the magnitude of body mass lost in the bathing period. Recruiting subjects to undergo 10% body mass outside of competition would be extremely difficult as RWL (especially to 10%) is not pleasant. I was able to recruit subjects for my studies as the duration of the studies was short and the disruption to the athletes training was minimal and a 4-5% body mass drop is not too arduous for athletes that are used to dropping 8-10% body mass.

Examining higher magnitudes of body mass reduction during RWL and their potential impact on indices of performance after RWG is also needed. This is important as some people will read that the 5% body mass reduction achieved in our studies was reversed, and interpret that as meaning that all body mass reductions (regardless of whether they are well above 5%) are negated with adequate recovery time and strategies.

Personal reflections on RWL from the lab and the field

Even though it was not objectively recorded in any of our studies, there was a definite difference between fresh water and salt water conditions in what I perceived as stress to the athletes during the hot water immersion. At no point did I feel that I needed to cut short any of the studies in the SWBs, and the contrast between FWB and SWB in this context was even more stark in the 5% solution of Study 4. On several occasions I was afraid that I might need to stop FWB trials when they appeared to be imparting too much distress to the athletes. Recurrent feedback that I received from the athletes across Studies 2, 3 and 4, was that the water did not feel as hot when it had the salt in it, although admittedly, these trials were not blinded so some form of expectation or bias from the fighters cannot be completely discounted.

All things considered, despite the lack of effect of salt to augment the magnitude of body mass lost during hot water immersion, I would still recommend using Epsom salt in a hot bath protocol, and additionally I would use the 5% solution. My rationale is that there is an apparent upside of reducing the severity of the experience of the hot bath, whereas the only drawback that I can see for the fighters is cost and convenience. For example, Holland & Barrett sell Epsom salt at €8 per kg i.e. €40 per 5 kg used in a 125 L bath, but I was able to purchase my Epsom salt in bulk at €1 per kg. If the fighter can only buy at the higher price, then the cost of even €40 would be prohibitive, or at least discourage, some fighters, and if a fighter has to travel for fights, it might be too hard to travel with or source the Epsom salt.

Other studies have shown an effect of having a higher %salt solution to be more effective for body mass reductions when the %salt has been from ~3.5% to 18% (Whitehouse et al. 1932; Buettner 1953; Peiss et al. 1956; Buettner 1959; Hertig et al. 1961; Brebner and Kerslake 1964; Hope et al. 2001). This difference in body mass reduction was not evident in my third study even with the 5% solution. As described in Chapter 6, this contrasting finding could have been due to the fact the osmolality of Epsom salt (which I used for my each of my studies) is lower than that of sodium chloride (which was used for the studies that did show a difference) when both salts are matched for %wt/vol. As shown in Table 7.1 there are very large differences between the calculated osmolality of the different hot salt water baths when type of salt is taken in to account. My first two studies had an osmolality of 130 mOsmol/kg, which is less than that of the human plasma (290-310 mOsmol/kg), and therefore does not produce an osmotic gradient that would increase fluid loss from the body. Even my higher solution (5%) Epsom salt hot bath was at an osmolality of 406 mOsmol/kg, which is contrast to Hertig et al. study of 5% sodium chloride that had an osmolarity of 1709 mOsmol/kg. The osmolality was obviously even higher in the studies by Whitehouse et al. (18% ~ 6152 mOsmol/kg) and Brebner & Kerslake (15% ~ 5127 mOsmol/kg). This lack of osmotic gradient could explain why there was no difference between the different conditions in my studies if it is true that the osmotic gradient was a causal factor in the effects observed in other hot salt water immersion studies.

Developing the Optimal Protocol

The following is a cumulation of experience and the studies listed in Table 7.1.

Before the athlete gets to the last few hours before a weight cut and is gonna use a hot bath there are a couple of very important prerequisites that need to be fulfilled. The athletes need to be as lean as possible as lower body fat means there is less heating of core temperature (Doupe et al. 1994). This not usually a problem for elite MMA athletes but it gives them an extra incentive to be as lean as possible for fight week. There also needs to be a heat acclimatisation period. This has been mentioned several times above to improve several factors related to the sweat glands, sweat rate and the comfort of cutting weight. I like my athletes to have a variety of methods to do this (saunas, training in heated room, grappling with skin mostly covered by tight clothing) but coming towards when it is time to cut weight then I want them to use hot baths. This causes heat acclimatisation but also gets them used to the very method they are going to use for the cut. It also must be recognised that there are certain people and populations that are just better at cutting body mass through sweating (Lee et al., 2011) so not everyone will have the same upper limit for body mass reduction in the hot baths.

The water temperature needs to hot enough to elicit a high sweat rate but not too hot so that the athlete reaches thermal discomfort and can not continue to reduce body mass (Collins & Weiner, 1962). This ideal temperature seems to be 39-40º C.

The body needs to be submerged up to the neck (Table 7.1). The studies that only submerged smaller areas of the body had high sweat rates in those areas but nowhere else.

The total duration appears to be at least 2 hours of continuous heat exposure. Whether this is a combination of bath and wraps. 20 minutes in the bath followed by 20-40 minutes in a wrap. Repeat this process until it is 2 hours of total exposure.

I would recommend adding Epsom salt to at least a 5% solution as it positively effects thermal comfort while not compromising body mass losses, whereas it remains possible that using sodium chloride at this concentration could augment body mass losses compared to fresh water. Concentrations greater than this 5% arguably become impractical for fighters in terms of the quantity of salt, its accessibility and the logistics of this approach when travelling to other cities or countries to compete and associated issues with hotel access.

Table 7.1. A summary of the most relevant hot water immersion (HWI) studies

Author	Year	Temp C	Time	Submerged	BM Loss kg	Salinity %	Osmolality mOsmol/kg
Whitehouse et al.	1932	91-100 F	5 h	to the neck	Max = 2.8	0-18	6152 (18%)

Author	Year	Temp C	Time	Submerged	BM Loss kg	Salinity %	Osmolality mOsmol/kg
Hertig et al.	1961	34-38	3-4 h	to the neck	NA	5 10 15	1709 3418 5127
Brebner & Kerslake	1964	41	5 h	to the neck	2kg/hr	15	5127
Ogawa et al.	1982	43	2 h	Arm to elbow	NA	0	
Fujishima	1986	43	8 min	to the neck	NA	0	
Alison & Rogers	1992	40	21 min	nipple line	NA	0	
Hope et al.	2001	34.5-38	4 h	to the neck	SW = 2.5 FW = 1.9	3.5 0	1000-1200
Lee et al.	2011	42	60 min	Feet and calves	25g	0	
Kraft et al.	2011	39	2 h	to the neck	3%	0	
Zurawlew et al.	2016	40	40 min	to the neck	NA	0	
Kasper et al.	2018	NA	20 min x 9 (180 min)	to the neck	5.3*	0	
Connor et al.	2020	37.8	20min x 2 (40 min)	to the neck	1.60 1.63	1.6 0	130
Connor & Egan	2021	39.0	20min x 2 (40 min)	to the neck	1.66 1.71	1.6 0	130
Connor et al.	2022	40.3	20min x 2 (40 min)	to the neck	2.24 2.17	5 0	406

* This was over several hours that included breaks and “wraps”.

Final thoughts

RWL can be extremely dangerous if done incorrectly, but our work has shown that under the proper supervision and with consistent protocols, RWL can be safe, effective and, according to the tests we used, not detrimental to health and performance when performed to a magnitude of ~5%body mass in 24 hours. The PhD process forced me to revise and reconsider some of my assumptions, and the evidence we have amassed, along with the protocols we have elaborated, make an important contribution to understanding how MMA athletes can safely cut weight without undermining performance. I intend to pursue further research in what remains a nascent field, but the experience of the PhD process and the sound conclusions it produced advance the field overall, and provide a solid foundation for developing my own research, and for supporting the research of others interested in the field.

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Appendices

Appendix A

Ollscoil Chathair Bhaile Átha Cliath
Dublin City University

DCU

Mr John Connor
School of Health and Human Performance

28 March 2017

REC Reference: **DCUREC/2017/055**

Proposal Title: **Exploration of Rapid Weight Cutting Practices by Mixed Martial Artists**

Applicant(s): **Mr John Connor, Dr Brendan Egan**

Dear John,

This research proposal qualifies under our Notification Procedure, as a low risk social research project. Therefore, the DCU Research Ethics Committee approves this project.

Materials used to recruit participants should state that ethical approval for this project has been obtained from the Dublin City University Research Ethics Committee.

Should substantial modifications to the research protocol be required at a later stage, a further amendment submission should be made to the REC.

Yours sincerely,

Donal O' Gorman

Dr Dónal O'Gorman
Chairperson
DCU Research Ethics Committee

DCU
Research & Innovation

Taighde & Nuálaíocht Tacaíocht
Ollscoil Chathair Bhaile Átha Cliath,
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Appendix B

REC Reference: **DCUREC/2019/021**

Proposal Title: **Effects of hot baths for acute weight loss in mixed martial artists.**

Applicant(s): Dr Brendan Egan, Mr John O'Connor, Mr Adam Shelly

Dear Colleagues,

Further to full committee review, the DCU Research Ethics Committee approves this research proposal.
Materials used to recruit participants should note that ethical approval for this project has been obtained from the Dublin City University Research Ethics Committee.

Should substantial modifications to the research protocol be required at a later stage, a further amendment submission should be made to the REC.

Yours sincerely,

Dr Dónal O'Gorman
Chairperson
DCU Research Ethics Committee

DCU Research & Innovation

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Appendix C

Ollscoil Chathair Bhaile Átha Cliath
Dublin City University

DCU

Mr John Connors
School of Human & Health Performance

21st June 2019

REC Reference: **DCUREC/2019/115**

Proposal Title: **Effect of hot baths at a self-adjusted temperature on acute weight loss in mixed martial artists**

Applicant(s): **MR John Connors, Dr Brendan Egan**

Dear Colleagues,

Further to full committee review, the DCU Research Ethics Committee approves this research proposal.
Materials used to recruit participants should note that ethical approval for this project has been obtained from the Dublin City University Research Ethics Committee.

Should substantial modifications to the research protocol be required at a later stage, a further amendment submission should be made to the REC.

Yours sincerely,

Mark Philbin

Dr Mark Philbin
Interim Chairperson
DCU Research Ethics Committee

DCU
Research & Innovation

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Appendix D

Ollscoil Chathair Bhaile Átha Cliath
Dublin City University

Mr. John Connor
School of Human Health and Performance

Dr. Brendan Egan
School of Human Health and Performance

29th September 2020

REC Reference:	**DCUREC/2020/186**
Proposal Title:	**Effect of hot water immersion in a 5% salt solution on acute weight loss and subsequent performance in mixed martial artists**
Applicant(s):	**Mr. John Connor, Dr. Brendan Egan, Mr. David Nolan, and Mr. Mark Germaine**

Dear Colleagues,

Further to full committee review, the DCU Research Ethics Committee approves this research proposal.

Materials used to recruit participants should note that ethical approval for this project has been obtained from the Dublin City University Research Ethics Committee.

Should substantial modifications to the research protocol be required at a later stage, a further amendment submission should be made to the REC.

Yours sincerely,

Geraldine Scanlon

Dr Geraldine Scanlon
Chairperson
DCU Research Ethics Committee

DCU Research & Innovation

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Appendix E

Data Processing of force plate data for CMJ and IMTP

Jump Height Calculation

Each sequence of 5 jumps generated a file contain the force (Left and Right foot) on the sensors every .002 second (500 Hertz frequency). The data for one jump is displayed in Figure E.1

1 = A jump consists of standing still (using the sensors to capture weight)

2 = A knee bend (resulting in a small to large drop in force on the sensors temporarily)

3 = A delivery of force as the participant straightens legs to jump upwards

4 = No force as the participant is off the sensor

5 = Large landing force as the participant lands

The raw data was examined and the jump time (time when the participant was deemed not to be generating any force or weight on the sensors was identified and noted).

This was determined in each jump as the first point where the force (after point 3) saw 0 or negative force (Figure E.2). This time was captured each of the five jumps and the middle jumps were used to calculate the jump performance, for each participant on the testing day. The time for the three jumps was then converted to distance using the formula (Moir, 2008):

Max Height Jumped = 9.81 x 0.5 x t2

Where t is the half the time between the start and end of the time off the force plate.

IMTP Calculation

Each sequence of data detailed 3 pull generated a file contain the force (Left and Right foot) on the sensors every .002 second (500 Hertz frequency). The data for one pull is displayed in Figure E.3. (this is the average of the left and right foot sensors).

1 = Each pull consisted of standing still/ready

2 = Short brace before the pull began

3 = The rise in force

4 = The maximum force applied during the test

5 = The end of the test and reduction in force

The max force applied by the participant was the max reading on the sensor during the test – the figures above are the average of Left and Right, so the max was doubled. This figure included their weight (standing still force).

For the purposes of calculating the force the participants employed, their calculated standing still force weight from the CMJ test on the same day was subtracted from the max force. The CMJ figure was used as the participants were holding handles and equipment in this test which caused variability in the sensors at rest

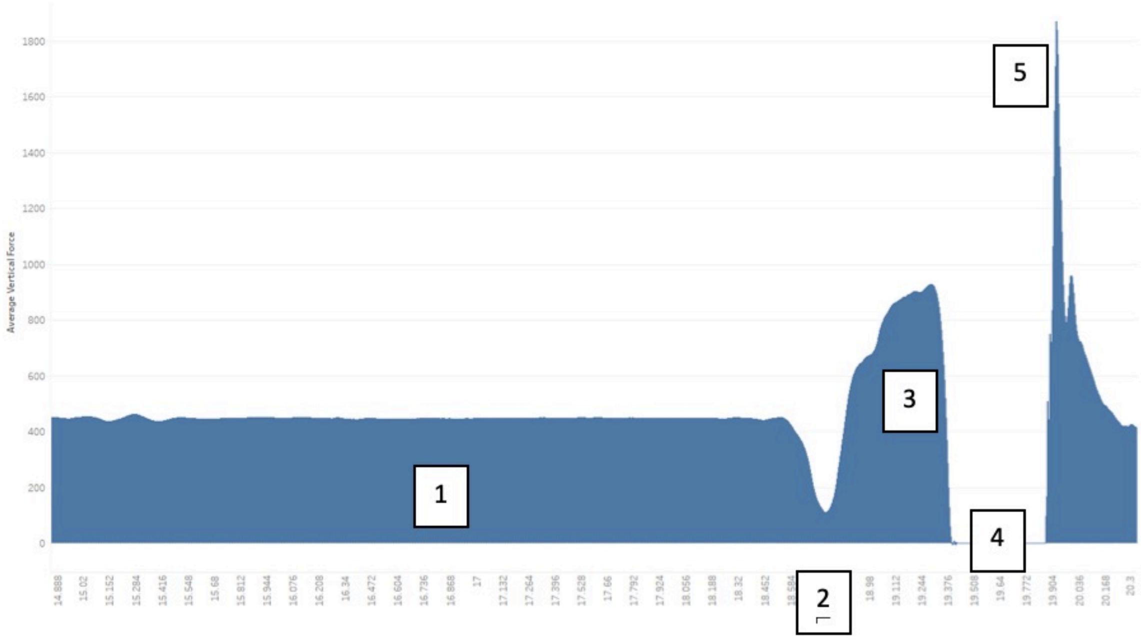

Figure E.1 Calculation of jump height from force plate data in the CMJ test. 1 = A jump consists of standing still (using the sensors to capture weight). 2 = A knee bend (resulting in a small to large drop in force on the sensors temporarily). 3 = A delivery of force as the participant straightens legs to jump upwards. 4 = No force as the participant is off the sensor. 5 = Large landing force as the participant lands.

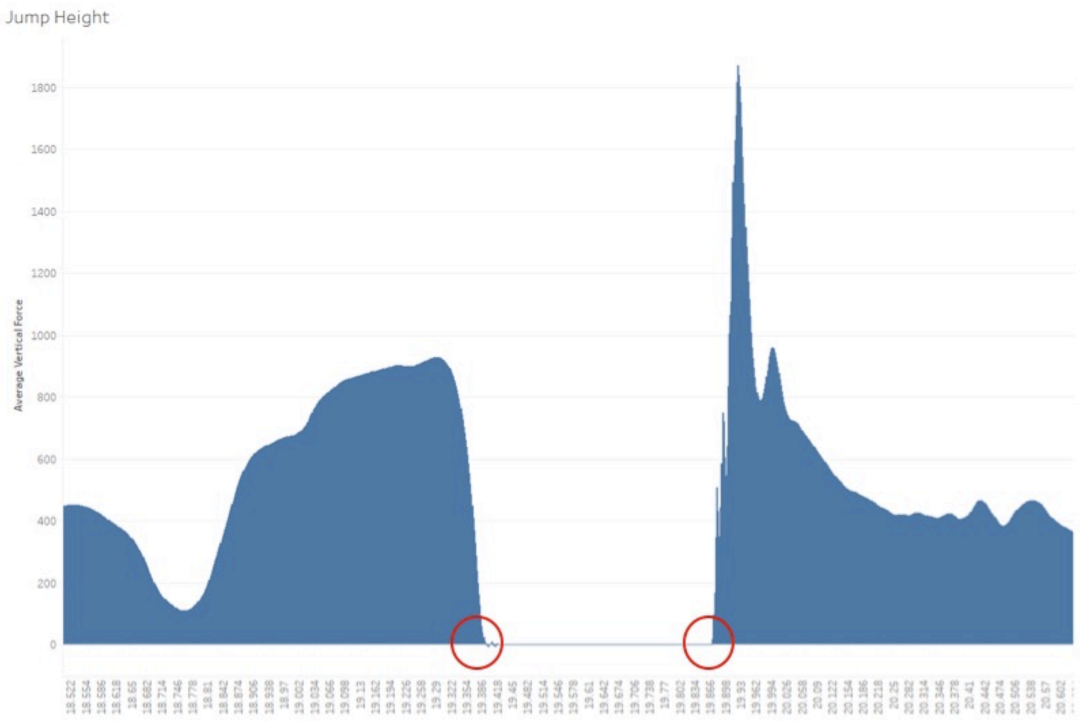

Figure E.2. Calculation of jump height from force plate data in the CMJ test. Take-off and landing points are circled above.

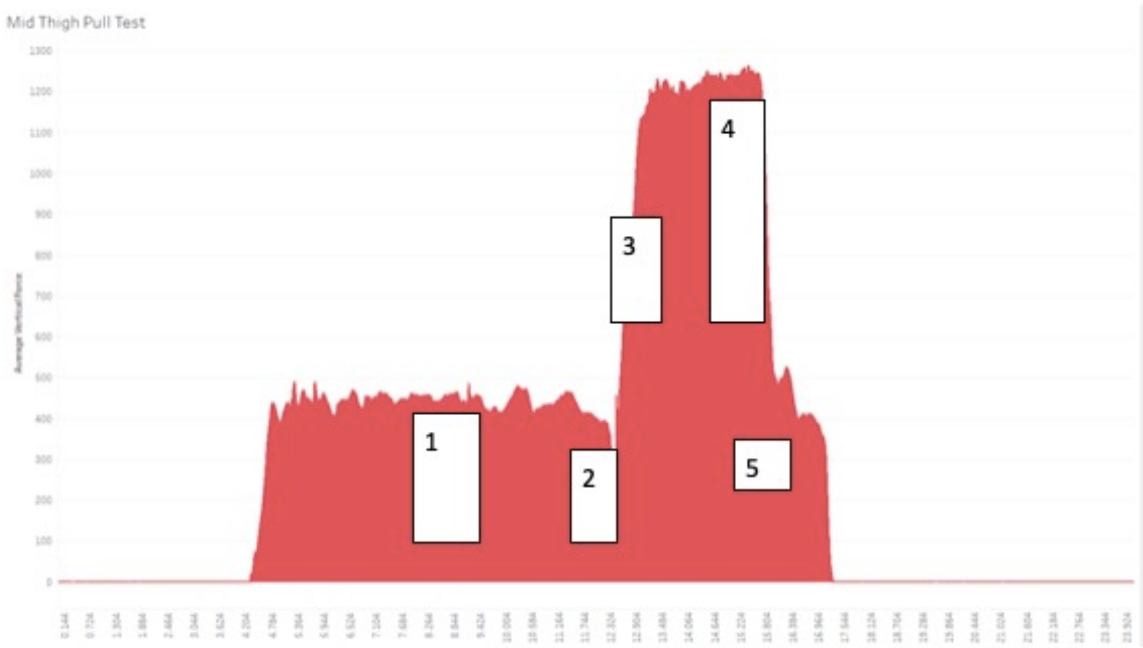

Figure E.3. Calculation of peak force from the force plate data in the IMTP test. 1 = Each pull consisted of standing still/ready. 2 = Short brace before the pull began. 3 = The rise in force. 4 = The maximum force applied during the test. 5 = The end of the test and reduction in force

Appendices F, G, H & I - Published Papers

Connor, J., & Egan, B. (2019). Prevalence, Magnitude and Methods of Rapid Weight Loss Reported by Male Mixed Martial Arts Athletes in Ireland. *Sports (Basel)* 7(9), 206. https://doi.org/10.3390/sports7090206

Connor, J., Shelley, A., & Egan, B. (2020). Comparison of hot water immersion at 37.8°C with or without salt for rapid weight loss in mixed martial arts athletes. *Journal of Sports Sciences* 38(6), 607–611. https://doi.org/10.1080/02640414.2020.1721231

Connor, J., Egan, B. (2021). Comparison of hot water immersion at self-adjusted maximum tolerable temperature, with or without the addition of salt, for rapid weight loss in mixed martial arts athletes. *Biology of Sport* 38(1), 89-96. https://doi.org/10.5114/biolsport.2020.96947

Connor, J., Germaine, M., Gibson, C., Clarke, P., & Egan, B. (2022). Effect of rapid weight loss incorporating hot salt water immersion on changes in body mass, blood markers, and indices of performance in male mixed martial arts athletes. European Journal of Applied Physiology, 10.1007/s00421-022-05000-7. https://doi.org/10.1007/s00421-022-05000-7

sports

Article

Prevalence, Magnitude and Methods of Rapid Weight Loss Reported by Male Mixed Martial Arts Athletes in Ireland

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Received: 28 June 2019; Accepted: 3 September 2019; Published: 9 September 2019

Abstract: Rapid weight loss (RWL) is frequently practiced in weight category sports, including Mixed Martial Arts (MMA). The aim of the present study was to describe self-reported methods of RWL in a sample of competitive MMA athletes comprising of both amateur and professional fighters. The previously-validated Rapid Weight Loss Questionnaire, with the addition of questions on water loading and hot salt baths, was completed anonymously online by athletes (n = 30; all male, n = 15/15 professional/amateur) from MMA clubs around Dublin, Ireland. All but one (97%) of the athletes surveyed lost weight in order to compete, with the average weight loss being 7.9% ± 3.1% of habitual body mass. The RWL score (mean ± SD) for this sample was 37.9 ± 9.6, and a tendency for higher [6.0 (95%CI; −1.1, 13.1) (p = 0.093; d = 0.64)] RWL scores for professional (40.8 ± 8.9) compared to amateur (34.8 ± 9.6) athletes was observed. Frequencies of "always" or "sometimes" were reported as 90% for water loading, 76% for hot salt baths and 55% for 24 h of fasting. Fellow fighters (41%) and coaches/mentors (38%) were "very influential" on RWL practices of these athletes, with doctors (67%), dietitians (41%), and physical trainers (37%) said to be "not influential". RWL is highly prevalent in MMA across both amateur and professional athletes, and RWL scores are higher than other combat sports. Water loading and hot salt baths are amongst the most commonly used methods of RWL despite little research on these methods for body mass reduction or effects on performance in weight category sports.

Keywords: combat sports; dehydration; hot bath; weight category; weight-making; water loading

1. Introduction

Rapid weight loss (RWL) is frequently practiced in sports that have weight class restrictions [1–5]. Many of these sports include combat sports such as wrestling, judo, boxing and taekwondo, as well as other mainstream sports such as horse riding and rowing [2,6]. RWL generally refers to the methods employed by an athlete in reducing body mass in the final one to two weeks before competition, and typically averages ~2% to 10%, depending on the sport [1–5]. Subsequent to the weigh-in, combat sport athletes generally proceed to regain often the majority of this weight from within a few hours up to 36 h before competing [7–9]. RWL followed by rapid weight regain is employed, especially in combat sports, as a means of gaining a size and/or strength advantage over an opponent as the heavier fighter is generally seen to have an advantage [1,3,8,10].

Mixed martial arts (MMA) is a combat sport comprised of styles of various martial arts and involves striking, grappling, wrestling and submission techniques [11]. MMA athletes are required to compete under specific weight categories, namely: atomweight, 105 lbs (47.6 kg); strawweight, 115 lbs (52.2 kg); flyweight, 125 lbs (56.7 kg); bantamweight, 135 lbs (61.2 kg); featherweight, 145 lbs

(65.8 kg); lightweight, 155 lbs (70.3 kg); welterweight, 170 lbs (77.1 kg); middleweight, 185 lbs (83.9 kg); light-heavyweight, 205 lbs (93.0 kg); heavyweight, 205–265 lbs (93.0–120.2 kg); and super-heavyweight, no limit. In professional bouts for MMA, the timeline between weigh-ins and fight time can vary depending on the organisation sanctioning the fight. All professional organisations have weigh-ins on the day before the fight. For the majority of organisations, weigh-ins are at least 24 h before the fight and up to 36 h beforehand. The timeframe for amateur MMA fights again depends on the organisation sanctioning the bout. Many organisations will follow the same outline as the professional bouts on their card (24 to 36 h before the fight), but under new rules set forth by the International Mixed Martial Arts Federation (IMMAF), weigh-ins for amateur fights are on the morning of competition.

MMA was established on the international stage as the Ultimate Fighting Championship (UFC) in 1993, but despite being one of the fastest-growing international sports [12], only recently have reports begun to emerge on the weight-making practices of these athletes [7,8,13–17]. One survey described MMA athletes losing 9% ± 2% of body mass in the week before a fight, and a further 5% ± 2% in the 24 h before weigh-in [16]. This is achieved due to employing one or all of the following methods: water loading, fluid restriction, prescription and over-the-counter diuretics, complete fasting or low carbohydrate diets in the final 3 to 5 days prior to weigh-in [16]. Such drastic methods for RWL result in 100% of athletes being dehydrated to various degrees at the official weigh-in [7,13], and 14% [7] and 39% [13] remaining dehydrated when measured in the final 2 h pre-fight.

Considering the increasing popularity of MMA, but documented adverse health outcomes and deaths attributed to RWL practices [16–18], the creation of bodies such as Safe MMA recognised that RWL practices may increase the risk of injury and health consequences. Indeed, there have been calls to ban RWL in combat sports, partly because of the potential health risk to the athlete [19]. Conversely, the case has been made that a well-designed RWL strategy supported by appropriate recovery and weight regain strategies—when the time from weigh-in to competition allows—may confer a performance advantage [5]. While the data across weight category sports as a whole remain equivocal [3,5], weight regain has been linked to a performance advantage in judo [10] and MMA [8]. Further studies are needed to characterize the prevalence and methods of RWL in MMA, with additional work then required to establish the safety, or otherwise, of these methods. Therefore, the aim of the present study was to describe self-reported methods of RWL in a sample of competitive MMA athletes comprising of both amateur and professional fighters based in Dublin, Ireland.

2. Materials and Methods

2.1. Study Design and Participants

The study was approved by the Research Ethics Committee at the Dublin City University (DCU), Ireland (permit: DCUREC 2017_055) in accordance with the Declaration of Helsinki. Participants, all of whom were male, were recruited from several MMA clubs around Dublin that are associated with Straight Blast Gym (SBG), the largest MMA gym franchise in Ireland. Participants were invited to participate in a survey of current and previous weight-making practices via the fighters' private page on Facebook. Participants clicked via a link that gave them access to the anonymous online questionnaire. A participant information leaflet was presented on arrival to the page, after which participants needed to provide consent via a tick box option in order to proceed to the questionnaire. Prior to commencing the questionnaire, RWL was defined to the participants as reducing body mass by 5% to 10% in seven days or less.

The private Facebook page is for active fighters only (i.e., have previously competed and are continuing to prepare for future fights), and has a membership of fifty athletes with an even distribution of amateur and professional fighters. Thirty athletes (60%) completed the online survey, with a final split of n = 15 amateur fighters, and n = 15 professional fighters. Professional and amateur status was self-reported and categorised based on the rules set under which they fought at the time of the questionnaire being administered. The major distinctions between the respective groups are that amateur fights consist of 3 × 3 min rounds (compared to 3 or 5 × 5 min rounds in professional fights), and amateur fighters wear shin guards and a rash guard, and are not permitted to perform certain strikes and holds that are permitted under professional MMA rules. Even though there can be different rule sets in amateur and professional MMA with regards to regulations around the timing of the weigh-in, all of the amateurs in this study competed under rules equivalent to professional MMA rules, i.e., with weigh-in on the day before competition.

2.2. Questionnaire

The questionnaire used in this study was a previously validated Rapid Weight Loss Questionnaire (RWLQ) [20] with slight modifications. The questionnaire has demonstrated good stability, reliability and discriminant validity [20,21], having been conducted with a relatively large and heterogeneous sample—including competitors of both genders—and a wide range of competitive levels and ages. This questionnaire was originally designed for the assessment of RWL in judo athletes, but was then modified and validated for other combat sports [22]. Subsequently, the questionnaire has been modified and utilised for MMA athletes [7,8,14,23,24], and other combat sports [25,26]. Similar to previous work [25], our modifications were to change all instances of "judo" to the combat sport of interest to this study, i.e., "MMA", and to add questions that better reflected current practices related to MMA such as water loading and hot salt baths [7,16,17,23–25,27]. Specifically, we added the option to answer "hot salt baths" and "water loading" under the question "How often did you use each one of the following methods to lose weight before competition?" with the same frequency options of always, sometimes, almost never, never used, and I don't use anymore. The questionnaire was recreated in Google Forms, and shared as a link to the aforementioned private Facebook page. The questionnaire was open for 8 weeks beginning 1 April 2017, with reminder requests for participation posted to the page once per fortnight.

2.3. Data Analysis

The RWLQ was scored as described previously to produce a Rapid Weight Loss Score (RWLS) for each athlete and frequency analysis was performed where appropriate [20]. Our additional questions on water loading and hot salt baths were not scored in the final calculation of RWLS. Therefore, the calculated RWLS remained directly comparable to other studies that employed the RWLQ. One amateur athlete indicated that he had never engaged in RWL and was excluded from the calculation of RWLS. Data were analysed and illustrated using PRISM v7 (GraphPad Software, San Diego, CA, USA). All data were assessed for normality using the Shapiro–Wilk test. For normal distributions, descriptive statistics are reported as mean ± SD, and differences between groups were assessed using an independent samples t-test. For non-normal distributions, descriptive statistics are reported as median (interquartile range) (IQR), and differences between groups were assessed using a Mann–Whitney U test. The significance level was set at $\alpha = 0.05$ for all tests. Differences between groups are reported as mean (lower 95% confidence interval, upper 95% confidence interval). Effect size was calculated using Cohen's d and interpreted using thresholds of <0.2, ≥0.2, ≥0.5 and ≥0.8 for trivial, small, moderate, and large, respectively.

3. Results

Of the n = 30 athletes surveyed, respondents had, on average, 4.7 ± 2.7 y of experience of formally competing in MMA (Table 1), and all but one athlete (97%) had previously engaged in RWL in preparation for competition. The percentage of habitual body mass usually lost in the overall weight cut preparation for a fight averaged 7.9% ± 3.1%, and 100% (85, 133)% of this weight loss was usually regained in the week after a fight (Table 1). In this cohort, the amateur fighters had a lower body mass index (23.6 ± 1.8 vs. 25.0 ± 2.2 kg m^{-2}; $p = 0.030$; $d = 0.70$), and tended to have fewer years of competitive experience (3.8 ± 2.6 vs. 5.6 ± 2.6 y; $p = 0.067$; $d = 0.69$).

Table 1. Participant characteristics [1].

	All (n = 30)	Amateur (n = 15)	Professional (n = 15)	Amateur vs. Professional p Value
Age (y)	25.5 ± 4.4	24.3 ± 4.2	26.7 ± 4.4	0.150
Years of experience competing in MMA (y)	4.7 ± 2.7	3.8 ± 2.6	5.6 ± 2.6	0.067
Weight category	AW, n = 0: SW, n = 0; FLW, n = 2; BW, n = 4; FEW, n = 4; LW, n = 10; WW, n = 6; MW, n = 3; LHW, n = 0; HW, n = 1	AW, n = 0: SW, n = 0; FLW, n = 1; BW, n = 2; FEW, n = 1; LW, n = 6; WW, n = 5; MW, n = 0; LHW, n = 0; HW, n = 0	AW, n = 0: SW, n = 0; FLW, n = 1; BW, n = 2; FEW, n = 3; LW, n = 4; WW, n = 1; MW, n = 3; LHW, n = 0; HW, n = 1	
Habitual body mass (kg)	78.3 ± 9.7	76.3 ± 7.3	80.3 ± 11.5	0.257
Height (m)	1.79 ± 0.07	1.80 ± 0.07	1.79 ± 0.08	0.743
Habitual body mass index (kg m^{-2})	24.3 ± 2.1	23.6 ± 1.8	25.0 ± 2.2	0.030
Fights in previous 12 months	2.5 (1.0, 3.3)	2 (1, 4)	1 (1, 3)	0.853
Usual weight cut (% of current body mass)	7.9 ± 3.1	7.2 ± 3.4	8.6 ± 2.8	0.397
Usual weight regain in week after fight (% of weight cut)	100 (85, 133)	100 (80, 131)	100 (91, 133)	0.612

[1] Data are reported as mean ± SD or median (IQR) for normal and non-normal distributions, respectively. Weight category abbreviations: AW, atomweight; SW, strawweight; FLW, flyweight; BW, bantamweight; FEW, featherweight; LW, lightweight; WW, welterweight; MW, middleweight; LHW, light heavyweight; HW, heavyweight.

The RWLS for this sample of MMA athletes was 37.9 ± 9.6 (Figure 1). Comparison of RWLS across codes revealed a tendency for higher RWLS [6.0 (−1.1, 13.1); p = 0.093] for professional (40.8 ± 8.9) compared to amateur (34.8 ± 9.6), with the magnitude of effect interpreted as 'moderate' (d = 0.64) (Figure 1).

While energy restriction strategies (i.e., gradual dieting, fasting) are frequently used, methods that reduce body water stores (i.e., water loading, fluid restriction, and hot salt baths) are also commonly employed for RWL by this cohort (Table 2). Water loading was the most commonly used method for RWL, with 90% of the athletes using water loading "sometimes" or "always". Of those that used water loading, 70% of the athletes start water loading between 5 and 8 days out from the weigh-in. When using water loading, 70% of the athletes consumed between 6 and 9 L of water for the high water intake days. Fluid restriction was used "sometimes" or "always" by 79% of the athletes, with 75% of this number employing the method at between 1 and 24 h prior to weigh-ins. Hot salt baths are commonly used, with 76% of athletes using the method "always" or "sometimes", compared to 48% of the athletes "always" or "sometimes" using saunas to dehydrate. Gradual dieting was used "sometimes" or "always" by 76% of the athletes, in addition to fasting for 24 h being used "sometimes" or "always" by 55%. Using winter or plastic suits, spitting, laxatives, diuretics, diet pills, and vomiting were the RWL methods that were least commonly used in this cohort.

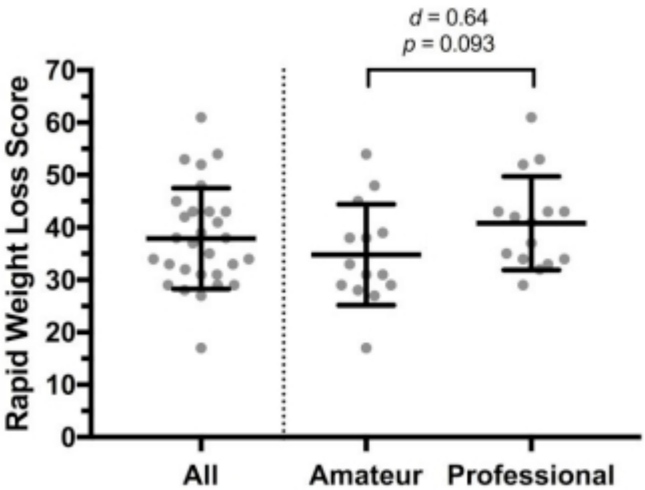

Figure 1. Rapid Weight Loss Score obtained by the RWLQ from the group as a whole (All, n = 29), and based on self-reported status as Amateur (n = 14) or Professional (n = 15). Data bars are mean values with error bars representing standard deviation.

(n = 29).

	(%)	(%)	(%)	(%)	Do Not Use
Skipping one or two meals	20.7	27.6	24.1	17.2	10.3
Fasting	31.0	24.1	10.3	24.1	10.3
Increased exercise	34.5	31.0	13.8	17.2	3.4
Heated training rooms	13.8	34.5	3.4	48.3	0.0
Hot salt baths	34.5	41.4	20.7	3.4	0.0
Training with rubber/plastic suits	31.0	13.8	20.7	24.1	10.3
Spitting	10.3	17.2	6.9	65.5	0.0
Laxatives	3.4	17.2	3.4	72.4	3.4

	(%)	(%)	(%)	(%)	(%)
Heated training rooms	13.8	34.5	3.4	48.3	0.0
Sauna	27.6	20.7	27.6	10.3	13.8
Hot salt baths	34.5	41.4	20.7	3.4	0.0

Table 3. Frequency analysis of the individuals who are influential on the weight-making practices reported by the mixed martial arts athletes.

Source	Very Influential (%)	Somewhat Influential (%)	Unsure (%)	A Little Influential (%)	Not Influential (%)
Online/written Material	18.5	14.8	14.8	14.8	37.0
Fellow fighter/training colleague	41.4	31.0	17.2	3.4	6.9
Physician/doctor	3.7	7.4	3.7	18.5	66.7
Physical trainer	14.8	11.1	29.6	7.4	37.0
Coach/mentor	37.9	24.1	20.7	6.9	10.3
Parents	0.0	3.7	3.7	11.1	81.5
Dietitian	14.3	14.3	10.7	14.3	46.4

4. Discussion

The present study establishes that a variety of methods for RWL are widely used by MMA athletes at amateur and professional levels. In addition to energy restriction by gradual dieting and short-term fasting, the methods most commonly being employed by this Irish cohort are those that reduce body water stores, i.e., water loading, fluid restriction, and hot salt baths. Even discounting water loading and hot salt baths, RWL scores were higher in these athletes than those reported in other combat sports, and a tendency existed for higher RWL scores in professional compared to amateur fighters. Fellow fighters and coaches are the dominant sources of information on methods of RWL in this cohort of athletes.

Despite the increasing popularity of MMA [12], and the concerns expressed around the safety of weight-making practices in the sport [16,19], there has been a scarcity of studies describing the prevalence and magnitude of RWL by these athletes, or indications of the personnel who are influencing these practices. During the execution of the present study, two other reports emerged describing weight-making practices in MMA in athlete cohorts of n = 70 [23] and n = 314 [24]. The findings of these studies are largely confirmed in our study, but in addition, we report an estimate of prevalence of the use of hot salt baths by MMA athletes.

Hot baths generally describe the practice of hot water immersion (e.g., >38 C), and supported by "wrapping" in warm towels or bedclothes for a period of time prior to further exposures to hot water immersion [17]. As part of the hot bath protocol, fighters will often add Epsom salts (magnesium sulfate) with the prevailing wisdom that this addition elicits greater loss of body mass through sweating-induced dehydration. Indeed, the addition of a salt to a hot water immersion to produce greater body mass loss does have some empirical evidence to support its practice [28]. Hot baths/hot salt baths have been briefly mentioned as part of weight-making practices in a number of case and small cohort studies [7,9,17,27], but to date, their prevalence in a larger cohort has not been documented. In the present cohort, 76% of the athletes reporting using hot salt baths "always" or "sometimes", with only one athlete reporting to have "never used" them. Clearly, there is a need for future work to explore the detailed protocols, and outcomes of this method for RWL given this prevalence.

Like other work [23,24], methods that reduce body water stores (i.e., water loading, fluid restriction, and hot salt baths) are the most commonly employed methods for RWL by this cohort. All but one (97%) of the n = 30 of those surveyed lost weight in order to compete, with water loading being the most prevalent method employed at a frequency of "always" or "sometimes" in 90% of respondents. The high prevalence of RWL is consistent with other reports in MMA athletes [23,24], and is greater than that reported, on average, in other combat and weight category sports [22,23,25]. The prevalence of RWL varies considerably between the various combat and weight category sports, with a number of reviews summarising the prevalence as between 50% and 80% [1,3,4]. Combat sports tend to report a higher prevalence of RWL compared to other weight category sports [1,3,4], and the prevalence of RWL in MMA is generally >95% of athletes [23,24]. Similarly, the prevalence of water loading observed in MMA athletes in the present study, and by others [23,24], appears to be higher than the prevalence of water loading reported in other combat sports [23,25]. Differences in methods of RWL between sports are not solely limited to methods to reduce body water stores; for example, the use of fasting "always" or "sometimes" was only reported by 24% of boxers compared to 70% of wrestlers [25]. The specific reasons for differences in methods of RWL between other combat and weight category sports remains to be explored. Several factors are likely to be at play, including the culture of the sport itself, the number of weight categories, and the duration of the time period between weigh-in and competition [1,3–5].

The level of competition, calibre of athlete and/or professional status have been observed to varying degrees to be influencing factors in the prevalence and/or magnitude of RWL in several studies [21,23,25], i.e., higher prevalence of RWL, greater %body mass lost, and/or higher RWL scores were associated with more elite performers. A similar tendency was noted in the present study, with a moderate effect size observed for higher RWL score in the professional fighters. The RWL score is an outcome based on scoring of the RWLQ as described by the original validation papers [20,21], which allows for direct comparison between studies. The RWL score for this sample of MMA athletes was 37.9 ± 9.6, which is higher than scores of ~31 reported in boxing, judo, taekwondo, and wrestling [25].

This scoring system and calculated RWL scores do not include a weighting attributed to water loading or hot salt baths, which are common practices by MMA athletes. Whether these methods are commonly used in other combat sports, or whether the prevalence of hot baths reported herein is similar in other MMA cohorts, remains to be confirmed. Nevertheless, separate to the RWL scoring system, it is generally accepted that the %body mass lost as part of the RWL process in greater in MMA than other sports [3,4]. In other combat sports, the %body mass lost during averages ~2% to 6% [22,23,25], whereas the average is ~5% to 10% in MMA [7,8,15,16,23,24]. The 7.9% ± 3.1% reported by our cohort is, therefore, consistent with the magnitudes in the latter studies cited.

Fellow fighters and coaches/mentors were the most influential sources of information for weight-making practices in this cohort of MMA athletes, whereas health and fitness professionals such as doctors, dietitians and physical trainers are generally reported to have limited influence. This finding is not exclusive to MMA, and in fact, is widely reported across a range of combat and weight category sports [21,23–25]. Whether it is possible to overcome ingrained practices in a sport such as MMA remains to be seen, but support staff should be aware of these key influencers of the practices of their athletes. Governing bodies should consider formal education modules for their coaches and athletes on the potential health, safety and performance consequences of methods for RWL.

Aside from the limitations generally associated with self-reported data, another limitation that must be acknowledged in the present study is that the cohort of athletes surveyed were part of the same larger MMA franchise, SBG. Although the athletes trained in several different MMA gyms, the convenience sampling approach using an internal social media page likely resulted in the recruitment of athletes with largely similar coaching and support staff. While circulation of nutrition and weight-making advice is not a feature of the social media page, given the described influence of coaches and fellow fighters on methods of RWL, the sampling approach in this study may have introduced a bias to the results. Specifically, the finding of a high prevalence of hot salt bath use will need to be confirmed in other MMA cohorts. However, the overall results in terms of prevalence, magnitude and methods of RWL are largely similar to that of surveys of larger MMA cohorts [23,24].

Therefore, we conclude that manipulation of body water stores through water loading, fluid restriction and hot salt baths, in addition to gradual dieting and short-term fasting, are the most common methods of RWL employed by MMA athletes. Given the greater degree of RWL in MMA compared to other sports, whether measured by prevalence, %body mass loss or RWL score, there is a need for research on the physiological responses to these methods of RWL in addition to understanding the safety and performance characteristics of athletes who have undertaken aggressive weight regain strategies subsequent to these weight-making practices. Such research will benefit fighters, coaches and administrators alike in developing evidence-based practices, recommendations and policies for the sport.

Author Contributions: Conceptualization, J.C.; B.E.; methodology, J.C.; B.E.; formal analysis, J.C.; B.E.; writing—Original draft preparation, J.C.; B.E.; writing—Review and editing, J.C.; B.E.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

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Journal of Sports Sciences

ISSN: 0264-0414 (Print) 1466-447X (Online) Journal homepage: https://www.tandfonline.com/loi/rjsp20

Comparison of hot water immersion at 37.8°C with or without salt for rapid weight loss in mixed martial arts athletes

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JOURNAL OF SPORTS SCIENCES
https://doi.org/10.1080/02640414.2020.1721231

Comparison of hot water immersion at 37.8°C with or without salt for rapid weight loss in mixed martial arts athletes

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ABSTRACT

Hot water immersion, known as a hot bath, is used by MMA athletes to produce rapid weight loss (RWL) by means of passive fluid loss. This study investigated the magnitude of body mass losses using a standardized hot bath protocol with or without the addition of salt. In a crossover design, eleven male MMA athletes (28.5 ± 4.6 y; 1.83 ± 0.07 m; 82.5 ± 9.1 kg) performed a 20-min immersion at 37.8°C followed by a 40-min wrap in a warm room. This bath and wrap was performed twice per visit. During one visit, only fresh water was used (FWB), and in the other visit, magnesium sulphate (1.6% wt/vol) was added to the bath (SWB). Prior to each visit, 24 h of carbohydrate, fibre, and fluid restriction was undertaken as part of the RWL protocol. Body mass losses induced by the hot bath protocols were 1.63 ± 0.75 kg and 1.60 ± 0.80 kg for FWB and SWB, respectively, and equivalent to ~2.1% body mass. Under the conditions employed, the magnitude of body mass loss in SWB was similar to FWB. However, further research should explore bathing in a temperature that is consistent with that habitually used by fighters, and/or higher concentrations of salt.

ARTICLE HISTORY
Accepted 14 January 2020

KEYWORDS
Body water; fluid balance; heat; hydration; magnesium; sweat

Introduction

Rapid weight loss (RWL) is frequently practised in sports that have weight class restrictions (Khodaee, Olewinski, Shadgan, & Kiningham, 2015; Reale, Slater, & Burke, 2017a), including combat sports such as mixed martial arts (MMA) (Barley, Chapman, & Abbiss, 2019; Matthews, Stanhope, Godwin, Holmes, & Artioli, 2019). The weight-making practices of MMA athletes have recently been a subject of much interest (Andreato et al., 2014; Barley, Chapman, & Abbiss, 2018; Connor & Egan, 2019; Coswig, Fukuda, & Del Vecchio, 2015; Coswig et al., 2019; Crighton, Close, & Morton, 2016; Hillier et al., 2019; Jetton et al., 2013; Kasper et al., 2019; Matthews & Nicholas, 2017). Notably, the prevalence and magnitude of the RWL process is greater in MMA than other combat and weight category sports (Barley et al., 2019; Matthews et al., 2019), with the %body mass loss usually ~5% to 10% in the week prior to competition (Barley et al., 2018; Coswig et al., 2015, 2019; Crighton et al., 2016; Hillier et al., 2019; Matthews & Nicholas, 2017). At both professional and amateur levels, these athletes are using strategies that reduce body water stores (e.g., water loading, fluid restriction, and increasing sweat losses through heat exposure) as the predominant methods of RWL (Barley et al., 2018; Connor & Egan, 2019; Hillier et al., 2019).

A means of passive fluid loss known as hot baths has been briefly mentioned as part of weight-making practices in a number of case and small cohort studies (Brandt et al., 2018; Kasper et al., 2019; Matthews & Nicholas, 2017; Pettersson, Ekstrom, & Berg, 2013). We recently identified hot baths as a highly prevalent method of RWL in MMA athletes with 76% of a cohort of n = 29 male fighters reporting using hot baths "always" or "sometimes" (Connor & Egan, 2019). Hot baths generally describe the practice of hot water immersion followed by wrapping in warm clothing for a period of time prior to further exposures to hot water immersion (Kasper et al., 2019). As part of the hot bath protocol, fighters will often add Epsom salts with the prevailing wisdom that the addition of salt augments the loss of body mass compared to that achieved by immersion in fresh water. The addition of salt to this end does have some empirical evidence to support its practice (Hertig, Riedesel, & Belding, 1961; Hope, Aanderud, & Aakvaag, 2001). For example, 4 h of immersion up to the neck in 38°C water produced ~0.6 kg more body mass loss in seawater (~2.5 kg/4 h) compared to fresh water (~1.9 kg/4 h) (Hope et al., 2001). While the loss of body mass by hot water immersion is primarily through sweating-induced dehydration, the addition of salt increases the osmotic pressure difference between the immersion medium and body fluids, which likely contributes to the greater fluid loss compared to fresh water (Hertig et al., 1961; Hope et al., 2001; Whitehouse, Hancock, & Haldane, 1932). However, a comparison of fresh versus salt water immersion has not been investigated in an athletic population as part of RWL practice.

Therefore, the aim of the present study was to determine the magnitude of body mass losses in MMA athletes using a standardized hot bath protocol, with or without the addition of Epsom salt.

Methods

Participants

Eleven male professional MMA athletes (age, 28.5 ± 4.6 y; height, 1.83 ± 0.07 m; body mass, 82.5 ± 9.1 kg) with previous experience

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Supplemental data for this article can be accessed here.

of RWL provided written informed consent to participate. The study was approved by the Human Research Ethics Committee of Dublin City University (permit number: DCUREC/2019/021).

Design

A crossover-repeated measures design was employed to compare the effects on passive fluid loss of hot water immersion under conditions of fresh water bathing (FWB) compared to salt water bathing (SWB). Participants performed two main experimental trials separated by at least 7 days, with the trials being identical with the exception of the water condition in which they were immersed. The bathing protocol comprised 20-min of hot water immersion ("bath") followed by 40-min wrap in heavy clothing and blankets in a warm room ("wrap"). This 60-min bath and wrap protocol was repeated twice per main experimental trial, i.e., 2 h total. On the day prior to bathing, participants were prescribed to eliminate carbohydrate- and fibre-rich foods from their diet and consume 22 kcal/kg body mass. Fluid intake was prescribed to be restricted to 15 mL/kg for the 24 h before bathing.

Change in body mass, measured to the nearest 0.05 kg (model #63667; Soehnle, Germany), was the primary outcome measure. Body mass was measured in minimal clothing, i.e., lower body short underwear in the form of briefs or boxer briefs, at several time points: (i) upon waking on the day prior to bathing (Morning Day −1), (ii) upon waking on the day of bathing (Morning Day 0), (iii) immediately prior to the first bath, (iv) immediately before the second bath, (v) immediately after the second wrap, and finally, (v) upon waking on the day after bathing (Morning Day +1).

Urine osmolality was measured (Osmocheck Portable Osmometer; Vitech Scientific, UK) at the same time points except immediately before the second bath and wrap. Participants were defined as dehydrated using a criteria of urine osmolality of >700 mOsmol/kg (Sawka et al., 2007).

Methodology

For each bath, participants were submerged up to the neck for 20-min bath at 37.8ºC. A floating thermometer (Avent Bath & Room Thermometer; Philips, UK) was checked frequently and the bath was topped up with hot water as needed to maintain the target temperature.

After 20-min of bathing, participants dried off in the bathroom and as quickly as possible put on a knitted wool hat, cotton t-shirt, hooded cotton sweatshirt, cotton tracksuit bottoms/sweatpants, and socks. Participants were then covered in blankets on a bed in an adjacent room with only their face exposed. This wrap was performed for 40-min. This 60-min bath and wrap protocol is considered one round and was repeated twice per main experimental trial.

Upon completion of the second round, participants began the weight regain process and were prescribed to consume fluids (in L) to the equivalent to 150% of total body mass loss (in kg) (Sawka et al., 2007) from Morning Day −1, and to consume 6 g/kg body mass of carbohydrate throughout the rest of the day.

For the FWB trial, only fresh tap water was used in the bath. For the SWB trial, Epsom salts (magnesium sulphate) were added to the bath with 160 L capacity at a concentration of 2 kg in 125 L of water (i.e., ~1.6% wt/vol). This quantity and type of salt was chosen based on our personal experiences of the practices of fighters making weight in combat sports and was subsequently confirmed as approximating general practices of this cohort in exit questionnaires completed by the study participants. Each participant completed the questionnaire upon completion of the second trial, which examined their experiences of the study and their habitual practices of hot baths for RWL.

Statistical analysis

Statistical analysis and graphical representation were performed using GraphPad Prism v8.1 (GraphPad Software, Inc., USA). Normality of data was assessed with the Shapiro-Wilk normality test, for which all data passed. All data are presented as mean±SD. A two-way (condition x time) repeated measures analysis of variance (ANOVA) was used to assess responses to the interventions. When a main or interaction effect was observed, pairwise comparisons were performed with Bonferroni's correction for which multiplicity-adjusted p-values are reported. The level of significance for all tests was set at $P < 0.05$. Standardised differences in the mean were used to assess magnitudes of effects between conditions. These were calculated using Cohen's d effect size (ES) and interpreted using thresholds of <0.2, ≥0.2, ≥0.5, and ≥0.8 for trivial, small, moderate, and large, respectively.

Results

For change in body mass in absolute (kg) (Table 1) and relative (%initial body mass) (Figure 1) terms, a main effect of time ($P < 0.001$), but neither a main effect of condition nor a condition × time interaction effect, was observed. Similarly, there was no difference between conditions for changes in urine osmolality at the various time points (Table 1).

Body mass losses induced by carbohydrate and fluid restriction were 2.29 ± 0.82 kg ($P < 0.001$; d= 0.26) and 2.25 ± 0.86 kg ($P < 0.001$; d= 0.25) in preparation for the FWB and SWB trials, respectively. Body mass losses induced by the hot bath protocols were 1.63 ± 0.75 kg ($P < 0.001$; d= 0.20) and 1.60 ± 0.80 kg ($P < 0.001$; d= 0.20) for the FWB and SWB protocols, respectively. FWB resulted in body mass loss of 0.85 ± 0.36 kg ($P < 0.001$; d= 0.10) during the 1st bath and wrap, and 0.79 ± 0.47 kg ($P < 0.001$; d= 0.09) during the 2nd bath and wrap. SWB resulted in body mass loss of 0.74 ± 0.44 kg ($P < 0.001$; d= 0.08) during the 1st bath and wrap, and 0.86 ± 0.43 kg ($P < 0.001$; d= 0.10) during the 2nd bath and wrap.

Total body mass losses induced by the entire RWL protocol were 3.92 ± 1.22 kg ($P < 0.001$; d= 0.44) and 3.84 ± 1.35 kg ($P < 0.001$; d= 0.42) for the FWB and SWB protocols, respectively. These values represented losses of initial body mass of 4.27 ± 1.50% and 4.29 ± 1.84% for the FWB and SWB protocols, respectively (Figure 2(a)).

Weight regain was 2.97 ± 1.15 kg ($P < 0.001$; d= 0.35) and 3.14 ± 1.04 kg ($P < 0.001$; d= 0.35) during recovery from the FWB and SWB protocols, respectively, resulting in a body mass deficit compared to Morning Day −1 of 0.95 ± 1.06 kg and 0.70 ± 1.03 kg, respectively. At Morning Day +1, 10 FWB

Table 1. Body mass (kg) and hydration status assessed by urine osmolality (mOsmol/kg) at time points during a rapid weight loss intervention featuring a hot bath protocol in fresh (FWB) or salt water (SWB).

	Morning Day −1	Morning Day 0	Before 1st bath	After 1st bath & wrap	After 2nd bath & wrap	Morning Day +1	P value
Body mass (kg)							Time, P = 0.001***
FWB	82.49 ± 9.14	80.69 ± 8.55	80.20 ± 8.66	79.36 ± 8.75	78.57 ± 8.73	81.55 ± 8.31	Condition, P = 0.271
SWB	82.04 ± 9.12	80.28 ± 9.00	79.79 ± 8.75	79.05 ± 8.92	78.20 ± 8.98	81.34 ± 8.82	Interaction, P = 0.817
Urine osmolality (mOsmol/kg)							Time, P = 0.004**
FWB	693 ± 235	894 ± 137	845 ± 115		928 ± 78	796 ± 219	Condition, P = 0.468
SWB	637 ± 204	871 ± 163	852 ± 157		925 ± 214	750 ± 314	Interaction, P = 0.737

Data are presented as mean ± SD, n = 11. **P < 0.01; ***P < 0.001.

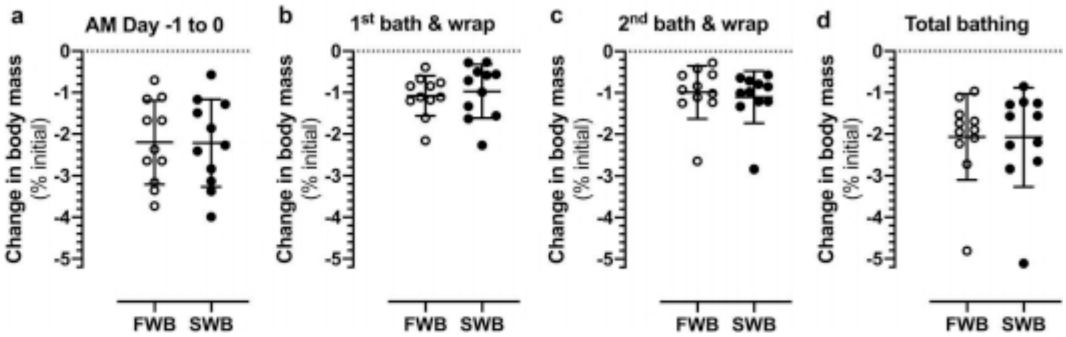

Figure 1. Percentage changes in body mass (relative to baseline recorded on Morning Day −1) induced by diet and fluid restriction, and a hot bath protocol in fresh (FWB) or salt water (SWB). Data are mean ± SD for changes observed within each time period that is defined above each panel.

trial and 8 SWB trial participants were in a body mass deficit compared to Morning Day −1, and 9 FWB trial and 6 SWB trial participants were defined as dehydrated (urine osmolality >700 mOsmol/kg).

Exit questionnaires were completed by the 10 of the 11 participants (Supplementary Table). Seven out of ten participants used hot baths "always" or "sometimes" as part of the RWL process, with six out of these seven participants using salt as part of the bath and wrap process. Six out of the seven participants usually spend 11 to 25-min immersed in hot water. Time spent wrapped in warm towels/bed clothes ranged from 6 to 60-min. Six out of eight participants who have previously used hot baths usually repeat the bath and wrap twice.

Body mass loss during the bath and wrap process was reported as usually being 1.1 to 1.5 kg. One participant reported a usual weight loss of 5.1 to 5.5 kg, with this individual reporting using two 60-min hot water immersions separated by a 15-min wrap. Another participant reported a usual weight loss of 3.6 to 4.0 kg, with this individual reporting using a 15-min hot water immersion followed by a 60-min wrap repeated for two rounds.

All but one participant found our bathing protocol at 37.8°C to be colder than the hot water immersion that they usually employ, but only two participants reported using a thermometer to measure the water temperature as part of their usual practice. All participants reported increasing the water temperature

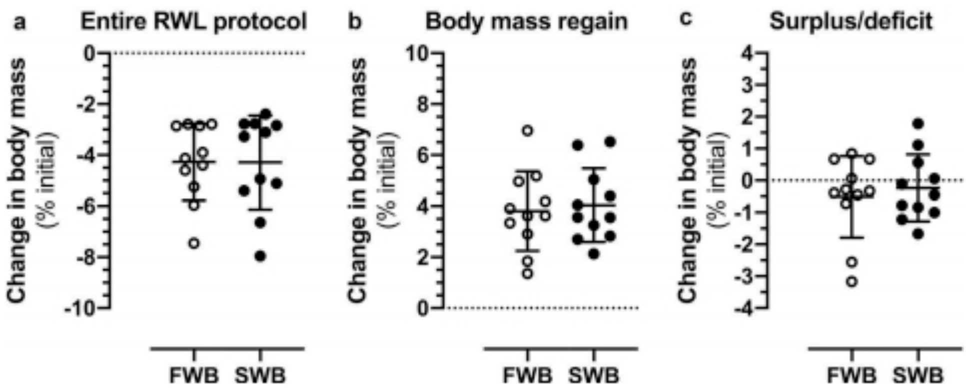

Figure 2. Percentage changes in body mass (relative to baseline recorded on Morning Day −1) during (a) the entire rapid weight loss intervention featuring a hot bath protocol in fresh (FWB) or salt water (SWB), (b) the period of weight regain prior to weigh-in on Morning Day +1, and (c) as a measure of total body mass deficit or surplus at on Morning day +1 compared to Morning Day −1. Data are mean ± SD.

throughout each immersion, either using hot tap water or boiled kettle water.

Seven out of eight participants reported adding salt to their hot baths, each of whom used Epsom salts, with the average quantity being 1 to 2 kg of salt. One participant reported using the salts for "muscle relaxation", whereas the remaining participants reported adding salts because they were led to believe that it enhanced the weight-cutting effect of a hot bath, with two participants referring to the concept of a potential osmotic effect in supporting weight loss.

Discussion

This is the first study to describe a standardized hot bath protocol in MMA athletes and investigate if adding salt to hot water immersion at 37.8ºC increases body mass loss during a RWL protocol. The main finding is that the body mass loss when bathing in a hot bath of fresh water (FWB) is similar to bathing in a hot bath with ~1.6% Epsom salt added (SWB).

The absence of difference between body mass loss during FWB compared to SWB is in contrast to previous work demonstrating ~32% greater body mass loss over 4 h of immersion at 38ºC in seawater compared to fresh water (Hope et al., 2001). The differences between the study protocols are most obviously the duration (4 h continuous immersion versus the present 2 × 20-min bath/40-min wrap protocol), the salt concentration (seawater being ~3.5% salt versus ~1.6% in our protocol), and the type of salt (seawater versus added Epsom salt). Whether the latter would make any difference to the outcome remains to be explored, but is unlikely. The contention is that in salt water immersion, the osmotic pressure difference between the immersion medium and body fluids results in greater fluid loss compared to fresh water (Hertig et al., 1961; Hope et al., 2001; Whitehouse et al., 1932). Such a difference was not observed in the present study, wherein body mass loss in both FWB and SWB trials averaged ~1.6 kg, or 2.1% body mass. However, the concentration of salt in the hot bath is an important factor to consider in this context. The present protocol employed a salt concentration of ~1.6% wt/vol magnesium sulphate. This quantity and type of salt was chosen for its ecological validity based on our personal experience of working with combat sports athletes during weight-making efforts and was confirmed during exit questionnaires to be the usual quantity and type of salt per bath used by this cohort of fighters. Early work established that even in thermoneutral water, i.e., in the absence of sweating, immersion in a strong salt solution (either 11.5% or 20.0% salt as sodium chloride) produces passive fluid loss (Whitehouse et al., 1932). In water heated to 36/37ºC, addition of 5% sodium chloride allowed for higher sweat rates during 3 h of immersion when compared to fresh water (Hertig et al., 1961). This effect was more pronounced at salt concentrations of 10% and 15%, with the authors suggesting that the salt did not serve as a stimulus for sweating, but rather served to remove an inhibitory influence on the decline in sweat rate that usually occurs with prolonged immersion in fresh water (Hertig et al., 1961). Therefore, it may be that the concentration of salt in a hot bath should at least 3.5% (Hope et al., 2001), or possibly greater (Hertig et al., 1961), if the aim is to augment the rate of passive fluid loss that would otherwise occur in fresh water.

Notably, there was a greater loss of body mass by the 24 h of restriction of carbohydrate, fibre, and fluid (~2.2 kg), than from either bathing protocol (~1.6 kg). This magnitude of body mass loss is consistent with the suggestion of a ~3% reduction in body mass to be expected by short-duration glycogen depletion and emptying of the intestinal contents (Reale, Slater, & Burke, 2017b).

All participants were classified as dehydrated when measured after the second wrap, a time point selected to be representative of weigh-in time for these fighters. This is consistent with typical methods of RWL resulting in 100% of MMA athletes being dehydrated to various degrees at an official weigh-in (Jetton et al., 2013; Matthews & Nicholas, 2017). For example, in preparation for a competitive bout, 57% and 43% of fighters were reported to be dehydrated (1033 ± 19 mOsmol/kg) and severely dehydrated (1267 ± 47 mOsmol/kg), respectively, at the weigh-in (Matthews & Nicholas, 2017). Moreover, 14% (Matthews & Nicholas, 2017) and 39% (Jetton et al., 2013) of fighters remained dehydrated when measured in the final 2 h prior to a competitive fight. In the present study, after a 20 h recovery period, 9 (FWB trial) and 6 (SWB trial) participants remained dehydrated.

Although mentioned briefly in a number of case and small cohort studies (Brandt et al., 2018; Kasper et al., 2019; Matthews & Nicholas, 2017; Pettersson et al., 2013), our recent survey reported the use of hot baths to be prevalent (76%) in MMA (Connor & Egan, 2019), but this present study suggests that the exact protocol varies considerably between individual fighters. Within the current cohort, duration of immersions varied from 11 to 60-min and duration of wraps varied from 6 to 60-min. Most fighters reported that the number of combined baths with wraps is two rounds for a "normal" weight cut. In contrast, one case study reported nine hot baths being used in the 20 h prior to weigh-in as part of one fighter's weight cut (Kasper et al., 2019). All but one participant found our bathing protocol at 37.8ºC to be colder than the hot water immersion that they usually employ. All participants reported increasing the water temperature throughout each immersion, either using hot tap water or boiled kettle water. Clearly, there are large variations in methods employed for hot baths, but the present study may act as a reference point for further research. For example, whether a higher water temperature and/or differences in salt type and/or concentration would reveal differences between FWB and SWB protocols.

In summary, hot baths are commonly used by MMA athletes and are an effective method of RWL, but there are large variations in protocols used by fighters in practice. Under the standardised conditions employed in the present study, the total amount of body mass loss during a hot bath in water supplemented with ~1.6% Epsom salt was similar to a hot bath performed in fresh water (~2.1% over 2 h of bathing and wrapping). However, further research should explore hotter bathing temperatures that are consistent with those habitually used by fighters, and higher concentrations of salt in order to produce a large osmotic gradient between the bath water and body fluids. Carbohydrate, fibre, and fluid restriction for 24 h prior to commencing the bathing protocol resulted in ~2.8% loss of body mass, suggesting that dietary manipulation should be considered as a method of RWL prior to employing aggressive dehydration strategies, particularly if the desired weight loss is less than ~3% of body mass.

Acknowledgments

The authors thank the fighters for their participation, and Ciaran Clarke and Alannah Hedderman for their assistance during data collection.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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 DOI: https://doi.org/10.5114/biolsport.2020.96947

Comparison of hot water immersion at self-adjusted maximum tolerable temperature, with or without the addition of salt, for rapid weight loss in mixed martial arts athletes

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ABSTRACT: Hot water immersion is used by athletes in weight category sports to produce rapid weight loss (RWL) by means of passive fluid loss, and often is performed with the addition of Epsom salts (magnesium sulphate). This study investigated the magnitude of body mass losses during hot water immersion with or without the addition of salt, with the temperature commencing at 37.8°C and being self-adjusted by participants to their maximum tolerable temperature. In a crossover design, eight male MMA athletes (29.4 ± 5.3 y; 1.83 ± 0.05 m; 85.0 ± 4.9 kg) performed a 20 min whole-body immersion followed by a 40 min wrap in a warm room, twice in sequence per visit. During one visit, only fresh water was used (FWB), and in the other visit, magnesium sulphate (1.6% wt/vol) was added to the bath (SWB). Prior to each visit, 24 h of carbohydrate, fibre and fluid restriction was undertaken. Water temperatures at the end of the first and second baths were ~39.0°C and ~39.5°C, respectively. Body mass losses induced by the hot bath protocols were 1.71 ± 0.70 kg and 1.66 ± 0.78 kg for FWB and SWB, respectively ($P = 0.867$ between trials, $d = 0.07$), and equivalent to ~2.0% body mass. Body mass lost during the entire RWL protocol was 4.5 ± 0.7%. Under the conditions employed, the magnitude of body mass lost in SWB was similar to FWB. Augmenting passive fluid loss during hot water immersion with the addition of salt may require a higher salt concentration than that presently utilised.

CITATION: Connor J, Egan B. Comparison of hot water immersion at self-adjusted maximum tolerable temperature, with or without the addition of salt, for rapid weight loss in mixed martial arts athletes. Biol Sport. 2021;38(1):89–96.

Received: 2020-05-29; Reviewed: 2020-06-26; Re-submitted: 2020-06-28; Accepted: 2020-06-30; Published: 2020-08-07.

Key words:
Body water
Fluid balance
Heat
Hydration
Magnesium
Sweat

INTRODUCTION

Rapid weight loss (RWL) is frequently practiced in sports that have weight class restrictions [1, 2]. For example, in mixed martial arts (MMA), the percentage of body mass lost by these athletes is usually ~5% to 10% in the week prior to competition [3–9]. To achieve losses of this magnitude, RWL strategies that reduce body water stores (e.g. water loading, fluid restriction, and increasing sweat losses through heat exposure) are the predominant methods of RWL [4, 5, 9].

A means of passive fluid loss known as hot baths is often employed as part of weight-making practices in combat sports [3, 9–13]. A recent survey of RWL practices by MMA athletes reported the use of hot baths to be highly prevalent, with 76% of fighters reporting their use either "always" or "sometimes" [9]. Hot baths generally describe the practice of hot water immersion followed by wrapping in warm clothing for a period of time prior to further exposures to hot water immersion and wrapping. However, there are large variations in how athletes perform a hot bath protocol [14]. For instance, in a cohort of 11 fighters, duration of immersions varied from 11 to 60 min and duration of wraps varied from 6 to 60 min, and the number of combined immersions with wraps is typically two rounds for a "normal" weight cut [14]. In contrast, one case study reported nine hot baths being used in the 20 h prior to weigh-in as part of one fighter's weight cut [10].

As part of their personal hot bath protocol, many of the fighters described the addition of 1 to 2 kg of Epsom salts to the water with the aim of augmenting the loss of body mass compared to that achieved by immersion in fresh water [14]. The addition of salt to this end does have some empirical evidence to support its practice [15–17], with the suggestion that the addition of salt increases the osmotic pressure difference between the immersion medium and body fluids, and/or removes the inhibitory effect on sweating, and thereby contributes to the greater fluid loss compared to fresh water [15–20]. We recently tested the addition of Epsom salts to produce a 1.6% salt solution but found no difference in body mass losses comparing fresh water and salt water immersion when the water temperature was maintained at 37.8°C. In the absence of previous studies in athletes, we used this fixed temperature in order to increase the internal validity of the experimental design. However, in an exit questionnaire, all but one participant found our bathing protocol at 37.8°C to be colder than the hot water immersion that they usually employ, and all participants reported that they usually increase the water temperature throughout each immersion, either using hot tap water or boiled kettle water. Therefore, in practice in MMA, a hot bath protocol is completed by starting at a warm water temperature and increasing temperature to the fighter's maximum tolerable temperature. This difference in protocol compared to our recent experiment is salient because there is a suggestion from previous work that water temperature and salt concentration may interact such that the effect of the addition of salt, if any, is greater at higher water temperatures [17, 19].

Therefore, the aim of the present study was to determine the magnitude of body mass losses in MMA athletes using a hot bath protocol with immersion in hot water with or without the addition of Epsom salt, and wherein participants were encouraged to increase bathing temperatures to that which they would use during their typical hot bath protocol during a weight cut.

MATERIALS AND METHODS

Participants

Eight male MMA athletes (age, 29.4 ± 5.3 y; height, 1.83 ± 0.05 m; body mass, 85.0 ± 4.9 kg) provided written informed consent to participate. Participants comprised both amateur and professional fighters, including two former Ultimate Fighting Championship (UFC) fighters. All participants were competing under professional weigh-in rules at the time of the study i.e. weigh-in 24 h before competition. Each participant had previous experience of RWL and the use of hot baths as part of that process, and each had made weight for competition on at least ten occasions prior to participation in the study. The study was approved by the Human Research Ethics Committee of Dublin City University (permit number: DCUREC/2019/115). This study protocol was based on our previous work [14], but was performed independent of that work, separated by 4 to 6 calendar months, and under a different ethics committee permit. However, n = 6 participants were common to both studies.

Protocol

A crossover-repeated measures design was employed to compare the effects on passive fluid loss of hot water immersion under conditions of fresh water bathing (FWB) compared to salt water bathing (SWB). Participants performed two main experimental trials separated by at least seven days, with the order of the FWB and SWB trials being assigned in a counterbalanced manner. The FWB and SWB trials were identical with the exception of the water condition in which they were immersed (Figure 1). On the day prior to bathing, participants were prescribed to eliminate carbohydrate- and fibre-rich foods from their diet and consume an energy intake of 22 kcal/kg body mass. Fluid intake was prescribed to be restricted to 15 mL/kg for the 24 hours before bathing. These dietary and fluid restriction

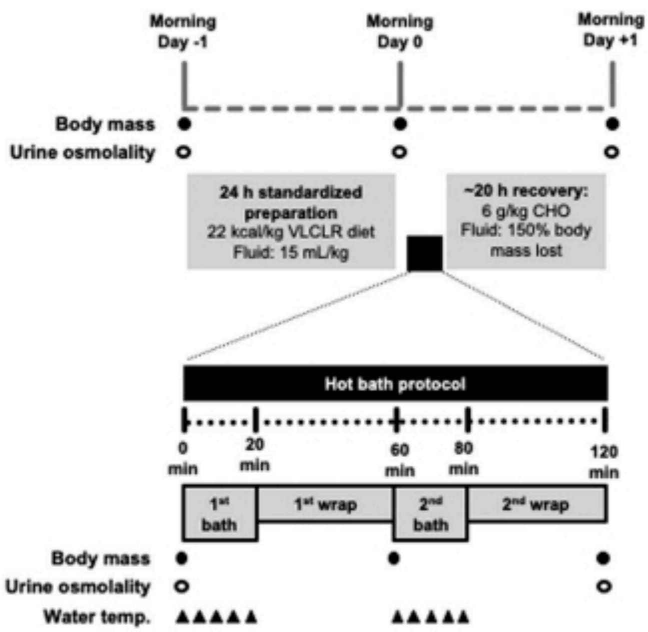

FIG. 1. Study design schematic. Experimental trials were identical with the exception of the water condition in which they were immersed being with fresh water bathing or salt water bathing on separate days. CHO, carbohydrate; VLCLR, very low carbohydrate, low residue.

protocols were typical of what was practiced by the participants in their previous RWL experiences, and compliance with the prescribed protocol was confirmed verbally on Morning Day 0. The bathing protocol was as previously described [14] and comprised of 20 min of hot water immersion ("bath") followed by 40 min wrapped in heavy clothing and blankets in a warm room ("wrap"). This 60 min bath and wrap protocol was repeated twice per main experimental trial i.e. 2 h total (Figure 1). All experiments took place in the same bath, bathroom, and adjacent bedroom of a private residential dwelling.

For each bath, participants were submerged up to the neck for 20 min. The initial water temperature of the bath was prepared to 37.8°C, but participants were encouraged to bath in a water temperature that was typical for a normal weight cut bath protocol. In practice, this process usually involves bringing the water temperature up to a fighter's maximum tolerable level, but this temperature will vary from fighter to fighter. To achieve this aim, participants requested from the researchers for the addition of boiling water from an electric kettle (1.5 L) to the bath *ad libitum*. The volume of additional boiling water per bath was noted. A floating thermometer (Avent Bath & Room Thermometer; Philips, UK) was checked at 4 min intervals for measurement of water temperature (Figure 1), but participants were not informed of the temperature during either bath or trial.

After 20 min of bathing, participants dried off in the bathroom and as quickly as possible put on a knitted wool hat, cotton t-shirt, hooded cotton sweatshirt, cotton tracksuit bottoms/sweatpants, and socks. Participants were then covered in blankets on a bed in an adjacent bedroom with only their face exposed. This wrap was performed for 40 min. Room temperature ranged from 24°C to 29°C during the trials. This 60 min bath and wrap protocol is considered one round and was repeated twice per main experimental trial (Figure 1).

Upon completion of the second round, participants began the weight regain process and were prescribed to consume fluids (in L) to the equivalent to 150% of total body mass lost (in kg) [21] from Morning Day -1, and to consume 6 g/kg body mass of carbohydrate throughout the rest of the day.

For the FWB trial, only fresh tap water was used in the bath. For the SWB trial, Epsom salts (magnesium sulfate) were added to the bath with 160 L capacity at a concentration of 2 kg in 125 L of water (i.e. ~1.6% wt/vol). This quantity and type of salt was used in our previous work and was chosen based on our personal experiences of the practices of fighters making weight in combat sports, and was subsequently confirmed as approximating general practices of that participant cohort in exit questionnaires completed by the study participants [14].

Change in body mass, measured to the nearest 0.05 kg (model #63667; Soehnle, Germany), was the primary outcome measure. Body mass was measured in minimal clothing, i.e. lower body short underwear in the form of briefs or boxer briefs, at several time-points (Figure 1): (i) upon waking on the day prior to bathing (Morning Day -1), (ii) upon waking on the day of bathing (Morning Day 0), (iii) immediately prior to the first bath, (iv) immediately before the second bath, (v) immediately after the second wrap, and finally, (vi) upon waking on the day after bathing (Morning Day +1).

Urine osmolality was measured (Osmocheck Portable Osmometer; Vitech Scientific, UK) at the same time points except immediately before the second bath and wrap. Participants were defined as dehydrated using a criteria of urine osmolality of >700 mOsmol/kg [21].

Sample size calculation

The primary outcome was change in body mass as a consequence of the 2 h bath and wrap protocol. Therefore, a sample size calculation was performed (G*Power v.3.1) based on previous research demonstrating an effect of salt water to augment the magnitude of body mass lost during hot water immersion when compared to fresh water [15]. Using the body mass lost after 2 h of that 4 h immersion protocol, a time point analogous to the present work, and that being 0.98 ± 0.44 kg and 1.24 ± 0.80 kg for fresh water and salt water respectively, and an assumed correlation between conditions of 0.90, the required sample size to detect a difference between FWB and SWB at a Type I error rate (a) of 0.05 and a power (1-b) of 0.8 was n = 26. However, because these data are based on a higher salt concentration of ~3.5%, and given the absence of effect in our previous research using a salt concentration of 1.6% [14], *a priori* we planned an interim data analysis for the assessment of futility, and therefore discontinuation, after completion of one-third (n~8) of the required sample size. In the absence of any difference between FWB and SWB for change in body mass with n = 8 (P = 0.867 between trials, d = 0.07; data reported below), we discontinued recruitment at that time.

Statistical analysis

Statistical analysis and graphical representation were performed using GraphPad Prism v8.3 (GraphPad Software, Inc., USA). Normality of data was assessed with the Shapiro-Wilk normality test, for which all data passed. All data are presented as mean ± SD. A two way (condition x time) repeated measures analysis of variance (ANOVA) was used to assess responses to the interventions. When a main or interaction effect was observed, pairwise comparisons were performed with Bonferroni's correction for which multiplicity-adjusted P-values are reported. Paired t-tests were used to assess differences between trials for the quantity of boiling water added, and differences in body mass lost during bathing between this study and our previous study for the n = 6 participants common to both studies. The level of significance for all tests was set at $P < 0.05$. Standardized differences in the mean were used to assess magnitudes of effects between conditions. These were calculated using Cohen's d effect size and are interpreted using thresholds of < 0.2, ≥ 0.2, ≥ 0.5 and ≥ 0.8 for *trivial*, *small*, *moderate*, and *large*, respectively.

RESULTS

After starting each bath temperature at 37.8°C, the participant's self-adjustment of bathing temperature resulted in progressive increases in water temperature in both the 1st and 2nd baths (main effect of time, $P < 0.001$) (Figure 2A & 2C). Average water temperature in the 1st bath of each trial was 38.41 ± 0.31°C and 38.16 ± 0.31°C for FWB and SWB, respectively ($P = 0.135$), and in the 2nd bath of each trial was 38.48 ± 0.36°C and 38.64 ± 0.22°C for FWB and SWB, respectively ($P = 0.341$). Final water temperature in the 1st bath of each trial was 38.94 ± 0.70°C and 38.93 ± 0.63°C for FWB and SWB, respectively ($P = 0.972$), and in the 2nd bath of each trial was 39.14 ± 0.70°C and 39.59 ± 0.45°C for FWB and SWB, respectively ($P = 0.154$). No condition or interaction effects were observed for the effect of salt (Figure 2A & 2C). The volume of boiling kettle water added to each bath was 4.50 ± 1.96 L for FWB and 5.44 ± 1.12 L for SWB during the 1st bath of each trial ($P = 0.305$), and 4.69 ± 2.03 L for FWB and 5.81 ± 0.96 L for SWB during the 2nd bath of each trial ($P = 0.080$) (Figure 2B & 2D).

For change in body mass in absolute (kg) (Table 1) and relative (%initial body mass) (Figure 3) terms, a main effect of time ($P < 0.001$), but neither a main effect of condition, nor a condition*time interaction effect, was observed. Similarly, there was no difference between conditions for changes in urine osmolality at the various time points (Table 1).

Body mass losses induced by carbohydrate and fluid restriction were 2.14 ± 0.78 kg ($P < 0.001$; $d = 0.44$) and 2.08 ± 0.96 kg ($P < 0.001$; $d = 0.40$) in preparation for the FWB and SWB trials, respectively. Body mass losses induced by the hot bath protocols were 1.71 ± 0.70 kg ($P < 0.001$; $d = 0.37$) and 1.66 ± 0.78 kg ($P < 0.001$; $d = 0.34$) for the FWB and SWB protocols, respectively. FWB resulted in body mass loss of 0.76 ± 0.53 kg ($P = 0.005$;

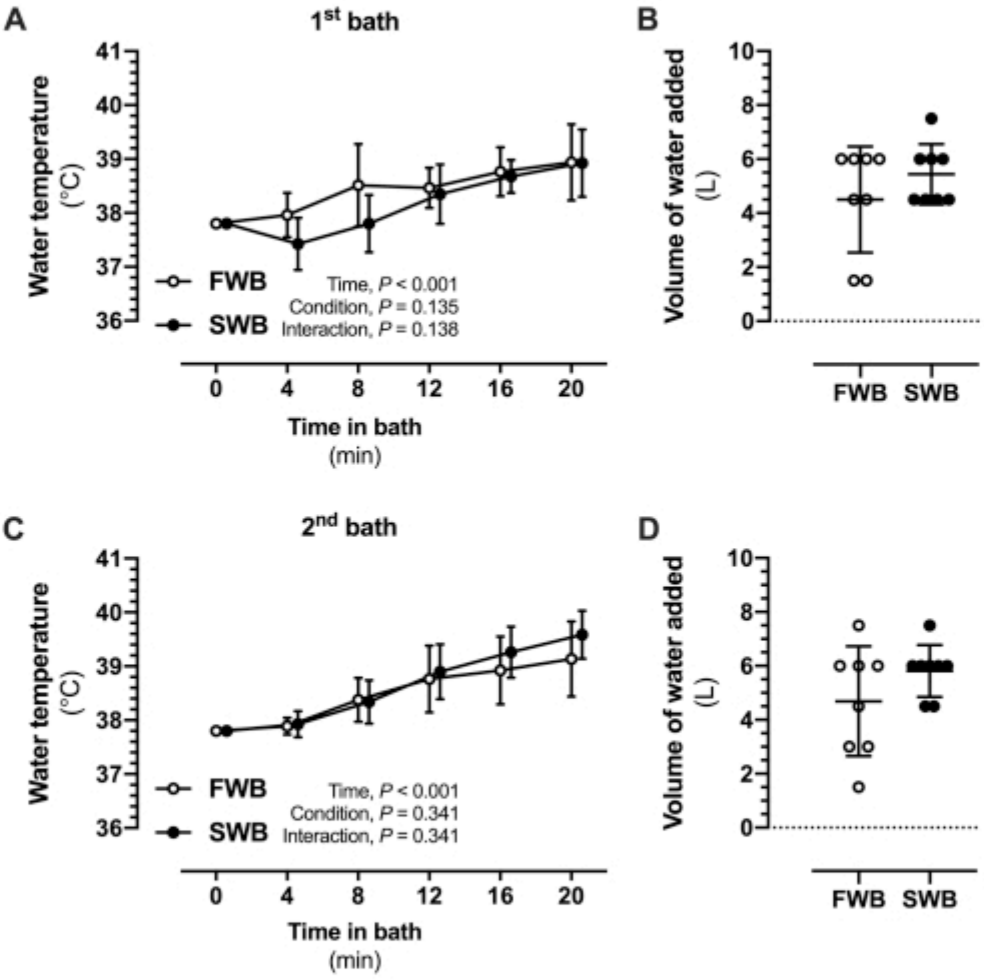

FIG. 2. Water temperatures measured at 4 min intervals during each bath (A, 1st bath; C, 2nd bath) during experimental trials of fresh (FWB) or salt water (SWB); and quantity of boiling kettle water added per bath (B, 1st bath; D, 2nd bath). White (FWB) and black (SWB) circles in panels B and D represent individual data points. Otherwise data are mean values with vertical bars representing SD.

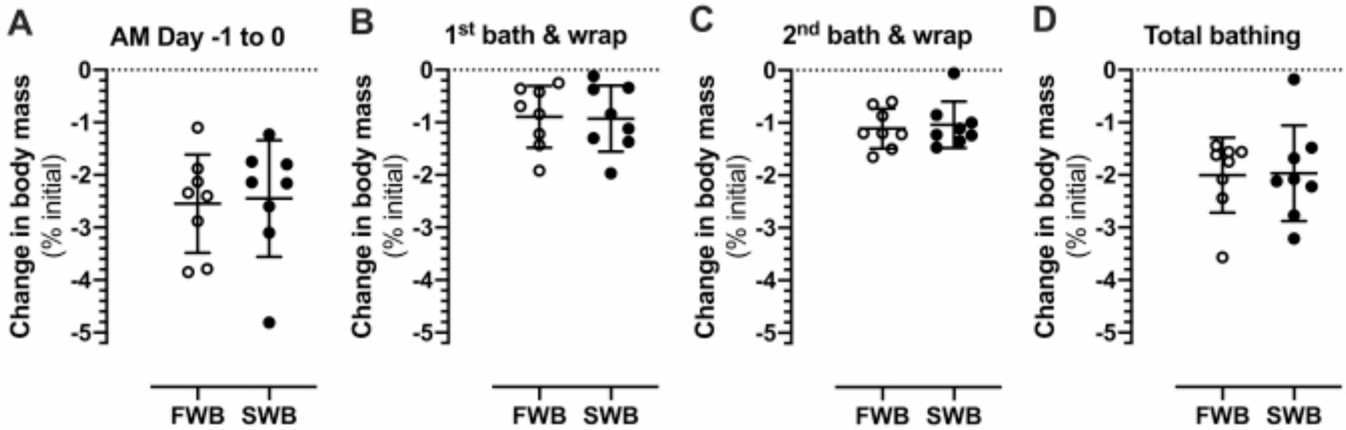

FIG. 3. Percentage changes in body mass (relative to baseline recorded on Morning Day -1) induced by diet and fluid restriction, and a hot bath protocol in fresh (FWB) or salt water (SWB) for (A) the period from Morning Day -1 to Morning Day 0, (B) the 60 min period comprising the first bath and wrap, (C) the 60 min period comprising the second bath and wrap, and (D) the 120 min period comprising both baths and wraps. White (FWB) and black (SWB) circles in each panel represent individual data points. Mean values are represented by the horizontal solid line with vertical bars representing SD for changes observed within each time period that is defined above each panel.

TABLE 1. Body mass (kg) and hydration status assessed by urine osmolality (mOsmol/kg) at time points during a rapid weight loss intervention featuring a hot bath protocol in fresh (FWB) or salt water (SWB).

	Morning Day -1	Morning Day 0	Before 1st bath	After 1st bath & wrap	After 2nd bath & wrap	Morning Day +1	P value
Body mass (kg)							Time, P < 0.001
FWB	85.03 ± 4.87	83.31 ± 4.86	82.89 ± 4.83	82.13 ± 4.61	81.18 ± 4.40	84.75 ± 4.72	Condition, *P* = 0.919
SWB	84.94 ± 5.45	83.34 ± 4.98	82.86 ± 4.85	82.09 ± 5.01	81.21 ± 4.87	84.60 ± 5.04	Interaction, *P* = 0.953
Urine osmolality (mOsmol/kg)							Time, P = 0.001
FWB	718 ± 137	880 ± 137	856 ± 117		989 ± 126	909 ± 134	Condition, *P* = 0.333
SWB	709 ± 234	939 ± 121	897 ± 152		943 ± 90	954 ± 133	Interaction, *P* = 0.615

Data are presented as mean ± SD, n = 8.

d = 0.16) during the 1st bath and wrap, and 0.94 ± 0.35 kg (P = 0.001; d = 0.21) during the 2nd bath and wrap. SWB resulted in body mass loss of 0.77 ± 0.52 kg (P = 0.004; d = 0.16) during the 1st bath and wrap, and 0.88 ± 0.40 kg (P < 0.001; d = 0.18) during the 2nd bath and wrap.

Total body mass losses induced by the entire RWL protocol were 3.84 ± 0.74 kg (P < 0.001; d = 0.83) and 3.74 ± 0.70 kg (P < 0.001; d = 0.72) for the FWB and SWB protocols, respectively. These values represented losses of relative to initial body mass on Morning Day -1 of 4.55 ± 0.77% and 4.44 ± 0.66% for the FWB and SWB protocols, respectively (Figure 4A).

Weight regain was 3.57 ± 0.86 kg (P < 0.001; d = 0.78) and 3.39 ± 0.87 kg (P < 0.001; d = 0.89) during recovery from the FWB and SWB protocols, respectively (Figure 4B), resulting in a body mass deficit compared to Morning Day -1 of 0.28 ± 0.44 kg and 0.34 ± 0.89 kg, respectively (Figure 4C). At Morning Day +1, 6 (FWB trial) and 5 (SWB trial) participants were in a body mass deficit compared to Morning Day -1, and all participants, regardless

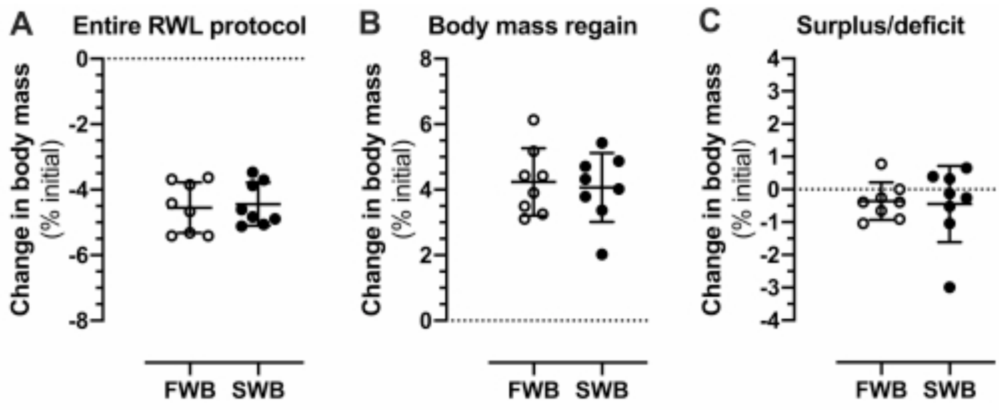

FIG. 4. Percentage changes in body mass (relative to baseline recorded on Morning Day -1) during (A) the entire rapid weight loss intervention featuring a hot bath protocol in FWB or SWB, (B) the period of weight regain prior to weigh-in on Morning Day +1, and (C) as a measure of total body mass deficit or surplus on Morning Day +1 compared to Morning Day -1. White (FWB) and black (SWB) circles in each panel represent individual data points. Mean values are represented by the horizontal solid line with vertical bars representing SD for changes observed within each time period that is defined above each panel.

of trial, were defined as dehydrated by having a urine osmolality >700 mOsmol/kg [21], both immediately after the 2nd bath and wrap, and at Morning Day +1.

Comparing the $n = 6$ participants common to the present study and our previous work [14], body mass lost during the bathing protocol using SWB was 1.57 ± 0.46 kg for bathing at 37.8°C, and 1.98 ± 0.47 kg for the present study of self-adjusted maximum tolerable temperature of ~39.0°C ($P = 0.152$; $d = 0.89$). Expressed as percentage of body mass prior to the 1st bath of each trial, this is equivalent to 2.07 ± 0.61% and 2.62 ± 0.62% for bathing at 37.8°C and ~39.0°C, respectively.

DISCUSSION

The present study demonstrates that the body mass lost when bathing in a hot bath of fresh water (FWB) is similar to bathing in a hot bath with ~1.6% Epsom salt added (SWB). This finding is consistent with our previous work using the same bathing protocol but performed at a fixed water temperature of 37.8°C [14]. The present study extends that work by investigating body mass lost when the water temperature is self-adjusted to a fighter's own maximum tolerable temperature. While there was greater body mass lost in hotter water temperatures in those participants common to both studies, there was again no effect of adding salt on the magnitude of body mass lost compared to fresh water.

Investigating body mass loss when the water temperature is self-adjusted to a fighter's maximum tolerable temperature was undertaken as a means to extend the ecological validity of our previous hot bath study [14]. An exit questionnaire performed during that study revealed that the most obvious difference between that study design and protocols that fighters were currently practicing was the temperature of the water i.e. all but one participant found our bathing protocol at 37.8°C to be colder than the hot water immersion that they usually employ, and all participants reported that in practice they increase the water temperature throughout each immersion. However, even at the increased water temperature of ~39.0°C, there was still no difference observed on body mass lost between the FWB and SWB trials. This finding, combined with our previous work, suggests that an interaction effect between water temperature and salt concentration, i.e. that addition of salt produces greater loss of body mass or body water at higher water temperatures, does not exist in the hot bath protocol employed. This is unsurprising given that of the work that previously suggested an interaction effect between water temperature and salt concentration, one study was performed with an *n*-size of one participant [17], and the other employed a forearm model of water exposure under rubber or neoprene sleeves [19].

That said, the addition of salt during hot baths is common practice in MMA athletes [14], and there is some empirical evidence of the effect of adding salt to increase body mass lost during immersion in water [15–17]. Early work established that even in thermoneutral water, i.e. in the absence of sweating, immersion in a strong salt solution (either 11.5% or 20.0% salt as sodium chloride) produces passive fluid loss [17]. In water heated to 36/37°C, addition of 5% sodium chloride allowed for higher sweat rates during 3 h of immersion when compared to fresh water, with the effect more pronounced at salt concentrations of 10% and 15% [16]. Lastly, during immersion in seawater compared to fresh water, ~32% greater body mass was lost in the former during 4 h of immersion at ~38°C [15].

Given that seawater is ~3.5% salt, it may be that the concentration of salt in a hot bath should be at least 3.5% [15], or possibly greater [16, 19], if the aim is to augment the rate of passive fluid loss that would otherwise occur in fresh water. Despite these indications, we employed a salt concentration of only ~1.6% wt/vol magnesium sulfate, but this quantity and type of salt was chosen for its ecological validity based on data from exit questionnaires in our previous work [14].

Future work should certainly investigate higher concentrations of salt in order to produce a larger osmotic gradient between the bath water and body fluids. The suggested mechanisms for how the addition of salt influences the loss of body mass during immersion are (i) that salt water serves to remove an inhibitory influence on the decline in sweat rate that usually occurs with prolonged immersion in fresh water, and/or (ii) that during immersion in salt water, the osmotic pressure difference between the immersion medium and body fluids results in greater fluid loss compared to fresh water [15–20]. However, in studies where an additive effect of salt has been observed, these have been 3 to 4 h immersions [15, 16], in contrast to the only 40 min of immersion time across the 2 h bath and wrap protocol that we employed. Moreover, whether the type of salt (i.e. seawater versus added Epsom salt) would make any difference to the outcome remains to be explored, but is unlikely. In previous work, when the osmotic gradient was produced by either sodium chloride, potassium chloride or cane sugar, the diffusion of water through the skin was similar in all conditions [19].

Absent an effect of the addition of salt under the conditions employed in our two studies, because there were six participants common to both studies, it was possible to explore the effect of self-adjusting the water temperature on body mass loss. Expressed as percentage of body mass prior to the respective 1st bath, the magnitude of loss was 2.07 ± 0.61% for the previous study at a water temperature of 37.8°C, and 2.62 ± 0.62% for the present study at ~39.0°C. While this difference was not statistically significant (P = 0.152), perhaps given the small n-size, the magnitude of the effect size was '*large*' (d = 0.89), and in practical terms translates to an extra ~410 g of body mass lost. As part of the process of making weight in weight category sports, this is a practically-meaningful amount of weight loss and speaks to the importance of water temperature in the hot bath process, but should be kept within safe limits, which remain to be defined. For illustration, water temperatures rarely exceeded 40°C across all participants and baths, and previous immersion studies have typically used temperatures of ~38/39°C [14–16, 22–25], but water temperatures of ~41°C acutely [18], and ~40°C repeated daily for six days [26], have also been employed without adverse effects being reported.

Despite the greater body mass loss with the higher water temperature in the present study, consistent with our previous work, there was a greater loss of body mass by the 24 h of restriction of carbohydrate, fibre, and fluid intake (FWB, -2.54 ± 0.93%; SWB -2.45 ± 1.11%), than from either bathing protocol (FWB, -2.00 ± 0.71%; SWB, -1.97 ± 0.91%). The loss of body mass with 24 h of such restriction is attributed to dehydration, short-duration glycogen depletion, and emptying of the intestinal contents [2], and like the present study is typically ~2–3% of body mass [2, 14, 27]. Therefore, while gradual weight loss using an appropriate calorie deficit is central to a weight loss strategy lasting several weeks or months [2], for the RWL period prior to weigh-in, acute (< 48 h) dietary manipulation (carbohydrate, fibre, and fluid intake) should be considered prior to employing aggressive heat-stimulated dehydration strategies, particularly if the desired weight loss is less than ~3% of body mass.

After the second wrap, a time point chosen to be typical of a weigh-in time for MMA athletes, total body mass lost including the 24 h restriction and 2 h hot bath protocol was ~4.5%. At this timepoint, all participants were classified as dehydrated based on a urine osmolality of >700 mOsmol/kg [21]. This finding is consistent with typical methods of RWL resulting in 100% of MMA athletes being dehydrated to various degrees at an official weigh-in [3, 28]. Body mass and hydration assessment performed on Morning Day +1 represents an ~20 hour recovery period after completing the second bath and wrap, and a body mass deficit and dehydration were observed at this timepoint. However, in practice the time from weigh-in until official competition in professional MMA is usually longer i.e. approximately 30 to 36 h. Even with a long time period for rehydration, the majority of MMA athletes remain dehydrated up to 2 h before competition [3, 28]. Therefore, regain of body mass alone is potentially not a good indicator of returning to euhydration, and indeed there remains some debate about the assessment of hydration status by spot analysis with urine measures [29].

The small sample size (n = 8) employed may be considered a limitation of the present study. However, this sample size was finalised based on a pre-planned interim data analysis for the primary outcome of change in body mass during the 2 h bath and wrap protocol. The small sample size may result in assessment of the secondary outcomes by ANOVA being underpowered, and thereby increase the likelihood of a type II error (i.e. false negative) for these outcomes. Another limitation of this study may be the heterogeneity in the experience of the participants with RWL practices. All participants had prior experience with making weight for competition and the use of hot baths in that process, but during either our recruitment or analysis, we did not account for the number of lifetime exposures to these practices. While speculative, it may be that the response to such practices changes over time, but with participants acting as their own control in this crossover design, we do not anticipate that this aspect had a meaningful impact on the results. Lastly, the magnitude of body mass lost during the entire RWL process averaged ~4.5% of body mass, whereas in practice losses of ~5% to 10% are typical in these athletes in the week prior to competition [3–9]. Therefore, whether there would be a differential effect of salt water when bathing has been preceded by RWL of greater magnitude cannot be excluded as a possibility.

CONCLUSIONS

In summary, hot baths are an effective method of RWL to produce a loss of ~2.0% body mass during 2 h of bathing and wrapping. When fighters self-adjusted the water temperature in the bath, temperatures were ~39.0°C. However, using this protocol, the total amount of body mass lost during a hot bath in water supplemented with ~1.6% Epsom salt was similar to a hot bath performed in fresh water. Future research should explore bathing in higher concentrations of salt, which likely need to be >3.5% in order to produce a sufficient osmotic gradient between the bath water and body fluids.

Acknowledgements

The authors thank the fighters for their participation. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors have no conflicts of interest, financial or otherwise, to disclose.

Conflict of interest

No conflict of interest, financial or otherwise, is declared by the authors.

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European Journal of Applied Physiology
https://doi.org/10.1007/s00421-022-05000-7

ORIGINAL ARTICLE

Effect of rapid weight loss incorporating hot salt water immersion on changes in body mass, blood markers, and indices of performance in male mixed martial arts athletes

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Received: 24 January 2022 / Accepted: 24 June 2022

Abstract

Purpose To investigate the effects of rapid weight loss (RWL), incorporating comparison of hot water immersion (HWI) in fresh or salt water, on changes in body mass, blood markers, and indices of performance in mixed martial arts athletes.

Methods In a crossover design comparing fresh water (FWB) to salt water (SWB; 5.0% wt/vol Epsom salt) bathing, 13 males performed 20 min of HWI (~ 40.3 °C) followed by 40 min wrapped in a heated blanket, twice in sequence (2 h total). Before bathing, ~ 26 to ~ 28 h of fluid and dietary restriction was undertaken, and ~ 24 to ~ 26 h of a high carbohydrate diet and rehydration was undertaken as recovery.

Results During the entire RWL process, participants lost ~ 5.3% body mass. Body mass lost during the 2 h hot bath protocol was 2.17 ± 0.81 kg (~ 2.7% body mass) and 2.24 ± 0.64 kg (~ 2.8% body mass) for FWB and SWB, respectively ($P = 0.647$ between trials). Blood urea nitrogen, creatinine, sodium, chloride, hemoglobin, and hematocrit were increased (all $P < 0.05$), and plasma volume was decreased (~ 14%; $P < 0.01$), but did not differ between FWB and SWB, and were similar to baseline values after recovery. No indices of performance (e.g., countermovement jump, isometric strength, and functional threshold power) were impacted when RWL was followed by the recovery process.

Conclusion Under the conditions of this hot bath protocol, fluid loss was not augmented by the addition of ~ 5.0% wt/vol of Epsom salt during HWI, and RWL of ~ 5.3% body mass followed by > 24 h of recovery did not impact indices of performance.

Keywords Body water · Fluid balance · Heat · Hydration · Magnesium · Sweat

Abbreviations

AKI	Acute kidney injury
BUN	Blood urea nitrogen
CMJ	Countermovement jumps
FTP	Functional threshold power
FWB	Fresh water bathing
HWI	Hot water immersion
IMTP	Isometric mid-thigh pull
MMA	Mixed martial arts
RWL	Rapid weight loss
SWB	Salt water bathing

Communicated by Narihiko kondo.

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Introduction

In sports that have weight class restrictions, athletes attempting to 'make weight' frequently practice short-term weight loss, termed acute or rapid weight loss (RWL), in the ~ 72 h before weigh-in (Reale et al. 2017; Burke et al. 2021). Methods of RWL focus on the acute reduction of the contents of the gastrointestinal tract, and of total body water, through methods such as a low carbohydrate and low-residue diet, fluid restriction, and increasing sweat losses through exercise and/or heat exposure (Barley et al. 2018a; Hillier et al. 2019; Connor and Egan 2019). In mixed martial arts (MMA) athletes, combinations of these methods typically induce losses of ~ 5% to ~ 10% of body mass in the week before weigh-in (Coswig et al. 2015, 2019; Matthews and Nicholas

Published online: 14 July 2022

2017; Barley et al. 2018a; Brechney et al. 2019; Connor and Egan 2019; Hillier et al. 2019).

One method of heat exposure used to induce passive fluid loss is hot water immersion (HWI), which is often employed as part of weight-making strategies in combat sports (Pettersson et al. 2013; Matthews and Nicholas 2017; Brandt et al. 2018; Connor and Egan 2019; Kasper et al. 2019; Park et al. 2019; Gordon et al. 2021). Colloquially known as hot baths, this method typically involves short-duration HWI followed by 'wrapping' in warm clothing for a period of time before further exposures to HWI and wrapping (Connor et al. 2020; Kasper et al. 2019). The HWI is often performed in salt water, usually by the addition of Epsom salt, with the aim of augmenting the loss of body mass compared to that achieved by immersion in fresh water (Connor et al. 2020). Indeed, there is some empirical evidence for salt water immersion to augment fluid loss when compared to fresh water immersion (Whitehouse et al. 1932; Hertig et al. 1961; Hope et al. 2001), albeit these studies involve prolonged (≥ 3 h) HWI. The mechanistic basis for this effect is proposed as the addition of salt increasing the osmotic pressure difference between the immersion medium and body fluids, and/or attenuating the inhibitory effect on sweating that occurs during prolonged HWI, and thereby resulting in the greater fluid loss compared to fresh water (Whitehouse et al. 1932; Buettner 1953, 1959; Peiss et al. 1956; Hertig et al. 1961; Brebner and Kerslake 1964; Hope et al. 2001).

Our recent work has investigated a hot bath protocol incorporating short-duration (2 × 20 min) HWI with an Epsom salt concentration of ~ 1.6%wt/vol, but found no difference in body mass losses comparing fresh water and salt water immersion either at a fixed water temperature of 37.8 °C (Connor et al. 2020), or when athletes self-adjusted the water temperature to the maximum temperature each could tolerate (~ 39.0 °C) (Connor and Egan 2021). Our choice of a ~ 1.6% salt solution using Epsom salt was chosen for its ecological validity based on our knowledge of applied practice, and responses to a questionnaire in which fighters described typically the addition of 1 to 2 kg of Epsom salt to a standard sized bath (Connor et al. 2020). However, higher concentrations of salt, which would induce a larger osmotic gradient between the bath water and body fluids, may be required to augment fluid loss when compared to fresh water. For example, even in thermoneutral water, i.e., in the absence of sweating, immersion in a strong salt solution (either 11.5 or 20.0% salt as sodium chloride) induced passive fluid loss (Whitehouse et al. 1932). In water heated to 36/37 °C, addition of 5% sodium chloride (~ 1709 mOsmol/kg) allowed for higher sweat rates during 3 h of immersion when compared to fresh water, with the effect more pronounced at salt concentrations of 10 and 15% (Hertig et al. 1961). Finally, immersion in seawater (~ 3.5% salt; ~ 1000 mOsmol/kg to ~ 1200 mOsmol/kg) resulted in ~ 32% greater loss of body mass compared to fresh water during 4 h of immersion at ~ 38 °C (Hope et al. 2001). Given these observations, it may be that the concentration of salt in a hot bath should be at least 3.5% (Hope et al. 2001), or possibly greater (Buettner 1953, 1959; Hertig et al. 1961), if the aim is to augment the rate of passive fluid loss that would otherwise occur during HWI in fresh water.

Therefore, the present study investigated the magnitude of body mass losses in MMA athletes using a hot bath protocol with immersion in fresh water or salt water at a concentration of ~ 5%wt/vol of Epsom salt. Extending our previous work (Connor et al. 2020; Connor and Egan 2021), we also investigated the effects of ~ 28 to ~ 30 h of RWL on blood markers (plasma volume, kidney function, and electrolytes) and indices of performance. Hypohydration is well established as negatively impacting indices of performance (Savoie et al. 2015; Deshayes et al. 2020), and importantly, recent evidence suggests this to be the case even when major confounders such as expectancy effects because of a lack of blinding, and inadequate familiarization with methods of dehydration, are addressed (James et al. 2019). While RWL inherently involves dehydration processes, in professional combat sports such as MMA and boxing the competitive bout typically takes place ~ 30 to ~ 36 h after weigh-in (Burke et al. 2021). This time-period may allow for rehydration and recovery of muscle glycogen (Burke et al. 2021), but several studies have observed a residual negative impact on indices of performance even after ~ 16 to ~ 24 h of recovery (Ööpik et al. 1996; Moghaddami et al. 2016; Alves et al. 2018; Yang et al. 2018; Barley et al. 2018b; Kurylas et al. 2019). Therefore, we also investigated the effect of RWL followed by ~ 24 to ~ 26 h of recovery in the form of a high carbohydrate diet and rehydration on body mass, hydration status, blood markers, and indices of performance.

Methods

Participants

Thirteen male MMA athletes (29.5 ± 6.7 y; 1.81 ± 0.07 m; 83.0 ± 8.8 kg) with previous experience of RWL provided written informed consent to participate. The study was approved by the Human Research Ethics Committee of Dublin City University (permit number: DCUREC/2020/186). Participants comprised both amateur and professional fighters, but all participants were competing under professional weigh-in rules at the time of the study, i.e., weigh-in ≥ 24 h before competition. Each participant had previous experience of RWL, and the use of hot baths as part of that process, and each participant had made weight for competition on at least five occasions before participating in the study.

Study design

A crossover-repeated-measures design was employed to compare the effects on passive fluid loss of HWI using fresh water bathing (FWB) compared to salt water bathing (SWB). Participants performed two main experimental trials separated by at least 7 days, with the order of the FWB and SWB trials assigned in a counterbalanced manner, and participants randomized to which trial they performed first. The FWB and SWB trials were identical with the exception of the water condition in which they were immersed during the bathing periods (Fig. 1).

On Day − 2, a performance test battery was performed consisting of tests of leg power (countermovement jumps; CMJ), maximal strength (isometric hand-grip strength and isometric mid-thigh pull; IMTP), and a 3 min all-out exercise test on a cycle ergometer to estimate functional threshold power (FTP). At least 72 h before the first trial commencing, a familiarization session for the performance test battery was performed, which involved the participants undertaking the test battery in its entirety, and in an identical manner to that undertaken during the main trials.

On Day − 1 (the day before bathing), participants were prescribed to restrict fluid intake to 15 mL/kg of body mass, eliminate carbohydrate- and fiber-rich foods from their diet, and consume an energy intake of 22 kcal/kg of body mass, which was tracked using the MyFitnessPal mobile phone application (UnderArmour, USA). These practices are similar to what these participants routinely undertake to make weight for competition, and compliance with the prescribed protocol was confirmed verbally on Day 0.

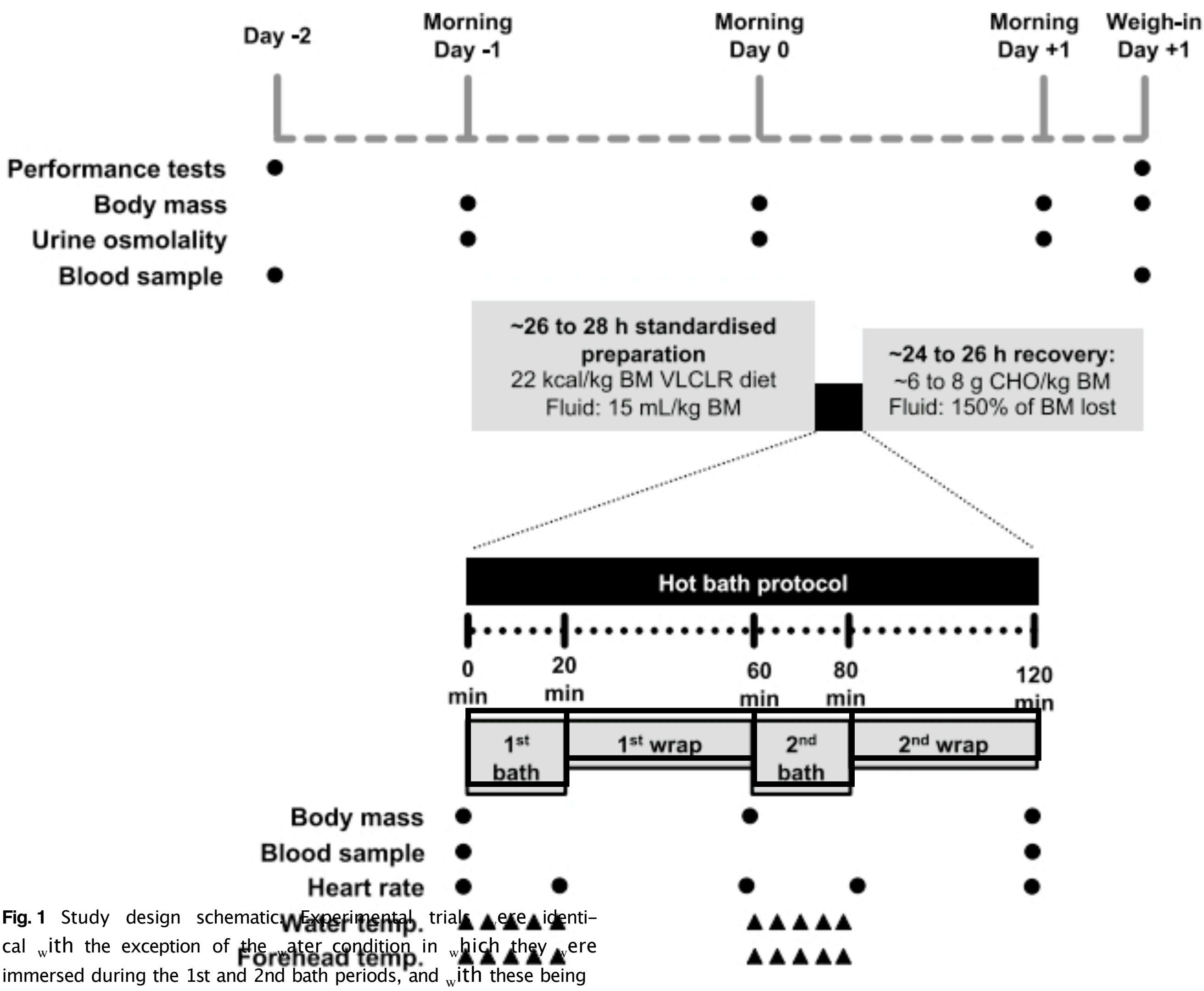

Fig. 1 Study design schematic. Experimental trials were identical with the exception of the water condition in which they were immersed during the 1st and 2nd bath periods, and with these being fresh water bathing or salt water bathing on separate days. BM, body mass; CHO, carbohydrate; VLCLR, very low-carbohydrate, low residue

On Day 0, participants arrived ~ 2 to ~ 4 h after waking to perform the bathing protocol. During this ~ 2 to ~ 4 h period, participants remained fasted and did not consume any fluids. Upon completion of the bathing protocol, the total body mass lost from Morning Day − 1 was calculated, and participants began the weight regain process by following the prescription to consume fluids (in L) to the equivalent to 150% of body mass lost (in kg) during the next 6 h (Sawka et al. 2007), and to consume 6 to 8 g/kg of body mass of carbohydrate during the rest of the day (Burke et al. 2021).

On Day + 1, participants were advised to follow their habitual fight day nutrition practices, and returned to undertake the performance test battery ~ 24 to ~ 26 h after completing the bathing protocol (Fig. 1). For their first trial, participants were asked to keep a record of what food and fluid they consumed from waking to before testing on both Day − 2 and Day + 1. For their second trial, participants were asked to repeat the timing and quantity of this intake for the respective days. Compliance with this approach was confirmed verbally upon arrival for testing on each day. To minimize the potential influence of circadian rhythms on indices of performance, the testing on Day + 1 was performed at the same time of day ± 1 h as performed on Day − 2. Participants completed their habitual training in the period between the main trials, but for the day before Day − 2, only low intensity training was allowed, and like dietary standardization was asked to be maintained consistent before each trial.

Bathing protocol

The bathing protocol comprised of 20 min of HWI ("bath") followed by 40 min wrapped in a rubberized sauna blanket ("wrap"). This 60 min bath and wrap protocol was repeated twice per main experimental trial, i.e., 2 h total, as described in our previous work (Connor et al. 2020; Connor and Egan 2021) (Fig. 1). One difference to these previous studies was that a sauna blanket (MiHIGH Infrared Sauna Blanket; MiHIGH Pty Ltd, Queensland, Australia) was used for the wrap periods, rather than a knitted wool hat, cotton t-shirt, hooded cotton sweatshirt, cotton tracksuit bottoms/sweatpants, and socks worn underneath several blankets in a heated bedroom. According to the manufacturer, the sauna blanket uses the same heating technology as an infrared sauna, emitting far infrared wavelengths.

For each bath, participants were submerged up to the neck for 20 min, i.e., head-out HWI. For the FWB trial, only fresh tap water was used in the bath. For the SWB trial, Epsom salt was added to the bath with 160 L capacity at a concentration of 6.25 kg in 125 L of water (i.e. ~ 5.0%wt/vol). Based on the chemical composition of Epsom salt (magnesium sulfate heptahydrate; $MgSO_4 \cdot 7H_2O$), 5.0%wt/vol of Epsom salt would result in the osmolality of the salt water being ~ 406 mOsmol/kg.

Another difference to our previous work in the present study was that the initial water temperature of the bath was prepared to ~ 40.3 °C rather than 37.8 °C (Connor et al. 2020; Connor and Egan 2021), and participants maintained the water temperature at their maximum tolerable level, rather than self-adjusting the water temperature upwards as previously (Connor and Egan 2021). To maintain the water temperature, participants requested from the researchers the addition of boiling water from an electric kettle (1.5 L) to the bath ad libitum. The volume of additional boiling water per bath was noted. Additional salt was not added to adjust for the additional boiling water, and therefore, during the bathing process, the %wt/vol was estimated to decrease from 5.0 to ~ 4.7%, whereas the osmolality of the salt water was estimated to concomitantly decrease from ~ 406 to ~ 381 mOsmol/kg.

A floating thermometer (Avent Bath & Room Thermometer; Philips, UK) was checked at 4 min intervals for measurement of water temperature, but participants were not informed of the temperature during either bath or trial. At the same 4 min intervals, forehead temperature was measured as the mean of two measures using a digital infrared thermometer (Model HTD8813; LPOW, USA), whose range of precision according to the manufacturer's instructions is ± 0.2 °C. After 20 min of bathing, the participants exited the bath, briefly dried themselves with a towel before entering the sauna blanket for the next 40 min. Heart rate was measured using an automated heart rate and blood pressure monitor (UA-611; A&D Company Limited, Japan) immediately before and after the 1st bath, immediately before and after the 2nd bath, and immediately after the 2nd wrap.

Body mass, urine, and blood sampling

Change in body mass, measured to the nearest 0.05 kg (model #63,667; Soehnle, Germany), was the primary outcome measure. Body mass was measured in minimal clothing, i.e., lower body short underwear in the form of briefs or boxer briefs, at several time-points: (i) upon waking on the day before bathing (Morning Day − 1), (ii) upon waking on the day of bathing (Morning Day 0), (iii) immediately before the 1st bath, (iv) immediately before the 2nd bath, (v) immediately after the 2nd wrap, (vi) upon waking on the day after bathing (Morning Day + 1), and finally (vii) on the day after bathing at "weigh-in" immediately to the performance test battery (Weigh-in Day + 1). Change in body mass induced by the entire RWL process, and whether a body mass deficit was present after recovery, were both calculated compared to body mass at Morning Day − 1.

Urine samples for the measurement of urine osmolality (Osmocheck Portable Osmometer; Vitech Scientific, UK) were taken upon waking on Day − 1, Day 0, and Day + 1. Participants were classified as hypohydrated using the criterion of urine osmolality of > 700 mOsmol/kg (Sawka et al. 2007).

Capillary blood was sampled at four time-points: (i) before undertaking the performance test battery on Day − 2, (ii) immediately before the 1st bath, (iii) immediately after the 2nd wrap, and finally (iv) before re-testing of performance on Day + 1. Participants were seated upright and stationary for ~ 3 min before a fingertip capillary blood sample (95 μL) was collected and analyzed for blood chemistry (glucose, blood urea nitrogen [BUN], creatinine, hematocrit, hemoglobin, the Anion Gap, sodium, potassium, chloride, ionized calcium, and total CO_2) using the i-STAT 1 point-of-care handheld blood analyzer and CHEM8 + cartridges (Abbott Laboratories, USA) according to the manufacturer's instructions. The CHEM8 + cartridges were the best available tool for point-of-care blood analysis, and our specific interest from this list of analytes were BUN and creatinine as indicators of acute kidney injury; sodium, potassium, and chloride as indicators of change in circulating electrolytes sensitive to sweat losses and dehydration; whereas the data for hemoglobin and hematocrit were used to calculate percentage change in plasma volume using the method of calculation described by Dill and Costill (1974). Due to technical issues resulting in missing data points, data for blood analysis are reported as $n = 10$ or $n = 11$ where appropriate.

Performance test battery

The performance test battery was identical on Day − 2 and Day + 1. After arrival and having a capillary blood sample taken, participants performed a standardized general warm-up. First, 10 min of cycle ergometry (Wattbike Pro; Wattbike Ltd., Nottingham, England) at a cadence of > 70 rpm and a self-selected moderate intensity (rating of perceived exertion of 12–15). Next, bodyweight exercises consisting of five squats, five split-squats each side, five push-ups, and five CMJs were performed, after which lastly, another 5 min of cycle ergometry and the same bodyweight exercises were performed.

Leg power was measured by CMJ for which five jumps in total were performed with 10 s of rest taken between each jump. The participants were instructed to jump with maximal effort on each jump, and were required to keep the hands firmly placed on the hips throughout the jump. Jumps were performed on a dual-force plate system sampling at 500 Hz (Pasco PS-2141; Pasco Scientific Corp, USA) and CMJ height was calculated as previously described (Jordan et al. 2018). Data are reported as jump height (in cm) calculated as the average of three jumps after the worst and best jumps of the five attempts were excluded. The coefficient of variation (%CV) for this parameter was 5.7% in this cohort of athletes.

Isometric hand-grip strength test was measured using a hand-grip dynamometer (TKK 5401 Grip-D; Takei Scientific Instruments Co, Japan). The dynamometer was held at shoulder height to start and the participants were instructed to apply maximum force while lowering their arm to their side while in full elbow extension (Savva et al. 2013). Two maximum efforts per hand were performed by alternating each side, with the best score for each hand being recorded and averaged as a composite score. The %CV for this parameter was 5.0% in this cohort of athletes.

IMTP was performed in a customized power rack (Grip Ltd.; Ireland) standing on a dual-force plate system using a standardized protocol as previously described (Halperin et al. 2016). Participants were positioned in a body position similar to completing the second pull of a power clean with a flat trunk position and their shoulders in line with the bar. This position allowed participants to maintain a knee angle of ~ 120 to ~ 130°. Two 3 s IMTP efforts were performed applying 50 and 80% of perceived maximum effort. After these priming efforts, 30 s of rest was taken before completing the three 3 s maximal efforts separated by seated rest for 150 s. Data are reported as peak force given that this measure is the most reliable measure from the data output (Brady et al. 2020). The %CV for this parameter was 7.2% in this cohort of athletes.

FTP was estimated using the 3 min all-out test (Burnley et al. 2006) performed on an electromagnetically and air-braked cycle ergometer (Wattbike Pro; Woodway Inc., USA) (Wainwright et al. 2017), using a previously validated protocol (Hanson et al. 2019). Handlebar and saddle position/height were recorded during the familiarization visit and replicated for each subsequent testing day. The warm-up was standardized as 5 min of cycling at cadence of > 70 rpm and the same self-selected moderate intensity as above. The goal of this test is then to maintain the highest power output possible for the 3 min of effort. Cadence was kept between 90 and 110 rpm for the duration of the test. On conclusion of the test, maximum heart rate (via telemetry; Polar, Finland) and FTP were extracted for analysis. The %CV for maximum heart rate and FTP were 3.5 and 3.0%, respectively, in this cohort of athletes.

The smallest worthwhile difference (SWD) for each of the performance tests was set at 0.2 between-subject SD, which is suggested to represent a practically relevant change in performance in athletes (Hopkins et al. 2009). Thus, in this study, the SWD corresponded to 0.6 cm for CMJ height, 1.5 kg for hand-grip strength, 56 N for IMTP peak force, 2.3 bpm for maximum heart rate, and 5.1 W for FTP.

Sample size calculation and early termination

The primary outcome was change in body mass as a consequence of the 2 h bath and wrap protocol. Therefore, a sample size calculation was performed (G*Power v.3.1) based on previous research demonstrating an effect of salt water to augment the magnitude of body mass lost during HWI when compared to fresh water (Hope et al. 2001). Using the body mass lost after 2 h of that 4 h immersion protocol, a time point analogous to the present work, and that being 0.98 ± 0.44 kg and 1.24 ± 0.80 kg for fresh water and salt water respectively, and an assumed correlation between conditions of 0.90, the required sample size to detect a difference between FWB and SWB at a Type I error rate (*α*) of 0.05 and a power (1–*β*) of 0.8 was *n* = 26. However, given the absence of effect in our previous research using a salt concentration of ~ 1.6% (Connor et al. 2020; Connor and Egan 2021), a priori we planned an interim data analysis for the assessment of futility, and therefore discontinuation, after completion of 50% of the required sample size, i.e., *n* = 13. In the absence of any difference between FWB and SWB for change in body mass with *n* = 13 (*P* = 0.647 between trials, *d* = 0.09; data reported below), we discontinued recruitment at that time.

Statistical analysis

Statistical analysis and graphical representation were performed using GraphPad Prism v9.1 (GraphPad Software, Inc., USA). Normality of data was assessed with the Shapiro–Wilk normality test for which all data passed. All data are presented as mean ± SD. A two-way (condition*time) repeated-measures analysis of variance (ANOVA) was used to assess responses to the interventions for variables with serial measurements. A one-way repeated-measures ANOVA was used to assess whether an order effect was present in the indices of performance from Trial 1 to Trial 2 regardless of salt condition. When a main or interaction effect was observed, pairwise comparisons were performed with Bonferroni's correction for which multiplicity-adjusted *P* values are reported. Paired *t* tests were used to assess differences between conditions for variables with two measurements, including to assess whether an order effect was present when comparing Trial 1 and Trial 2. The level of statistical significance for all tests was set at *P* < 0.05. Standardized differences in the mean were used to assess magnitudes of effects between conditions. These were calculated using Cohen's *d* effect size and are interpreted using thresholds of < 0.2, ≥ 0.2, ≥ 0.5 and ≥ 0.8 for *trivial*, *small*, *moderate*, and *large*, respectively.

Results

Water temperature

The starting water temperature did not differ between trials (1st bath, *P* = 0.374; 2nd bath, *P* = 0.133). The starting water temperature was 40.31 ± 0.32 °C and 40.62 ± 0.37 °C for the 1st and 2nd baths, respectively, in FWB (*P* = 0.240), and 40.46 ± 0.44 °C and 40.42 ± 0.31 °C for the 1st and 2nd baths, respectively, in SWB (*P* = 0.744). No interaction effect was observed for the effect of salt (1st bath, *P* = 0.343; 2nd bath, *P* = 0.297), and average water temperature remained above 40 °C throughout the bathing periods (Fig. 2A and B). The volume of boiling kettle water added to each bath was 4.39 ± 1.14 L for FWB and 3.46 ± 1.42 L for SWB during the 1st bath of each trial (*P* = 0.055), and 2.31 ± 1.80 L for FWB and 2.65 ± 1.64 L for SWB during the 2nd bath of each trial (*P* = 0.513).

Forehead temperature and heart rate response to the bathing protocols

Forehead temperature increased in response to the hot bath protocol in both the 1st and 2nd bath periods (main effect of time, *P* < 0.001 for both) (Fig. 2C and D). Resting heart rate was similar for each trial before the 1st bath (FWB, 67 ± 18 bpm; SWB, 65 ± 11 bpm). Heart rate increased in response to the hot bath protocol (main effect of time, *P* < 0.001) and reached a measured peak of 128 ± 19 bpm and 127 ± 21 bpm after the 2nd bath period during FWB and SWB, respectively, but no main effect of condition (*P* = 0.166) or interaction effect (*P* = 0.762) was observed (Fig. 3).

Changes in body mass

For change in body mass in absolute (kg) (Table 1) and relative (%initial body mass) (Fig. 4) terms, a main effect of time (*P* < 0.001), but neither a main effect of condition, nor a condition*time interaction effect, was observed. Similarly, there was no difference between conditions for changes in urine osmolality at the various time-points (Table 1).

Body mass losses induced by carbohydrate and fluid restriction were 2.18 ± 1.18 kg (*P* < 0.001; *d* = 0.26) and 1.98 ± 0.95 kg (*P* < 0.001; *d* = 0.24) in preparation for the FWB and SWB trials, respectively. These values represented losses of relative to initial body mass on Morning Day − 1 of 2.72 ± 1.47% and 2.47 ± 1.18% for the FWB and SWB protocols, respectively.

Body mass losses induced by the hot bath protocols were 2.17 ± 0.81 kg (*P* < 0.001; *d* = 0.28) and 2.24 ± 0.64 kg (*P* < 0.001; *d* = 0.28) for the FWB and SWB protocols, respectively, which corresponded to 2.70 ± 1.01% of initial body mass for FWB, and 2.78 ± 0.79% of initial body mass for SWB (Fig. 4A). Analysis for the presence of an order effect demonstrated no difference (*P* = 0.704) in body mass losses induced by the hot bath protocols when analyzed as Trial 1 (2.27 ± 0.71 kg) versus Trial 2 (2.14 ± 0.75 kg). FWB resulted in body mass loss of 1.15 ± 0.63 kg (*P* = 0.001;

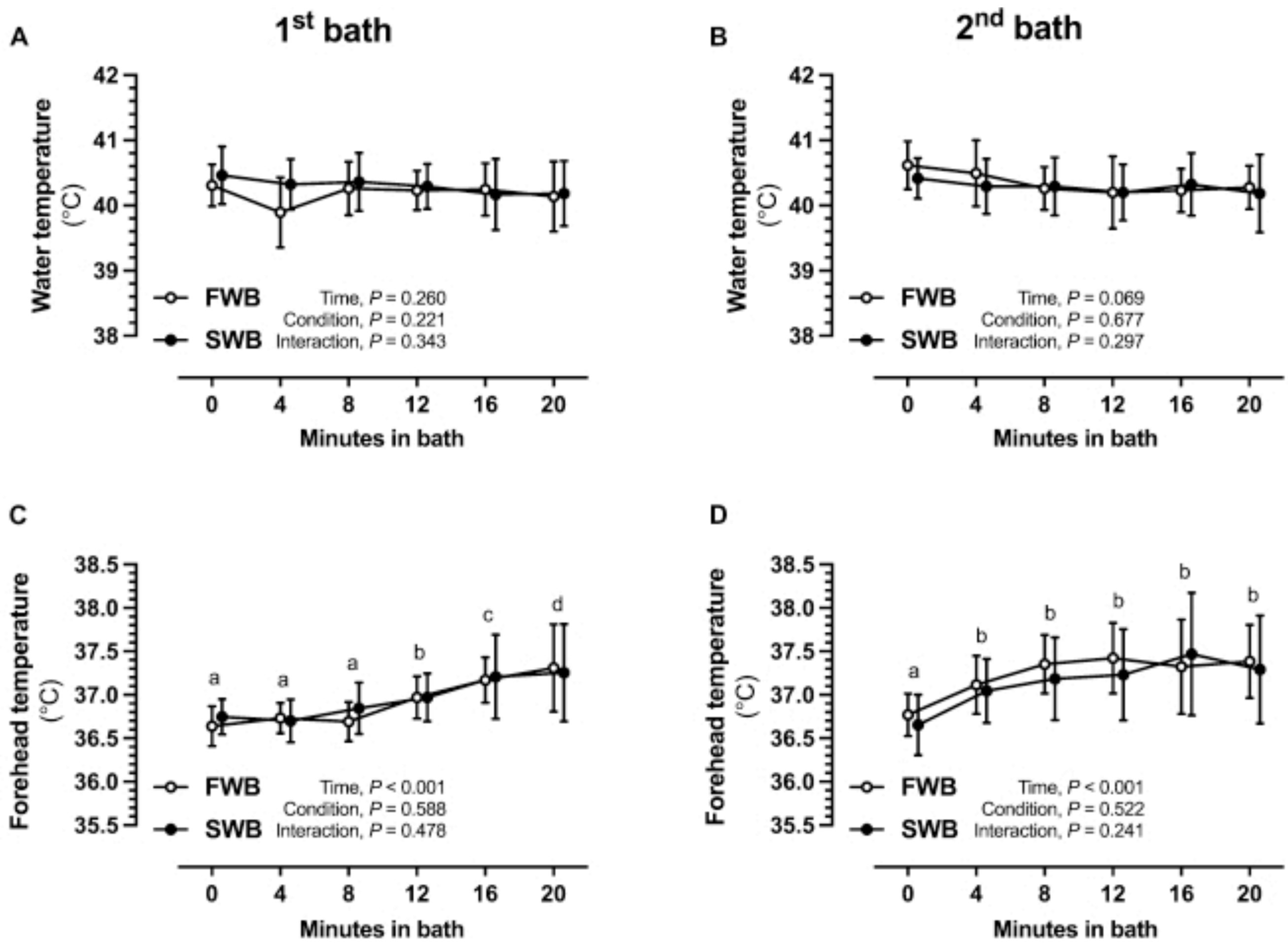

Fig. 2 Water temperatures measured at 4 min intervals during each bath (**A** 1st bath; **B** 2nd bath) during experimental trials of fresh (FWB) or salt water (SWB), and forehead temperatures measured at 4 min intervals during each bath (**C** 1st bath; **D** 2nd bath). Data are mean values ($n = 13$, all male) with vertical bars representing SD

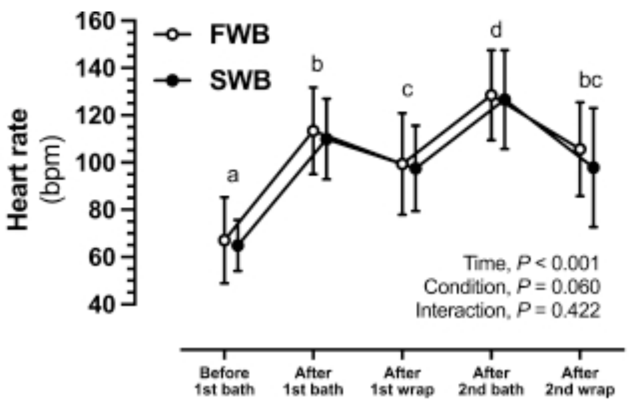

Fig. 3 Heart rate responses to hot water immersion during experimental trials of fresh (FWB) or salt water (SWB). Data points are mean values ($n = 13$, all male) with vertical bars representing SD. Differences *within* conditions are noted by different letters representing significant differences ($P < 0.05$) between respective time-points, whereas time-points with the same letter are not different to each other

$d = 0.15$) during the 1st bath and wrap, and 1.02 ± 0.31 kg ($P < 0.001$; $d = 0.13$) during the 2nd bath and wrap. SWB resulted in body mass loss of 1.18 ± 0.25 kg ($P < 0.001$; $d = 0.15$) during the 1st bath and wrap, and 1.06 ± 0.45 kg ($P < 0.001$; $d = 0.13$) during the 2nd bath and wrap.

Total body mass losses induced by the entire RWL protocol were 4.35 ± 1.60 kg ($P < 0.001$; $d = 0.53$) and 4.22 ± 1.17 kg ($P < 0.001$; $d = 0.51$) for the FWB and SWB protocols, respectively. These values represented losses of relative to initial body mass on Morning Day − 1 of 5.42 ± 1.99% and 5.25 ± 1.46% for the FWB and SWB protocols, respectively (Fig. 4B).

On Morning Day − 1, 9 (FWB trial) and 7 (SWB trial) were classified as hypohydrated with a urine osmolality of > 700 mOsmol/kg (Sawka et al. 2007). On Morning Day + 1, 7 (FWB trial) and 9 (SWB trial) were classified as hypohydrated, and 8 (FWB trial) and 6 (SWB trial) participants were in a body mass deficit compared to Morning Day − 1. However, at Weigh-in Day + 1, i.e., before

Table 1 Body mass (kg) and hydration status assessed by urine osmolality (mOsmol/kg) at time-points during a rapid weight loss intervention featuring a hot bath protocol in fresh (FWB) or salt water (SWB)

	Morning Day − 1	Morning Day 0	Before 1st bath	After 1st bath & wrap	After 2nd bath & wrap	Morning Day + 1	Weigh-in Day + 1	*P* value
Body mass (kg)								Time, $P < 0.001$***
FWB	82.95 ± 8.78^{a}	81.09 ± 7.89^{b}	80.76 ± 7.79^{b}	79.62 ± 7.70^{c}	78.59 ± 7.64^{d}	82.45 ± 7.83^{a}	83.42 ± 7.84^{a}	Condition, $P = 0.754$
SWB	82.86 ± 8.69^{a}	81.16 ± 8.24^{b}	80.88 ± 8.02^{b}	79.70 ± 8.00^{c}	78.64 ± 7.99^{d}	82.73 ± 8.49^{a}	83.56 ± 8.63^{a}	Interaction, $P = 0.655$
Urine osmolality (mOsmol/kg)								Time, $P = 0.002$**
FWB	762 ± 217$_{a}$	955 ± 145$_{b}$				674 ± 269$_{a}$		Condition, $P = 0.570$
SWB	695 ± 252$_{a}$	845 ± 185$_{b}$				769 ± 230$_{a}$		Interaction, $P = 0.067$

Data are presented as mean ± SD, $n = 13$. Differences *within* conditions are noted by superscripted letters where different letters represent significant differences ($P < 0.01$) between respective time-points, whereas time-points with the same letter are not different to each other

**$P < 0.01$ and ***$P < 0.001$ for main and interaction effects from the two-way (condition*time) ANOVA analyses

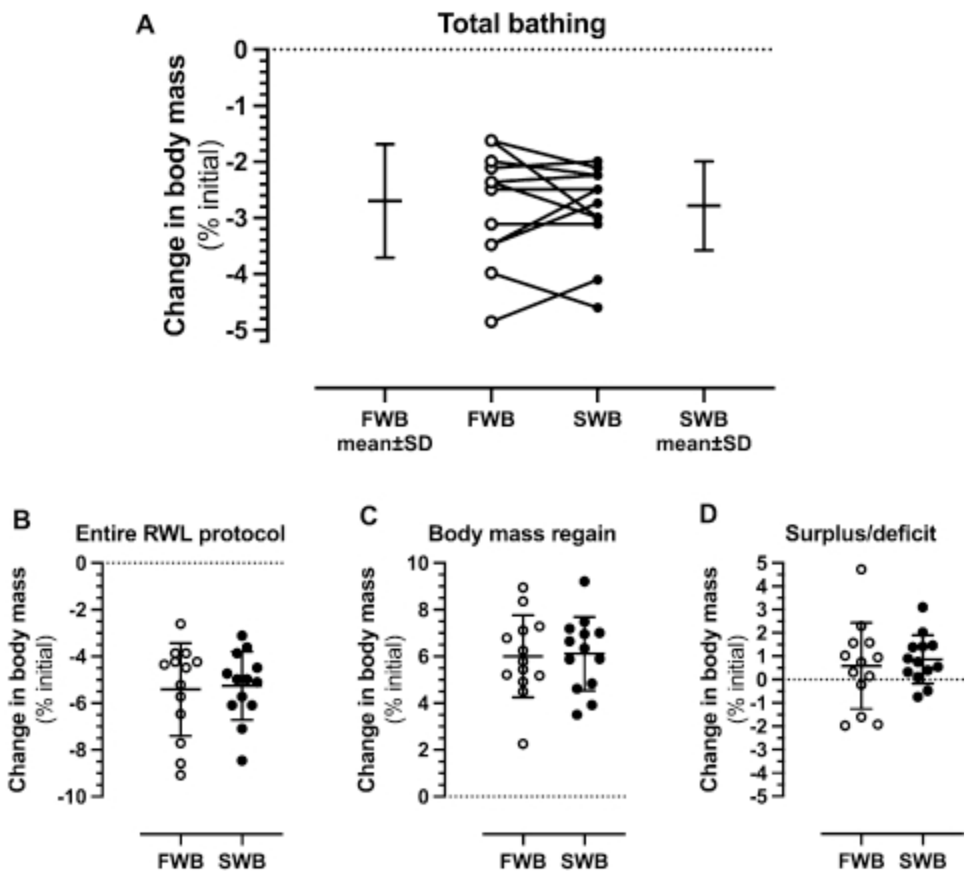

Fig. 4 Percentage changes in body mass (relative to baseline recorded on Morning Day − 1) induced during **A** a hot bath protocol in fresh (FWB) or salt water (SWB) for a 2 h period comprising both baths and wraps, **B** the entire rapid weight loss (RWL) intervention, **C** the period of weight regain before weigh-in on Day + 1, and **D** as a measure of total body mass deficit or surplus at weigh-in on Day + 1 compared to Morning Day − 1. White (FWB) and black (SWB) circles in each panel represent individual data points. Mean values ($n = 13$, all male) are represented by the horizontal solid line with vertical bars representing SD for changes observed within each time-period that is defined above each panel

the performance test battery, only 4 (FWB trial) and 2 (SWB trial) participants were in a body mass deficit compared to Morning Day − 1. Overall, weight regain from the end of the 2nd wrap period to Weigh-in Day + 1 was 4.83 ± 1.41 kg ($P < 0.001$; $d = 0.62$) and 4.92 ± 1.27 kg ($P < 0.001$; $d = 0.59$) during recovery from the FWB and SWB protocols, respectively (shown as % of initial body mass in Fig. 4C), resulting in a body mass surplus compared to Morning Day − 1 of 0.47 ± 1.48 kg and 0.69 ± 0.83 kg, respectively (shown as % of initial body mass in Fig. 4D).

Indices of performance

Across the five time-points measured (FAM, Trial 1 Day − 2, Trial 1 Day + 1, Trial 2 Day − 2, Trial 2 Day + 1), there was no order effect observed for any of indices of performance, i.e., CMJ height ($P = 0.907$), hand-grip strength ($P = 0.722$), IMTP peak force ($P = 0.537$), maximum heart rate ($P = 0.284$), and FTP ($P = 0.874$). Comparing between FWB and SWB trials, in the 3 min all-out test, the absence of interaction effects or main effects of time are indicative of there being no significant differences in FTP or maximum heart rate either between or within conditions (Table 2). Similarly, no differences in CMJ height, hand-grip strength, or IMTP peak force either between or within conditions were observed (Table 2).

Blood markers

For blood markers, the absence of interaction effects or main effects of condition are indicative of there being no significant differences between conditions on these markers (Table 3). A main effect of time (all $P \leq 0.01$) was observed for several markers (BUN, chloride, creatinine, hemoglobin, hematocrit, and sodium) with each being increased in blood samples taken immediately after the 2 nd wrap period, but returning to values similar to Day − 2 when measured after the period of recovery up to sampling at Weigh-in Day + 1 (Table 3). Declines in plasma volume induced by the entire RWL protocol were estimated as being −14.1 ± 12.1% for FWB ($P = 0.003$; $d = 1.64$) and −13.0 ± 11.4% for SWB ($P = 0.004$; $d = 1.62$).

Discussion

Given that our previous work using ~ 1.6% salt solutions did not reveal an effect of salt to augment body mass loss during a hot bath protocol (Connor et al. 2020; Connor and Egan 2021), the present study investigated body mass losses when the salt concentration is increased to ~ 5.0%wt/vol. This higher concentration is more similar to immersion studies where an effect of salt to augment the loss of fluid and/or body mass has been observed (Whitehouse et al. 1932; Hertig et al. 1961; Hope et al. 2001). However, the present study demonstrates that the body mass lost during a hot bath protocol using fresh water (FWB) is similar to a protocol using ~ 5.0%wt/vol of Epsom salt (SWB).

Body mass losses induced by ~ 26 to ~ 28 h of restriction of fluid intake combined with a low-residue, low-carbohydrate diet were ~ 2.6% of body mass. This is similar in magnitude to the suggestion of ~ 3% reduction to be expected by short-duration restriction of carbohydrate and fluid, and emptying of the gastrointestinal contents using a low-residue diet (Reale et al. 2017; Burke et al. 2021), and is also similar to our previous work (Connor et al. 2020; Connor and Egan 2021). However, the percentage of body mass lost during

Table 2 Countermovement jump (CMJ) height, hand-grip strength, isometric mid-thigh pull (IMTP) peak force, functional threshold power (FTP), and maximum heart rate (HR) measured before (Day − 2) and ~ 28 h after (Day + 1) a rapid weight loss intervention featuring a hot bath protocol in fresh (FWB) or salt water (SWB)

	FAM	Day − 2	Day + 1	*P* value
CMJ height (cm)	31.11 ± 4.36			Time, $P = 0.572$
FWB		32.93 ± 2.71	32.86 ± 3.30	Condition, $P = 0.080$
SWB		31.81 ± 3.86	32.29 ± 3.06	Interaction, $P = 0.435$
Hand-grip strength (kg)	46.7 ± 6.5			Time, $P = 0.503$
FWB		48.0 ± 6.8	48.9 ± 7.9	Condition, $P = 0.739$
SWB		48.8 ± 8.0	47.5 ± 7.6	Interaction, $P = 0.066$
IMTP peak force (N)	1766 ± 280			Time, $P = 0.435$
FWB		1690 ± 267	1757 ± 276	Condition, $P = 0.785$
SWB		1739 ± 288	1729 ± 287	Interaction, $P = 0.152$
3 min all-out test Maximum HR (bpm)	182.5 ± 9.4			Time, $P = 0.374$
FWB		183.5 ± 10.1	185.2 ± 11.4	Condition, $P = 0.228$
SWB		185.2 ± 12.4	186.1 ± 11.4	Interaction, $P = 0.790$
3 min all-out test FTP (W)	213.8 ± 21.0			Time, $P = 0.642$
FWB		219.6 ± 22.9	220.6 ± 26.4	Condition, $P = 0.068$
SWB		222.2 ± 25.4	223.7 ± 27.8	Interaction, $P = 0.854$

Data are presented as mean ± SD, $n = 13$. FAM, familiarization trial. Data for FAM are included for descriptive purposes. *P* values are obtained from two-way (condition*time) ANOVA analyses on the FWB and SWB data

Table 3 Blood markers measured at time-points during a rapid weight loss intervention featuring a hot bath protocol in fresh (FWB) or salt water (SWB)

	Pre-testing Day − 2	Before 1st bath	After 2nd bath & wrap	Weigh-in Day + 1	P value
Glucose (mg/dL)					Time, $P=0.041$*
FWB	94.3 ± 9.2	88.1 ± 9.1	91.8 ± 14.8	100.2 ± 13.1	Condition, $P=0.290$
SWB	94.8 ± [illegible]	90.7 ± 6.1	104.9 ± 16.1	94.9 ± 11.5	Interaction, $P=0.024$*
BUN (mg/dL)					Time, $P<0.001$***
FWB	22.2 ± 4.4	26.9 ± 6.9	27.8 ± 7.3	20.9 ± 6.6	Condition, $P=0.030$*
SWB	18.8 ± 5.2	25.5 ± 7.4^{a}	27.3 ± 6.7^{aa}	20.0 ± 5.8	Interaction, $P=0.481$
Creatinine (mg/dL)					Time, $P<0.001$***
FWB	0.99 ± 0.16	1.03 ± 0.14	1.19 ± 0.23	1.13 ± 0.18	Condition, $P=0.125$
SWB	0.96 ± 0.11	1.00 ± 0.11	1.20 ± 0.12^{aaa}	0.94 ± 0.10	Interaction, $P=0.071$
Hematocrit (%)					Time, $P=0.001$**
FWB	44.2 ± 3.7	45.5 ± 3.1	48.2 ± 4.0	45.1 ± 3.4	Condition, $P=0.517$
SWB	44.3 ± 3.2	46.2 ± 2.1	47.9 ± 2.3^{a}	46.1 ± 2.7	Interaction, $P=0.644$
Hemoglobin (g/L)					Time, $P=0.001$**
FWB	15.0 ± 1.3	15.5 ± 1.1	16.4 ± 1.4^{a}	15.3 ± 1.2	Condition, $P=0.536$
SWB	15.1 ± 1.1	15.7 ± 0.7	16.3 ± 0.8^{a}	15.7 ± 0.9	Interaction, $P=0.640$
Change in plasma volume (%)					Time, $P=0.001$**
FWB		− 5.1 ± 7.4	− 14.1 ± 12.1^{a}	2.7 ± 13.6	Condition, $P=0.630$
SWB		− 7.0 ± 10.0	− 13.0 ± 11.4^{a}	− 6.3 ± 12.8	Interaction, $P=0.657$
Anion gap (mM)					Time, $P=0.805$
FWB	16.4 ± 1.8	16.4 ± 2.7	16.0 ± 2.4	15.2 ± 2.8	Condition, $P=0.851$
SWB	15.7 ± 1.6	15.7 ± 2.1	16.3 ± 2.6	16.6 ± 1.9	Interaction, $P=0.173$
Sodium (mM)					Time, $P<0.001$***
FWB	139.9 ± 2.2	141.3 ± 1.6	143.5 ± 2.8^{aa,b}	139.7 ± 1.7	Condition, $P=0.361$
SWB	140.5 ± 1.5	141.3 ± 1.3	144.1 ± 2.5^{a}	140.1 ± 1.8	Interaction, $P=0.814$
Potassium (mM)					Time, $P=0.642$
FWB	4.63 ± 0.43	4.87 ± 0.37	4.75 ± 0.39	4.67 ± 0.41	Condition, $P=0.227$
SWB	4.82 ± 0.36	4.80 ± 0.27	4.80 ± 0.32	4.90 ± 0.24	Interaction, $P=0.272$
Chloride (mM)					Time, $P<0.001$***
FWB	103.6 ± 2.7	105.5 ± 2.5	108.3 ± 3.1^{a,bb}	103.6 ± 1.6	Condition, $P=0.547$
SWB	103.6 ± 1.6	105.5 ± 1.5	107.8 ± 2.9^{a}	102.7 ± 1.7	Interaction, $P=0.507$
iCalcium (mM)					Time, $P=0.311$
FWB	1.25 ± 0.07	1.29 ± 0.08	1.29 ± 0.07	1.35 ± 0.23	Condition, $P=0.983$
SWB	1.29 ± 0.07	1.26 ± 0.08	1.32 ± 0.10^{a,bbb}	1.31 ± 0.07	Interaction, $P=0.583$
Total CO_2 (mM)					Time, $P=0.125$
FWB	25.5 ± 1.6	25.7 ± 2.3	25.3 ± 1.6	26.5 ± 2.2	Condition, $P=0.283$
SWB	27.1 ± 1.6	25.8 ± 1.2	25.6 ± 1.4	26.6 ± 1.6	Interaction, $P=0.334$

Data are presented as mean ± SD, $n=10$ or 11

BUN blood urea nitrogen, *CO_2* carbon dioxide

*$P<0.05$; **$P<0.01$; ***$P<0.001$ for main and interaction effects from the two-way (condition*time) ANOVA. Where a main effect of time was indicated, differences *within* conditions are noted by $^{a}P<0.05$, $^{aa}P<0.01$, and $^{aaa}P<0.001$ compared to Pre-testing Day − 2, and $^{b}P<0.05$, $^{bb}P<0.01$, and $^{bbb}P<0.001$ compared to Before 1st bath

the entire RWL process was greater in the present study at ~ 5.3% compared to those previous studies where ~ 4.3% (Connor et al. 2020) and ~ 4.5% (Connor and Egan 2021) were observed. The larger magnitude is explained by differences in percentage of body mass lost in the bathing protocol, which was ~ 2.7% loss of body mass in this study compared to ~ 2.1% in the other studies. This difference may simply reflect inter-individual differences in RWL between studies. Alternatively, commencing bath in water of higher temperature (e.g. ~ 40.3 °C versus 37.8 °C), and using a sauna blanket for the wrap periods rather than cotton clothing in a warm room, may result in more efficient loss of body mass per unit of time invested in such a protocol.

Together, these findings across three studies suggest that the addition of salt to HWI does not augment the loss of body mass compared to fresh water, at least in the hot bath protocol employed. The caveat that this conclusion only applies to the hot bath protocol employed is important, because several prior studies do indeed demonstrate an effect of salt to augment immersion-induced loss of fluid and/or body mass in various experimental models including whole-body immersion, and localized immersion of an arm/hand or leg/foot (Whitehouse et al. 1932; Buettner 1953, 1959; Peiss et al. 1956; Hertig et al. 1961; Brebner and Kerslake 1964; Hope et al. 2001). There are two suggested mechanisms for this phenomenon. First, that during immersion in salt water, the osmotic pressure difference between the immersion medium and body fluids results in greater fluid loss compared to fresh water, and/or second, that salt water serves to attenuate an inhibitory influence on the decline in sweat rate that usually occurs with prolonged immersion in hot fresh water (Whitehouse et al. 1932; Buettner 1953, 1959; Peiss et al. 1956; Hertig et al. 1961; Brebner and Kerslake 1964; Hope et al. 2001).

The absence of an effect of salt in our work may be explained by duration of immersion being much shorter than those previous studies observing an effect. For example, those studies have used immersion times of 3 h (Hertig et al. 1961), 4 h (Hope et al. 2001) and 5 h (Whitehouse et al. 1932; Brebner and Kerslake 1964). Despite our protocol comprising of 2 h of passive heating, HWI only accounts for 2 × 20 min of this time-period. Hope et al. (2001) observed a difference of ~ 600 g of body mass lost in 4 h when comparing immersion in fresh water to salt water (sea water) at 38°C (Hope et al. 2001). This difference between conditions would be the equivalent of ~ 2.5 g per minute assuming linearity in the response. Translating this rate into our 40 min of total time spent immersed in water would result in an expected difference of just 100 g between FWB and SWB. Therefore, for the addition of salt to have the desired impact of augmenting loss of body mass through passive fluid loss, much longer immersion times than the 2 × 20 min employed in this study may be required.

Another consideration, however, is the osmolality of the salt water given the proposed mechanism around the osmotic pressure difference between the immersion medium and body fluids. While the %wt/vol of salt is most commonly used as the descriptor of the salt water condition, the osmolality will be a function of both the concentration and type of salt. Our previous work using 1.6%wt/vol of Epsom salt (Connor et al. 2020; Connor and Egan 2021), and the present study using 5.0%wt/vol of Epsom salt, would result in an osmolality of ~ 130 and ~ 406 mOsmol/kg, respectively, which would decline somewhat with the addition of boiling water to maintain or increase the water temperature while bathing. Thus, these salt water baths were, respectively, hypotonic and only mildly hypertonic relative to the osmolality of body fluids (i.e., ~ 280 to ~ 295 mOsmol/kg). In contrast, when augmented body mass losses have been previously observed, these salt water baths were markedly hypertonic, i.e., 5%wt/vol of sodium chloride (Hertig et al. 1961) being ~ 1709 mOsmol/kg, and seawater (Hope et al. 2001) being ~ 3.5% salt and ~ 1000 to ~ 1200 mOsmol/kg. Therefore, while Epsom salt was used for its ecological validity, a salt such as sodium chloride may be more effective on a %wt/vol basis. Alternatively, Epsom salt would need to be used at > 12.3%wt/vol to produce an osmolality of > 1000 mOsmol/kg. These points assume that the osmotic gradient is an important mechanism by which salt water augments loss of body mass during HWI, and tentatively suggest that > 1000 mOsmol/kg is a valid threshold above which these effects would be observed.

The present study extends our previous work by measuring heart rate during the hot bath protocol, and measuring changes in blood markers during the RWL process, in addition to investigating effects of the RWL followed by ~ 24 to ~ 26 h of recovery on indices of performance. The heart rate data during the hot bath protocol demonstrate that a moderate degree of cardiovascular stress was induced as indicated by heart rate averaging ~ 110 bpm throughout the 2 h period and a measured peak at 128 ± 19 bpm and 127 ± 21 bpm during FWB and SWB, respectively. These values are equivalent to ~ 68% of the participants' age-predicted maximum heart rate.

The concentrations of several analytes in blood were increased during the hot bath protocol. Specifically, in blood samples taken immediately after the 2nd wrap period, concentrations of BUN, chloride, creatinine, hemoglobin, and sodium were each increased, as was the hematocrit value, but each returned to values similar to baseline by weigh-in on Day + 1. Calculation of plasma volume from hemoglobin and hematocrit revealed an average decrease in plasma volume induced by RWL of ~ 14% when measured upon completion of the 2nd wrap. This value is somewhat greater than that observed by (Hope et al. 2001) of ~ 7 to ~ 12%, but perhaps unsurprising given that the overall loss of body mass during the RWL protocol in the present study was approximately double of that previous work. In contrast, when elite amateur boxers undertook RWL in which a similar quantity of body mass was lost (5.6 ± 1.7%), the reduction in plasma volume was smaller at 8.6 ± 3.9% (Reljic et al. 2013). In that study, the RWL process was over a 5 day period, which potentially suggests that a shorter time frame of RWL and/or exposure to HWI may lead to greater loss of plasma volume or differential effects on different compartments of body water. There are two caveats that apply to the interpretation of these data for plasma volume. First, the i-STAT blood analyzer derives the value for hemoglobin using a proportionality constant after the measurement of hematocrit by a conductometric method, so the plasma volume data are based on an estimation of hemoglobin rather than direct measurement. Alternatively, using hematocrit only and thereby calculating loss of blood volume (Dill and Costill 1974), RWL resulted in a decrease in blood volume by ~ 8% in both conditions upon completion of the 2nd wrap. Second, postural changes are known to acutely influence measures of plasma volume (Pivarnik et al. 1986; Lippi et al. 2015), and a reduction in plasma volume of ~ 4.8% was previously observed within the initial 5 min after moving from a supine to seated (Pivarnik et al. 1986). Although the seated posture and rest period was consistent before blood sampling on Day − 2, before the 1st bath on Day 0, and on Day + 1, the sample taken upon completion of the 2nd wrap was preceded by 40 min in a supine position and only ~ 3 min of equilibration in a seated position. Therefore, the change in posture from a supine to seated position may have also contributed to decrease in plasma volume observed in response to the hot bath protocol.

Also of note is the observation of increased BUN and creatinine concentrations as these are often used as biomarkers of acute kidney injury (AKI) (Edelstein 2008; Kellum and Lameire 2013; Ostermann et al. 2020). RWL of > 4% of body mass consistently results in an increase in BUN and creatinine, which has been suggested as an indication of AKI being caused by RWL (Lakicevic et al. 2021). AKI has been previously defined as an increase in serum creatinine concentration by ≥ 0.3 mg/dL within 48 h (Kellum and Lameire 2013), a threshold which just two of our participants exceeded and which occurred within the 2 h bathing period. Additionally, the utility of circulating BUN and creatinine concentrations as sensitive and specific markers of AKI has been questioned (Edelstein 2008; Ostermann et al. 2020), whereas traditional measures of AKI are limited in their ability to classify AKI during heat stress, especially when combined with dehydration and/or exercise (Chapman et al. 2021). BUN and creatinine concentrations are indirect measures of AKI rather than direct measures of tissue injury such as with creatine kinase and cardiac troponin from skeletal muscle and heart, respectively. Direct measures of AKI in the circulation remain to be firmly established, especially those that can differentiate between 'pre-renal' and 'intrinsic' causes of change in circulating markers (Edelstein 2008; Ostermann et al. 2020; Chapman et al. 2021). Moreover, changes in BUN and creatinine concentrations are generally delayed in their response to AKI rather than acutely responsive (Edelstein 2008; Ostermann et al. 2020). Our data indicate an acute response in creatinine concentration to increase over the 2 h bathing period, whereas BUN concentration was already increased after the diet and fluid restriction, and increased further during bathing. Hence, it remains unclear whether these increases can indeed be considered to be evidence of AKI, or whether these simply reflect the well-established hemoconcentration effect of an acute decrease in plasma volume (Harrison 1985). In favor of the former is that it is well established that heat stress, especially when combined with physical exertion, can result in AKI (Chapman et al. 2021). Especially relevant to the present study is that heat stress-associated AKI is also influenced by hydrostatic pressure of water when HWI is used to apply the heat stress in experimental contexts (Chapman et al. 2021). Therefore, changes in markers of AKI during RWL and comprehensive assessment of kidney function should continue to be investigated by future research to better understand this phenomenon given its implications for the welfare of athletes who repeatedly undertake RWL.

Immediately before the performance testing on Day + 1 represented a ~ 24 to ~ 26 h recovery period at which point only 4 (FWB trial) and 2 (SWB trial) participants remained in a body mass deficit compared to Morning Day − 1. On average, there was a body mass *surplus* of 0.47 ± 1.48 kg and 0.69 ± 0.83 kg compared to Morning Day − 1 after recovery from FWB and SWB, respectively. This surplus is in contrast to the deficit observed on average in our previous work (Connor et al. 2020; Connor and Egan 2021), but is explained by the ~ 2 to ~ 4 h longer recovery time in the present study due to the inclusion of the performance tests. Therefore, despite the loss of ~ 5.3% of body mass in ~ 28 to ~ 30 h, blood markers had returned to values similar to baseline after ~ 24 to ~ 26 h of recovery. In practice, the time from weigh-in until official competition in professional MMA is usually longer, i.e., ~ 30 to ~ 36 h, but even with a longer time-period for rehydration, the majority of MMA athletes have been observed to be hypohydrated up to 2 h before competition (Jetton et al. 2013; Matthews and Nicholas 2017). Based on these observations, regain of body mass alone was suggested as potentially not being a good indicator of returning to a euhydrated state, but there is some debate about the validity of the classification of hypohydration through assessment of hydration status by spot analysis with urine measures (Cheuvront et al. 2015; Barley et al. 2020). For example, an alternative to the criterion of urine osmolality of > 700 mOsmol/kg being classified as hypohydration (Sawka et al. 2007) has been proposed as ≥ 925 mOsmol/kg (Armstrong et al. 2010). Using the Armstrong et al.'s threshold, only 3 (FWB trial) and 3 (SWB trial) participants were classified at hypohydrated at Morning Day + 1 compared to 7 (FWB trial) and 9 (SWB trial) using the Sawka et al.'s threshold.

No indices of performance were impacted by the RWL and recovery process when compared to pre-RWL values in either the FWB or SWB conditions. These results are in contrast to studies that have demonstrated a residual negative impact on indices of performance after ~ 24 h of recovery

(Alves et al. 2018; Barley et al. 2018b; Kurylas et al. 2019). In one study, athletes were dehydrated by ~ 5% of body mass through exercise in a heated room, and performance tests were completed 3 and 24 h after the intervention (Barley et al. 2018b). Vertical jump was unaffected by dehydration and recovery; hand-grip strength was weaker at 3 but not 24 h; medicine ball chest throw distance was shorter at 24 h, but not 3 h; and repeated sled push performance was worse at both 3 and 24 h after dehydration (Barley et al. 2018b). Therefore, there are likely to be time course-specific effects on performance in response to RWL and recovery, and which may also be impacted by whether passive or active methods of dehydration are employed, and the choice of performance test. Active methods of dehydration, i.e., involving exercise, may lead to residual fatigue and depletion of energy stores (Savoie et al. 2015), and can produce divergent responses in relation to changes in plasma volume, serum and urine osmolality, and performance, compared to passive dehydration (Nielson et al. 1981; Caldwell et al. 1984; Muñoz et al. 2013). Moreover, if a chosen performance test is not sensitive enough to detect physiological and performance changes, if any, that may be happening in response to RWL, the conclusion that there are no negative performance consequences of RWL when followed by adequate recovery and rehydration may be a type II error, i.e., false-negative finding.

Relatedly, this study was powered using the primary outcome of change in body mass as a consequence of the 2 h bath and wrap protocol. Given the absence of effect in our previous research using a salt concentration of ~ 1.6% (Connor et al. 2020; Connor and Egan 2021), like our previous approach (Connor and Egan 2021) a priori we planned an interim data analysis for the assessment of futility, and therefore discontinuation. However, the sample size was based on data derived from pre-to-post differences in a crossover design, and therefore, it is likely that the sample size is underpowered for the analysis of serial time point data such as those analyzed by ANOVA. In this scenario, again a type II error for observing the lack of differences between FWB and SWB cannot be fully discounted. Additionally, there are several methodological limitations that could be addressed in future work including the measurement of body temperature with a valid measure of core temperature (either esophageal or rectal), and the inclusion of a body mass measurement immediately after each period of HWI to isolate the effects of salt versus fresh water during HWI specifically rather than the entire hot bath protocol including wrapping periods.

In summary, short-duration HWI combined with periods under an infrared sauna blanket is an effective method of RWL to induce a loss of ~ 2.7% of body mass during 2 h of bathing (2 × 20 min) and wrapping (2 × 40 min). Using this protocol, the total amount of body mass lost when the water was supplemented with ~ 5.0%wt/vol of Epsom salt was similar to fresh water. When an appropriate refueling and rehydration strategy was followed, the ~ 5.3% loss of body mass during the overall ~ 28 to ~ 30 h RWL period was not detrimental in terms of blood markers or indices of performance measured after the ~ 24 to ~ 26 h recovery period.

Acknowledgements The authors thank the fighters for their participation, and Mr. Damian Martin (Sports Institute Northern Ireland) for providing us access to the i-STAT analyzer. This research was supported by funding from Enterprise Ireland (grant number: IV/2019/1192).

Author contributions JC and BE conceived and designed research. JC, MG, CG, and BE conducted experiments. JC, PC, and BE analyzed data. JC and BE wrote the manuscript. All authors read and approved the final manuscript.

Funding Open Access funding provided by the IReL Consortium. Enterprise Ireland, IV/2019/1192, Brendan Egan.

Declarations

Competing interests No conflict of interest, financial or otherwise, is declared by the authors.

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