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**Research Protocol**

**Study Title**

Effect of repetitive cold water immersion on structural and inflammatory biomarkers during a training camp and following a mixed martial arts contest

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16. **Introduction**

The purpose of this research is to investigate the effects of cold water immersion in comparison to passive recovery on structural, inflammatory and oxidative stress biomarkers during an 8 week MMA training camp and following a mixed martial arts contest. Currently a lack of information is available on the level of physiological stress accompanying mixed martial arts, while equivocal evidence is presented on the effectiveness of cold water immersion on systemic markers of inflammation and muscle damage and the effects of its long term use. This research hopes to recruit elite mixed martial artists and identify changes in select biomarkers at appropriate and selective time points to evaluate cold water immersion following full contact contests and an 8 week training camp. Should cold water immersion be an effective strategy at attenuating the hypothesized changes in each biomarker and reflect signs of overreaching, it could provide valuable information on post-exercise or trauma interventions. This may lead to decreased recovery times and subsequent increased performance.

1. **Background**

Mixed martial arts (MMA) is a relatively new sport that incorporates several forms of martial arts from Jiu-Jitsu to kick-boxing. It has gained notoriety through its combative nature which has drawn interest from multiple disciplines and champions of their own respective martial art. In a sport that revolves around grappling, striking and wrestling with impacts known to generate 90 – 120 G [[1](#_ENREF_1)], the level of associated muscle damage and inflammation is relatively unexplored, despite studies having identified MMA fights having injury rates ranging from 23.6 - 40.3 % to at least one of the competitors [[2](#_ENREF_2), [3](#_ENREF_3)].

Whilst several studies have been conducted on the diverse martial arts disciplines [[4](#_ENREF_4)], little has been conducted specifically on MMA. Other physical impact sports such as rugby union, rugby league, American football and jiu-jitsu have been shown to cause substantial increases in markers of physiological stress [[5-8](#_ENREF_5)], which suggests the force and frequency of the impacts accompanying this sport will induce similar trauma. Buse has identified 14.1 % of all MMA contests end because of musculoskeletal stress [[9](#_ENREF_9)] while jiu-jitsu, Tae Kwon Do and Greco-Roman wrestling training/competition cause significant elevations in haematological stress parameters [[8](#_ENREF_8), [10](#_ENREF_10), [11](#_ENREF_11)]. It is therefore plausible that significant elevations in oxidative stress, inflammation and muscle damage may transpire. Neopterin (NP), total NP (NP + 7,8-dihydroneopterin) and myoglobin are key physiological markers of these respective processes which have been used to assess exercise induced physiological stress [[5](#_ENREF_5)] and whose measureable changes present credible and reliable information pertaining to immune system activation and loss of muscle membrane integrity.

Recovery from impact induced physiological trauma is critical for sustained training and improved performance without risking the development of non-functional overreaching or over-training syndrome. Cold water immersion (CWI) has been shown to be an effective post-exercise intervention following impact trauma [[12](#_ENREF_12)] and resistance training induced muscle damage [[13](#_ENREF_13)] that is more readily available and similarly efficient at cooling [[14](#_ENREF_14)] in comparison to more modern whole body cryotherapy (WBC) strategies. Commonly used for its analgesic properties and shown to reduce muscle temperature by ~ 6°C [[13](#_ENREF_13)], CWI is capable of alleviating symptoms of delayed onset muscle soreness (DOMS), improve muscle power recovery, reduce post-exercise increases in creatine kinase [[15](#_ENREF_15)] through reduced peripheral blood flow [[16](#_ENREF_16)] and enhance the adaptation response through mitochondrial biogenesis [[17](#_ENREF_17)]. However, there is equivocal evidence surrounding the positive effect of CWI on systemic markers of inflammation and muscle damage [[18](#_ENREF_18), [19](#_ENREF_19)] which suggests further investigation is required.

1. **Aim of study**

The aims of this study are to identify whether cold water immersion is a more effective recovery intervention than passive recovery at attenuating hypothesized increases in markers of inflammation, oxidative stress and muscle damage following a mixed martial arts contest, and identify whether its continuous use throughout a training block has any effect on levels of these markers and performance measures.

1. **Objectives**

* Quantify whether muscle damage, inflammation and oxidative stress accompany a mixed martial arts contest.
* Identify whether cold water immersion is a more effective than passive recovery at attenuating changes in physiological stress.
* Quantify the extent to which cold water immersion reduces skin and core body temperature that may explain a potential reduction in each biomarker.
* Identify whether a relationship may exist between changes in neopterin production and myoglobin concentration following the recently observed relationship in rugby union.
* Identify whether ratings of perceived soreness are diminished following cold water immersion in comparison to passive recovery.
* Identify changes in oxidative stress and inflammation during an 8 week MMA training camp
* Identify if neopterin and total neopterin are capable of predicting overreaching/training
* Identify whether cold water immersion twice per week following full contact training sessions reduces inflammation and oxidative stress and increases performance

1. **Hypotheses**

* Significant muscle damage, inflammation and oxidative stress will accompany a contest
* Cold water immersion will significantly attenuate the change in inflammatory and muscle damage markers (neopterin and myoglobin) in comparison to passive recovery
* Cold water immersion will significantly reduce skin and core temperature
* The more muscle damage a fighter experiences, the greater the change in neopterin (oxidative stress).
* Ratings of perceived soreness will become reduced following CWI in comparison to CWI.
* Levels of neopterin and total neopterin will significantly increase throughout the 8 week training camp
* Subjects with higher levels of neopterin and total neopterin will perform less efficiently
* Repetitive cold water immersion will attenuate rises in neopterin and total neopterin during the 8 week training camp

1. **Study design**

This study will monitor elite mixed martial artists during an 8 week training camp culminating in a sanctioned competition.

1. **Study Location**

This study will be a multi-centre study. The mixed martial arts contest and training camp will be conducted at the gym where fighters train and compete. Here samples will be collected, stored on ice and transported back to the laboratory for analysis. All analysis will be conducted at the Free Radical Biochemistry Laboratory, School of Biological Sciences, University of Canterbury.

1. **Study Population**

There will be no exclusion/inclusion criteria based on ethnicity or sex. The population will be recruited from a mixed martial arts affiliated club whose fighters are over 18 years of age and compete in sanctioned provincial, national and international competitions. If fighters are currently recovering from a form of concussion, have a disease (which they are already independently tested for within the club) or to their knowledge are pregnant, they will be excluded.

The total number of subjects recruited will be a minimum 10 and a maximum of 20 to give good overall statistical power. No sub-group analysis will be conducted unless an equal number of men and women participate.

1. **Study Outcomes**
2. The primary outcome of this research is identifying the effectiveness of cold water immersion at attenuating the change in physiological stress accompanying a mixed martial arts contest
3. Assess the level of physiological stress enabling comparison with other impact related sports.
4. Identify whether repetitive cold water immersion reduces changes in physiological stress throughout an 8 week training camp
5. **Study Procedures**
   1. ***Participant Recruitment***

Potential participants will be identified/selected for recruitment through their active participation in sanctioned mixed martial arts contests in both Christchurch and Dunedin. All subjects will be approached through the managing director of the mixed martial arts clubs. They will then inform potential subjects of the impending study and will subsequently be approached individually by the CI. They will be given an information sheet containing all the relevant information pertaining to the study including, purpose, subject participation requirements, risks and ethical considerations. After consideration of the study information, subject’s written consent will be obtained.

Due to the increasing popularity of mixed martial arts, there is an ever increasing number of people participating in the sport. Recruitment of 10 – 20 fighters (allows for participant drop-out) should therefore be feasible. If participation numbers are below that required from fighters associated with Strikeforce Gym Canterbury or Academy of Combat, recruitment of Dunedin based fighters from Team Hammerhead will be conducted in the same manner. The time frame for this recruitment should take no more than four weeks based on the number of fighters available for recruitment in Christchurch and Dunedin.

***10.2 Randomization***

Half the group will be randomly assigned to a cold water immersion protocol and the other half to a passive recovery protocol during the 8 week training camp and contest.

***10.3 Study Procedure***

All data collection and analysis will be conducted by the CI (Angus Lindsay) under the supervision of Dr Carl Petersen and Assoc. Prof Steven Gieseg.

For the 8 week training camp, subjects will fight and train as per usual. A diary of their training schedule that will include sparring, strength and conditioning and full contact sessions will be recorded.

Prior to the contest, subjects enrolled in the study will be asked to abstain from any form of exercise 24 hours prior to the contest, as well as 48 hours following. This should provide us with the physiological stress response to the fight itself without any influence from an outside source.

Data collection requires subjects to complete the contest, remain at the contest location for 2 hours following their contest, and return to the contest location 24 and 48 hours later.

On the day of the contest, participants will be required to ingest a CorTemp® temperature pill (Sensor) 3 hours prior to each fight [[20](#_ENREF_20)] that will be provided to them on the day written consent was obtained. A reminder sent by text will be done the morning of the contest by a member of the research team. Core temperature will be measured pre-contest, immediately post-contest, immediately following the cold water immersion, and every 15 minutes for two hours. Similarly, skin temperature will also be assessed at each of these time points simultaneously.

All subjects will be weight-matched by the managing director of the clubs based on his competitive record and compete in a sanctioned competition. Each contest will consist of three rounds of five minutes. If a participant receives a lesion or any form of concussion that is deemed too dangerous to continue with (diagnosed by a clinical physician onsite associated with the club), the contest will be halted immediately.

All subjects will be randomly (name picked from a hat) assigned to one of two (CWI and passive) recovery protocols following the contest. Each protocol requires consumption of 750 mL H2O, a carbohydrate-protein beverage (50 g carbohydrate, 30 g protein, 6 g fat) and minimum eight hours sleep following the contest that is customarily completed following training and contests. The CWI protocol requires subjects to complete a CWI immediately following, 12 hours post, and 36 hours post-contest to observe the repeated effects of the strategy. Subjects will be asked to completely submerge themselves, except the neck and head, for 15 minutes in a temperature controlled 10°C bath at the contest location. This temperature was chosen because of its common use in CWI studies [[13](#_ENREF_13)] and its verified ability to reduce muscle temperature, blood flow, energy demand, micro-vascular perfusion and metabolic activity following exercise [[17](#_ENREF_17), [21](#_ENREF_21)].

For the 8 week training camp, subjects will once more be randomly assigned (name picked from a hat) to one of two protocols. Following both the full contact training sessions (Tuesday, Thursday and Saturday of their normal schedule), half the group will complete a CWI as described above, while the other half will sit passively for 15 minutes.

A questionnaire (McGill short form pain questionnaire (SF-MPQ)) will be required for subjects to complete at every sample collection following the contest. This questionnaire has three aspects that identify the level of pain or discomfort a subject is experiencing as a result of the contest and how CWI may affect these response. Both the descriptor and affective dimension of the SF-MPQ will be tallied to give an overall score, while visual analogue scale (VAS) of discomfort and present pain intensity (PPI) will also be collected.

Subjects will also fill in a questionnaire (same as above) before the Tuesday, Thursday and Saturday training sessions. The coaches (four of them) will also be asked to grade the performance of each fighter during those two training sessions to observe whether the pre-training concentrations of the physiological markers correspond. This will be a visual analogue scale as above, rating performance on a scale of 1-10.

Subjects will also complete a mixed martial arts specific performance test at the beginning of the Tuesday, Thursday and Saturday training sessions that can be used to gauge what effect repetitive cold water immersion has on sustainable performance. This performance test will require subjects to complete a circuit fitness test including jumping, push-ups, squatting, bag punching, bag kicking and shuttle runs.

Urine samples will be collected pre, immediately post, and one, two, 24 and 48 hours post-contest. Each participant will provide a specimen mid-stream (from the bladder) into a 70 mL collection pottle. All samples will be placed on ice immediately and transported to the laboratory for analysis. For myoglobin quantification, 5 mL of urine will be aliquoted at time of collection into a sterile 15 mL tube containing 1 mL 0.2 mol/L NaOH that adjusts the pH to approximately 7 – 9 [[5](#_ENREF_5)].

For the 8 week training camp, urine samples will be collected before the training sessions on Tuesday and Thursday and transported and analysed (neopterin and total neopterin only) as above.

Notational analysis will be performed on the video-recordings from a referee head-mounted camera that quantifies takedowns, standing punches (including elbows), kicks (including knees) attempted and respective percentage of each landed [[22](#_ENREF_22)] for correlation analysis with any change in physiological marker.

***10.4 Marker Selection***

The specific set of markers selected for this study include neopterin (oxidative stress), total neopterin (neopterin + 7,8-dihydroneopterin) (inflammation) and myoglobin (muscle damage).

Neopterin, a marker of cellular immune system activation, ratio indicator of associated oxidative stress and indicative of a pro-inflammatory immune status, is produced from macrophages as a product of 7,8-dihydroneopterin (78NP). As a marker of exercise-induced muscular tissue damage, neopterin has been used to identify the onset and severity of inflammation post-exercise. Several studies have used and shown neopterin to increase significantly following intense physical exercise [[23-30](#_ENREF_23)] making it an ideal marker for this study.

Myoglobin (Mb) is only found within the blood stream following muscle injury making it is diagnostically relevant to the nature of this study [[31](#_ENREF_31)]. Due to its small size (17kDa), it is leaked from the muscle following injury. It is also rapidly cleared from circulation due to renal excretion which leads to a sharper response than most other systemic markers of muscle damage [[32](#_ENREF_32)]. This trend has been demonstrated in the aftermath of downhill running [[33](#_ENREF_33)] and cycling [[32](#_ENREF_32)] with significant concentration increases immediately after exercise being observed at the completion of a rugby match as well. This makes Mb an optimal marker for muscle damage and this particular type of research.

***10.5 Measurement Tools***

Urine will be collected by a 70 mL collection pottle provided to subjects at each sample collection time point. They will be asked to provide the specimen mid-stream to ensure the sample is from the bladder.

Neopterin, total neopterin (inflammatory marker and indicator of immune system activation and oxidative stress) and specific gravity (used to control for urine volume) analysis will be conducted using strong cation exchange High Performance Liquid Chromatography (SCX-HPLC) [[34](#_ENREF_34)] and refractometry in the Free Radical Biochemistry Laboratory at the University of Canterbury under the supervision of A/Prof. Steven Gieseg. These methods are well established and published by the CI in the corresponding laboratory.

Myoglobin (muscle damage) will be assayed by reverse phase HPLC (RP-HPLC) in the same laboratory under the same supervision. The method used was recently established as a reliable assay for urinary myoglobin and currently under review for publication.

Core temperature will be measured using the CorTemp® telemetry system (HQ Inc., Palmetto, FL, U.S.A). It includes an ingestible temperature sensor (HT150002) (2 cm by 1.3 cm in diameter) accurate to ± 0.1°C, FM antenna and data system recorder (HT150001). The calibrated temperature sensor includes a silver oxide battery (1.5 V) which provides the power for sensing and transmitting temperature. The components of the sensors are encapsulated in epoxy and covered with silicone rubber. Temperature is transmitted through the body to a double bandoleer-type antenna and recorded by a CorTemp® data logger.

Skin temperature will be measured with a thermistor (YL-T11, Grant Instruments Ltd, Camebridgeshire, UK) placed 10 cm above the most superior aspect of the patella indicating the inferior horizontal line of the quadrilateral [[35](#_ENREF_35)] with data recorded simultaneously (Squirrel SQ2020 Series Data Logger, Grant Instruments Ltd, Camebridgeshire, UK).

Notational analysis will be performed using a Go Pro® (CA, U.S.A) attached to the referees head. This quantifies takedowns, standing punches (including elbows), kicks (including knees) attempted and respective percentage of each landed.

The short-form McGill pain questionnaire (SF-MPQ) [[36](#_ENREF_36)] will be used to gauge the level of pain or discomfort a subject is in at each stage of sample collection. Both the descriptor and affective dimension of the SF-MPQ were tallied to give an overall score, while visual analogue scale (VAS) and present pain intensity (PPI) were also collected.

***10.6 Patient Safety***

The foreseeable risks in the participation of this study other than the risk of injury from the contests themselves which would be occurring for training purposes whether this study was going ahead or not, is the unlikely possibility of pill temperature malfunction. Because the pill is encapsulated in epoxy and covered with silicone rubber, there is a very minor chance this will cause any adverse effects. If a participant were injured in this study from the study techniques, which is unlikely, they would be eligible for compensation from ACC just as they would be if they were injured in an accident at work or at home.

All participants will be provided with an information sheet outlining the risks and benefits of this study, and what would happen if they were to get injured and who to contact.

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1. **Statistical Considerations and Data Analysis**

Statistical analysis will be conducted by the CI under the supervision of Dr. Jason Tylianakis (biological statistician) at the University of Canterbury.

The effect of contest and training camp variables (number and type of impacts, contest outcome, recovery protocol and soreness questionnaire) on the change in biomarker concentrations will be tested in a linear mixed effects model fitted with restricted maximum likelihood, conducted in the lme4 package in R version 3.1.1. P values for coefficients of fixed effects will be calculated using Satterthwaite's method of denominator synthesis, conducted in the lmerTest package for R. Each marker will be analysed as the response variable in a separate model. The fixed predictors in each model will be number and type of impacts sustained by the participant during the contest, outcome of the contest (win or loss) and recovery protocol. Participant identity will also be included as a crossed random effect to account for the non-independence of marker measures from each participant. A repeated measures ANOVA will also be conducted with Tukey’s test post-hoc analysis to identify any differences between core and skin temperature, perceived ratings of pain from the SF-MPQ, VAS and PPI with each recovery protocol over time, and changes in neopterin and total neopterin during the 8 week training camp. Spearmen’s rank will be used to test the correlation between percentage changes in NP and total NP at each time point with temperature measurements and SF-MPQ, VAS and PPI scores and correlation with performance measures and urinary neopterin and total neopterin.

1. **Ethical Considerations**

Informed consent will be completed for every participant after they have been given a copy of an information sheet, understand it, and have any questions answered.

Participation in this study is of a voluntary nature. Subjects are free to decline participation, or to withdraw from the research at any time without experiencing any disadvantage, including the withdrawal of any information provided.

All participants can request a copy of any publication of this research. They will also be told any new information about adverse or beneficial effects related to the study that becomes available which may have an impact on their health.

The information gathered will remainconfidential between the research team and each individual participant. In addition, they will be provided with a summary of your results and an interpretation of the data.

The results of the project may be published and a summary of findings made available to each participant, but it is assured that the data gathered in this investigation is completely confidential. The identity of participants will not be made public. To ensure anonymity and confidentiality, all data will be kept on the CI’s password protected personal computer in a locked office.

All biological specimens collected (urine) will be destroyed using autoclaving or returned to the participants if they choose.

1. **Budget**

The consumables of this project will be made available from the Free Radical Laboratory, School of Biological Sciences, University of Canterbury.

As a result of the newly developed HPLC methods, consumables for this project are minimal and require no outside funding.

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