Title:
The effect of cold water immersion on electrocardiogram recordings in healthy male rugby union players

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DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted in fulfilment of the requirements for the degree of MSc Sport Science in the University of the Witwatersrand, Johannesburg. It has not been submitted for any degree or examination in any other university.

(Signature of candidate)

15th day of February 2021.

ABSTRACT

Introduction: Although cold water immersion therapy (CWI) is a popular recovery therapy used to accelerate the recovery process in rugby players, the cardiovascular response to cold water immersion places strain on the heart. However, little is known about the cardiac electrical effects due to cold water temperatures. The aim of this study, therefore, was to investigate the effects of cold water immersion therapy at two different water temperatures on cardiac electrical activity in male university rugby players.

Methods: A prospective, randomised crossover study design was used to investigate the effects of CWI therapy at two different water temperatures (8ºC and 14ºC) measuring cardiac electrical effects and heart rate variability of male university rugby players. An electrocardiogram (ECG) was used to measure the cardiac electrical effects and a Zephyr™ BioHarness™ was used to measure heart rate variability in the participants. Heart rate variability, blood pressure, and heart rate data were tested for normality. Continuous data were analysed using Statistica 12 and presented as means and standard deviations. Electrocardiogram data was assessed and presented as frequencies and proportions. Significance was set at $p<0.05$.

Results: Significant lower root mean square of successive RR interval differences (RMSDD) ($P <0 .00001$), and standard deviation of normal-to-normal RR intervals (SDNN) ($P<0 .00001$) were observed during the cold water immersion period at both 8ºC and 14ºC compared to pre-immersion period. Additionally, the stress index was significantly lower during the cold water immersion period compared to the pre-test values ($P=0.000754$) at both cold water temperatures. This indicates that there was a greater parasympathetic response during the cold water period. With regards to systolic blood pressure, the initial cold water immersion readings were significantly higher compared to pre-tests for both water temperatures. During the post cold water immersion period (or the post-test period), recorded measurements returned to baseline measures. Importantly, no ECG abnormalities were detected during the cold water immersion tests.

Conclusion: These findings showed no cardiac electrical activity abnormalities with cold water immersion therapy in rugby players. Heart rate variability during cold water immersion is significantly higher compared to the non-immersion pre-test period, due to a larger parasympathetic response. This study showed that the parasympathetic system has a predominant effect on the body compared to the sympathetic nervous system during cold water immersion therapy.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF DEFINITIONS

Cold water immersion:

"Is a training regimen usually following a period of intense exercise in which a substantial part of a human body is immersed in a bath of ice or ice water for limited duration (1)".

Heart rate variability:

"Heart rate variability is a measure which indicates how much variation there is in your heartbeats within a specific time frame (1)".

Blood pressure:

"The pressure of the blood in the circulatory system, often measured for diagnosis since it is closely related to the force and the rate of the heartbeat and the diameter and elasticity of the arterial walls (22)".

Kubios heart rate variability (HRV):

"Kubios HRV is a scientific tool for studying the variability of heartbeat intervals analysis" (40).

Standard deviation of normal-to-normal RR intervals (SDNN):

A fundamental measure that indicates standard deviation of normal to normal R-R Intervals in relation to HRV (40).

Root mean square of successive RR interval differences (RMSSD):

Root mean square of the successive difference is a domain tool used for the assessment of heart rate variability (40).

LIST OF ABBREVIATIONS

AV node:	Atrioventricular node
BP:	Blood pressure
BPM:	Beats per minute
CWI:	Cold water immersion
HR:	Heart rate
ECG:	Electrocardiogram
HRM:	Heart rate maximum
HRV:	Heart rate variability
LQTS:	Long QT Syndrome
MI:	Myocardial infarction
PNS:	Parasympathetic nervous system
PNSI:	Parasympathetic nervous system index
SI:	Stress index
SNS:	Sympathetic nervous system
SNSI:	Sympathetic nervous system index

CHAPTER ONE: PROBLEM IDENTIFICATION

Rugby Union (hereafter referred to as “rugby”) is a collision sport that can cause physical and non-physical stress to players’ bodies. Physical stress can occur due to impact through tackling, rucking, sprinting, and contact during the game (1). Non-contact physical stress can include travel, injuries, and weather conditions. Cold water immersion (CWI) therapy is a recovery strategy used to reduce acute trauma sustained during a game to specific body regions and to assist recovery (2,3,4). However, there is little research on the cardiac electrical effects of cold water on the heart. Anecdotal evidence indicates that full cold water immersion in events such as open-water swimming and deep diving can cause cardiac abnormalities (10).

In CWI therapy, athletes are partially submersed or immersed waist-deep in water with a temperature below 15 degrees Celsius (˚C) for a period usually ranging between five to fifteen minutes. The physiological mechanisms of recovery have been attributed to temperature and hydrostatic effects. These mechanisms can cause vasoconstriction of blood vessels around the affected limbs, stimulating the return of venous blood to the heart and assisting in the removal of metabolites from the affected areas to the kidney, skin, and lungs to be excreted (4-15).

Cold water immersion increases heart rate, blood pressure, and cardiac output (52). These initial responses to immersion cause a “cold shock” response and account for most immersion deaths in open-water swimming (52). These deaths are caused by drowning due to the physiological response of uncontrollable gasps and hyperventilation, initiated by the dynamic response of the cutaneous receptors, resulting in the aspiration of water (52). Fatalities due to cold water immersion have been reported in events such as open water swimming and deep-sea diving (14). These fatalities have been attributed to autonomic conflict causing myocardial infarctions (14,15,18). Autonomic conflict can cause arrhythmias, due to the simultaneous trigger of the sympathetic and the parasympathetic systems which oppose each other. Furthermore, it has been suggested by Shattock and Tipton (2017) that a larger number of deaths may be attributed to arrhythmias on immersion but these fatalities seem to occur in individuals who have predisposing factors such as Long QT Syndrome (LQTS), ischaemic heart disease or myocardial hypertrophy (52,54).

However, it is unknown whether autonomic conflict may occur in CWI therapy (52,53,54). It is necessary to determine if there are any additional risks alongside predisposing risk factors

(52,53,54). In addition, different water temperatures may also have different cardiac electric effects during cold water immersion therapy such as arrhythmias or myocardial infarction (5,7,9). These effects are caused from rapid increases in blood pressure and heart rate which creates additional strain on the heart (28).

There are many reports of cold water immersion and its beneficial physiological responses, however, research on the cardiac abnormalities due to CWI therapy is lacking (47,48). Despite the lack of research, coaches continue to implement this recovery strategy without knowing the full extent of its potentially harmful effects and if it has the same effects as cold water immersion in sports such as cold water swimming. Because of the unknown factor of the effect of CWI therapy on the heart, research into the subject is even more important to ensure players' safety. Therefore, this dissertation aims to determine electro-cardiac changes when using cold water immersion therapy as a recovery strategy in rugby players.

Research question

What are the effects of cold water immersion therapy at two different temperatures on the cardiac electrical activity in male university rugby union players?

Aim

To investigate the effects of cold water immersion therapy at two different water temperatures on the cardiac electrical activity of male university rugby union players.

Objectives

1. To determine the heart rate, rhythm, blood pressure, heart rate variability, and electrocardiogram (ECG) at baseline (pre-test) in male university rugby players.
2. To determine the heart rate, rhythm, blood pressure, heart rate variability and electrocardiogram (ECG) features during cold water immersion at 8°C and 14°C in male university rugby union players.
3. To compare the cardio electrical tracings at pre-test using an electrocardiogram (ECG) to cold water immersion at 8°C and 14°C in male university rugby union players.

4. To compare the blood pressure, heart rate, rhythm, and heart rate variability (using a Zephyr™ BioHarness™ 3 system) at pre-test with cold water immersion at 8°C and 14°C in male university rugby union players.

Null hypothesis

Cold water immersion therapy will have no effects on cardiac electrical conduction of male university rugby players and there will be no variability in blood pressure. Heart rate variability will reflect changes in variables and there will be an increase in heart rate.

Alternative hypothesis

Cold water immersion therapy will have an effect on the cardiac electrical conduction with the greatest effect at the lower water temperature and there will be variability in blood pressure. Additionally, heart rate variability is not expected to change.

CHAPTER 2: LITERATURE REVIEW

Rugby

Rugby is a collision sport that places a large amount of physical stress and non-physical stress on a player's musculoskeletal system. Physical stress is caused through activities such as tackling, rucking, sprinting, and contact in the game. Non-physical stress can include temperature variations, travel fatigue, injuries, and weather conditions including hot, cold, and rain. The physical stresses cause acute trauma to the muscle, tendons, and ligaments. This trauma, in the form of micro-tears, bruising, and soft tissue damage will undergo the process of reparation. The reparation process, which includes acute inflammation, repairing, and remodelling, can take between 72 to 96 hours post-training (3).

Physical conditioning (or training) of rugby players is important to enable players to withstand these forces and stresses. Players will undergo certain physical training regimens during pre-season training, which will cause physiological adaptations to help players cope better with the aforementioned stresses. These physiological adaptations include muscular hypertrophy, a reduction in resting heart rate and an increase in stroke volume and cardiac output (16). There are three phases to adaptations (16). The first phase is the shock or alarm phase, followed by the super-compensation phase, and then the exhaustion phase (16). Training periodisation is also used to enhance and manage players to avoid overtraining. (16).

Certain physiological responses such as delayed onset of muscle soreness (DOMS), causes pain and swelling in the muscle which may hinder optimal performance (35). Cold water immersion therapy is used to help reduce DOMS (35). Delayed onset of muscle soreness occurs after unfamiliar training exercises and generally twenty-four to seventy-two hours after training sessions and matches (35). The mechanism of DOMS is caused by eccentric training, or overloaded training sessions (35). Eccentric training involves movements that move with gravity, while concentric training is movement against gravity (35). Delayed onset muscle soreness can affect a variety of performance indicators in the body (35). A performance indicator is used to measure the limitations experienced by the body from exercise (16). These include a decrease in the economy of movement, impairment of glycogen repletion, reduction in biomechanical movement, and reductions in strength and power (16).

Matches and vigorous training sessions can cause exercise-induced muscle trauma resulting in a reduction in the force produced from muscular function (1). The physical demand and stress placed on rugby players during training or matches should be managed appropriately. The importance of recovery techniques such as active recovery, massage therapy, electrical stimulation, hyperbaric oxygen therapy, and cold water immersion therapy is implemented to reduce acute trauma and assist with recovery (2,3,4). Cold water immersion therapy is a widely used recovery strategy in certain sports, such as rugby. Cold water immersion therapy is most commonly used after a physically challenging session and matches, to help the athlete’s recovery faster. The process of cold-water immersion therapy and its effectiveness will be discussed in more detail.

Cold water immersion therapy

Recovery is essential to minimise the risk of over-training while promoting physical readiness for an early return to physical activity. This is vital in competition phases as high standards of performance need to be maintained. Cold water immersion (CWI) therapy is a popular method used in competitive sport to aid an athlete's recovery after exercise (5-9) and is useful in aiding the recovery of acute trauma caused to muscles during vigorous exercise sessions. Cold water immersion therapy involves an athlete submerging the affected limbs or sitting waist-deep in ice water for a short period of time (2). No standard duration of immersion has been identified in CWI therapy for effective recovery (2). Roberts, Jeff, Peake (2013) has suggested that players should submerge themselves in water between five to fifteen minutes depending on water temperature (2). In Roberts, Jeff, Peake's (2013) study they also concluded that the numerical value for the temperature of the water (in degrees Celsius) should also be the same numerical value for the time (in minutes) of the duration participants remain submerged (2). For example, at a water temperature of 5ºC, participants should be submerged for five minutes (2). The most commonly reported duration of immersion is between five to fifteen minutes depending on the water temperature (2,4,5,7,10).

Tipton (2017), investigated whether cold water immersion is a cure or a killer, and reported that cold water immersion duration effects can be broken down into four stages (52): the initial immersion, short term immersion, long-term immersion, and circum–rescue collapse. The initial immersion is the first three minutes that cools the skin (52). The short-term immersion is three or more minutes where the superficial neuromuscular cooling occurs (52). The current study examined this stage determining the superficial neuromuscular effects and analysing the effect on heart rate, blood pressure, and HRV in particular. Long-term immersion is 30 minutes or more during which deep tissue cooling occurs which can cause hypothermia (52). Lastly, circum–rescue collapse happens immediately before, during, or after a rescue from the cold water (52).

In studies completed by Ascensao, Rebelo, Leite et al. (2011), Pointon and Duffield et al. (2012) and Higgens, Trevor, Cameron et al. (2012), participants were immersed in cold water for 10 minutes at a temperature of 10°C. The researchers recorded a decrease in recovery time. The intensity of training sessions and matches also plays a role in the effectiveness of cold water immersion therapy. Pointon and Duffield (2012); Ascensao, Rebelo, Leite (2011); Elias, Wreyckelsma, Varley, et al. (2013); Brophy-Williams, Landers and Wallman (2011) Leeder, Gissane, Van Someren et al. (2011) subjected their participants to perform high-intensity workloads. The researchers found that their participants' recovery time and muscular damage was reduced. Furthermore, the participants perceived less discomfort in extremities which aided in the faster recovery of neuromuscular function and were more successful in restoring physical performance (6,8,10,11,12).

In contrast, studies have reported that cold water immersion did not influence players' recovery (19,20). Sellwood, Brukner and Williams (2007) examined the effect cold water immersion had on untrained athletes in reducing delayed onset muscle soreness (DOMS). Sellwood, Brukner, and Williams (2007) found that their participants perceived that cold water immersion did not influence reducing DOMS in players' limbs. However, these participants did acknowledge that there was a reduced muscle soreness (19,20). Analgesic effects of CWI are the reduced perception of pain caused from the decreased nerve conduction from the cold water immersion (16). Pain perception was reduced immediately after submersion in the cold water. The cold water inhibits the nerve receptors conduction, creating the perception of less pain (16).

Cold water immersion provides many benefits for the male rugby player. Through these benefits, physiological adaptations can take place to improve performance such as increases in heart rate, blood pressure and cardiac output.

Using precooling to improve performance benefits rugby players in hot environments which can affect performance (Galloway & Maughan, 1997) (56). The pre-cooling effect underpins the ergolytic effect of heat and can contribute to the direct consequences of hyperthermia (56). The intervention to reduce heat content in the body should enable greater storage of heat in the body before hyperthermia sets in, thus precooling can be enhancing to an athlete during exercise. (56). Athletes use deliberate cooling to cool themselves between bouts of exercise (55). This is to prevent hyperthermia and heat illness, especially in hot conditions, for example competing in conditions hotter than 30C (52). Barwood et al. (2009) investigated different cooling techniques using ice-vests, air- and water-perfused vests (55). The researchers found that hand immersion in cold water and whole-body fanning were the most preferential (55).

Further benefits of CWI therapy include the reduction in inflammation (57). Inflammation has an ever-expanding body of evidence that is linked to health and diseases (57). These include atrial fibrillation and diabetes (57). Cold water habituation, which is the process of undergoing cold water immersion and desensitise yourself from the cold water, has shown to improve insulin sensitivity (Hanssen et al. 2015) (58). Thus, habitual exercise is recognised as a therapeutic intervention for type 2 diabetes (58). Health benefits from CWI therapy and cold water immersion swimming are believed to be caused from physiological responses to cold water (57,58).

Therapeutic intervention for inflammation is used to reduce the magnitude of pre-inflammatory triggers through repeated immersions (52,59). Anecdotal evidence suggests that CWI benefits the body by reducing inflammation (52,59). Cold water immersion enhances serotonin secretion (59). Serotonin is a chemical nerve cells produce. It sends signals between nerve cells (52). It is found in blood plasma throughout the nervous system (52). This enhancement of secretion reduces the symptoms of inflammation (52,59).

These multiple benefits for cold water immersion can help athletes perform at their best (52-59). In heated conditions, the athlete's ability to cool off before, during and after events will improve their chances of competing at their best (53,54). This along with the reductions in DOMS and inflammation, athletes will be able to maintain their performance over competitions period lasting two to three months (14,54). Therefore, coaches and trainers have access to what is regarded as a very successful athlete maintenance therapy (52).

Physiological responses

Research has shown that participants that use cold water immersion therapy (CWI) recover significantly faster and had less perceived soreness than control groups (5,7,9,11,12,13). The immersion of an athlete's body in the cold water causes physiological changes within the body. These changes include intracellular and intravascular fluid shifts, reduction in muscle oedema and increases in cardiac output and stroke volume, which is the volume of blood excreted by the left ventricle per beat, which accelerates the recovery process (5-11,15). Cold water immersion has been associated with an increase in blood pressure, metabolism and vasoconstriction of blood vessels around the affected limbs, which stimulates the venous return of blood to the heart and also assists in the removal of metabolic waste from the affected areas to the kidney and lungs to be excreted (15,16).

Metabolism is the series of chemical reactions that cells use to maintain structure and balance in the body (28) and has two processes: catabolism and anabolism. Catabolism is the breakdown of molecules from food into parts of energy for cellular use, while anabolism is the construction of structures from smaller units i.e. from proteins into muscles (28). Metabolic wastes are substances left over from excretory processes in the human body and include salts, phosphates, sulphates, and nitrogenous wastes like urea (28). These substances cannot be used and are, therefore, removed from the body. Cold water immersion will remove metabolic waste substances through the vasoconstriction of blood vessels which also increases blood pressure and blood flow (35). Vasoconstriction narrows the blood vessels thus increasing the blood pressure which will speed up the process to remove these metabolic waste materials (35).

Cold water immersion therapy can cause vasoconstriction in the blood vessels. Vasoconstriction is the narrowing of blood vessels to limit blood flow to a region. This is caused from the cold water activation of the thermal nerve receptors, causing a change in the sympathetic nervous system response. This helps to reduce oedema and inflammation of the affected area (16). The vasoconstriction causes an increase in blood pressure, forcing more blood to flow through at a faster rate.

After a training session, the cells in the body require energy to repair structural damages, such as microscopic muscle tears and replace depleted energy stores (35). Yanagisawa, Niitsu and Takahashi et al. (2003) used a spectroscope to investigate the effects of fifteen minutes CWI at 5ºC following strenuous exercise (34,35). The researchers found muscle pH (potential hydrogen) values to be higher than those who did not use CWI therapy (34-35). Muscle acidity is a contributing factor to fatigue and muscular impairment (34-35). Therefore, CWI therapy helped reduce the muscular acidity in muscles through vasoconstriction on the blood vessels, which allowed the faster removal of waste (34-35).

CWI therapy can reduce oedemas (1,4,34). Oedema caused by increased permeability of sarcolemma can cause pain and impair oxygen delivery (1,4,34). The cold reduces the blood flow to affected muscle fibres, which decreases the possibility of swelling (1,4,34). During CWI therapy, the reduced blood flow acts as an anti-inflammatory influence (1,4,34). This is due to the increased affinity of alpha-adrenergic receptors for norepinephrine in vascular walls (1,4,34). With these restrictions, oedema can be reduced. Internal factors such as vasoconstriction can alter vascular adrenergic receptors which can help with the reduction of oedemas during CWI therapy (1,4,34,35). Tipton (1989) analysed the initial response to cold water immersion in male athletes. In his research, he reported that heart rate increases initially in cold water (two to three seconds) and then drops down below the normal average. The researcher also reported that cardiac output increases after one minute (28). Cooper Martin and Riben et al. (1976) reported that heart rate on average increased by 20 beats per minute during cold water immersion (29). The cardiovascular response to cold water immersion, therefore, places significant strain on the heart (28). This is from a rapid increase in blood pressure and cardiac output which requires more work output from the heart (28).

Researchers found that respiratory and ventilator response increases are noted in cold water immersion (30-33). The shock response of cooling skin temperature from cold water immersion can cause participants to gasp, hyperventilate and can experience a tachycardia (30-33). Inspiratory gasps occur on immediate immersion which can lead to individuals holding their breath (30-33). Tipton and Datta (2006) found that the duration of holding their breath was on average 23 seconds which causes tachycardia for this duration. After this, the participants adapted to the water temperature and resumed normal breathing (32). Keatinge (1961) found that when testing men during cold water immersion at temperatures of 5ºC and 15ºC, their pulmonary ventilation through partial pressure of carbon dioxide (pCO_2) would increase when compared to their baseline measure taken at the beginning of the test (30). During the test, the $pC0_2$ would become normal again (30). This shows that ventricular fibrillation and hyperventilation can cause tachycardia (30).

The vagus nerve has important physiological roles because of its location and multiple functions it has in the body (65,66,67). The nerve has sensory and motor pathways (65,66,67). This nerve transmits a wide range of signals to the brain including cardiac, digestive, and immunological signals (65,66,67). These signals are interpreted, and the correct signals are sent back to the correct organs (65,66,67). The motor nerve activity is measured non-invasively through heart rate variability (65,66,67). An increase in vagus nerve activity will decrease heart rate through inhibition of sinoatrial node by acetylcholine (65,66,67)

Heart rate variability (HRV) has been a non-invasive measure for the vagus nerve stimulation (65,66,67). It is used as a measure for morbidity and mortality in diverse diseases (65,66,67). A low HRV has been shown to predict the onset of cardiovascular diseases, cancer, and metabolic syndrome (65,66,67). The stimulation of this nerve and the response it creates can help assist with drawing link as to the physiological effect CWI therapy has had on participants (65,66,67). With the stimulation of the vagus nerve through the motor stimulation of the cold shock response to the cold water, heart rate and blood pressure will drop due to the release of acetylcholine (65,66,67). This is indicated with low RMSSD values and lower HRV values (65,66,67).

Blood pressure

Blood pressure is shown as a measure with two numbers, a numerator and denominator, which relate to systolic and diastolic pressures respectively (44). Systolic pressure is the pressure in the blood vessels after cardiac systole and diastolic pressure is the pressure in the blood vessels when the heart is not contracting (44). For the classification of blood pressure refer to Table 1. High blood pressure places strain on the heart. This can result from immersion in cold water which causes a shock response (a physiological reaction which can increase blood pressure). Other factors that cause high blood pressure include chronic age, overweight (excessive weight), not enough sleep (fatigue), stress and inadequate exercise (44).

Table 1: Blood pressure classification table (44)

Blood pressure classification	SBP (mm Hg)	DBP (mm Hg)
Normal	<120	<80
Pre-hypertension	120–139	80–89
Stage 1	140–159	90–99
Stage 2	⩾160	⩾100

One of the main causes of increased blood pressure in cold water immersion is vasoconstriction of blood vessels (44). When skin receptors are exposed to cold water, the body's cold shock response causes the blood vessels to constrict (44). This occurs as the body attempts to limit thermo excretion of heat (33). With blood flow being limited to the skin and limbs most of the blood flow is redirected to the vital organs (33).

Tipton (1989) researched the initial response to cold water immersion in humans (28) and found that blood pressure increased from peripheral vasoconstriction and therefore caused reductions in peripheral blood flow (28). Additionally, Keatinge (1961) measured the systolic and diastolic pressure in participants on entry into cold water and found that on average the participants' blood pressure increased from 130/76mmHg to 175/93mmhg (30).

Heart rate

Heart rate is the number of times a heart will beat in a minute (49). Heart rate is recorded in the following ways: resting heart rate and maximal heart rate (49). Resting heart rate is the number of heartbeats per minute while at rest (49). The best time to record resting heart rate is first thing in the morning (49). Maximal heart rate is the maximum number of beats a heart can reach per minute when exercising (49). To estimate maximal heart rate, one may use the following formula: 220 minus participant's age (49).

A normal heart rate will vary from person to person (51). Resting heart ranges between 60-100bpm (51). There are a few factors that can affect a person's heart rate. For example, if a person is fit and exercises regularly they will have a lower resting heart rate (51). This is because the heart ejects more blood per beat (i.e. greater stroke volume) and therefore requires fewer beats per minute to transport nutrients and oxygen around the body (51). Consuming certain foods can also increase or decrease heart rate, for example, caffeine increases an individual's heart rate (51). Furthermore, emotions can cause an increase in heart rate especially if an individual is anxious or gets a fright (51).

Heart rate has been measured in many studies with CWI (5,7,9,11,12,13,28,30,31,32,33, 42). Heart rate in both CWI and CWI therapy increased rapidly on entry into cold water due to the activation of the sympathetic nervous system and the "cold shock response" (5,7,9,11,12,13, 28,30,31,32,33,42). This is known to cause arrhythmias. However, as the physiological response changes during the immersion period and the parasympathetic nervous system takes over, the heart rate decreases to below resting heart rate (5,7,9,11,12,13, 28, 30,31,32,33,42).

Heart Rhythm

Heart rhythm is the pattern in which the heartbeats (50). Normal heart rhythm, which is sinus rhythm, is generated through the sinus node in the right atrium (50). The sinus node is a person's natural pacemaker (50). The sinus node produces an electrical charge that is spread through the heart cells causing the heart to contract (50). The electrical impulses travel through the atrium and into the atrioventricular node (AV node) (50). The electrical impulse then travels from the AV node through the ventricles, causing ventricles to contract (50).

If irregularities occur in the heart's rhythm, this can cause serious harm, such as damage to the heart, or even be fatal (50). Irregularities include sinus arrhythmias where the heart rate increases from a breath (50) as well as sinus bradycardia, which is a rhythm slower than 60bmp (50). Other symptoms include palpitations, which are the perceived abnormality of the heartbeat characterised by awareness of cardiac muscle contractions in the chest and is further characterised by hard, fast, and irregular beatings of the heart. Other irregularities include ectopic beats which is a premature heartbeat; arrhythmias which is irregular heartbeats and includes atrial flutters, atrial and ventricle fibrillation; AV blocks and bundle branch blocks (50). If an individual suspects or feels fluttering and pain in the chest region, a doctor should be consulted immediately to conduct an ECG test to assess the rhythm of the heart and to provide a diagnosis (50).

Cold water immersion therapy versus cold water swimming

There is an assumption that athletes who are immersed in cold water will have similar effects regardless of whether it is in a controlled therapeutic manner such as CWI therapy, or in open water events (17). This, however, is not the case. Cold water immersion may influence the heart's rhythm causing irregularities in the heartbeat and therefore affect the heart and cause arrhythmias (60). Open water swimmers with predisposing risk factors are most at risk (60). There is little research about how athletes would be affected in a controlled environment and it is, therefore, important to compare CWI therapy to open water swimming.

Open water swimming and diving has been known to increase blood pressure and heart rate (14). In open water sporting events, the body and head are partially immersed in water and this is combined with physical exertion such as swimming. In very rare cases this can lead to what is termed autonomic conflict. Autonomic conflict refers to the trigger of responses from the sympathetic and the parasympathetic nervous systems that oppose each other and are reported to cause arrhythmias (10,11). During rapid submersion in cold water, such as diving, two powerful autonomic responses are activated, the cold water shock and the diving response (11). Triathlon athletes or long-distance swimmers have reported cases of autonomic conflict during swimming events (10,11). The cold-water shock response is a pattern of reflexes driven by cutaneous cold thermo receptors in the face and is characterised by tachycardia, respiratory gasps, and hyperventilating (10,11). The diving response, which is a physiological response to immersion that overrides the basic homeostatic reflexes, is the opposite and is characterised by bradycardia, and vasoconstriction in the trunk and limbs (10,11). A study conducted by Shattock (2012) examined the evidence and hypothesised that autonomic conflict may cause sudden cardiac death (10,11). These two responses (cold-water shock and the diving response) when stimulated together may cause conflict and cause dysrhythmias (abnormality in a physiological rhythm) (11). A larger sympathetic response will occur in swimmers in open-water events due to an increase in splashing and kicking, and even swallowing of water, placing the body under more stress (11).

Cardiac abnormalities have been found in long-distance swimmers and divers who have Long QT Syndrome (a condition that affects the repolarisation of the heart) (9). The abnormalities can be triggered by the shock of the cold water. The initial shock of the low water temperature can cause arrhythmias (9). This arrhythmia can be caused by congenital Long QT Syndrome (LQTS) and is characterised by a delay in the repolarisation of the myocardium (9). Congenital LQTS is a disorder of the heart's electrical activity (10). Normally this repolarisation abnormality has no consequences, however in some cases and when a trigger is introduced, this can create an electrical misfire (9). Electrical misfires in the heart can lead to a potential life-threatening dysrhythmia (9). In most of these incidences, not relating to only water immersion, the heart’s rhythm returns to normal. It is noted that only five per cent of individuals die from arrhythmias caused by such events (9).

Long QT Syndrome refers to an abnormal pattern seen on an electrocardiogram (ECG) (10). An ECG is a test performed to measure the electrical activity of a person's heart (10). A person with LQTS will have a much longer QT interval on an ECG (10). This extended interval causes disruptions in the timing of the heart and can cause harmful dysrhythmias. There are seven different types of LQTS, but the most common ones are LQTS1,2,3. LQTS1 is caused by emotional or exercise stress. LQTS2 is caused by severe emotions or surprises. LQTS3 is caused by having a slow heart rate while you sleep (10).

It is important to note that LQTS is hereditary and can be diagnosed with an ECG (18). A person with LQTS can be at risk of dysrhythmias which can be triggered by shock, stress, or exercise (10). Autonomic conflict can cause arrhythmias and possible heart failure (18). Persons that have LQTS may have a greater risk of cardiac abnormalities, however, there are very few reported adverse cardiac effects from CWI therapy (47). Nevertheless, this study will be conducted to objectively assess potential cardiac risk for athletes during CWI therapy. This will include the different risk factors that athletes may face during training; heart rate and heart rate variability will be discussed and reviewed to understand the known effect CWI therapy has on the heart, and whether there is the need for further research.

Heart rate variability

Heart rate variability (HRV) describes the variations between consecutive inter-beat-intervals in a heart rate (23). Heart rate variability is linked to the autonomic nervous system. The autonomic nervous system controls both the parasympathetic and sympathetic systems (23). Heart rate variability is an indicator of the autonomic system and how it regulates itself through the parasympathetic and sympathetic systems (23). The higher the heart rate variability, the slower the heart rate becomes. As the heart rate variability lowers, the heart rate increases (32).

Heart rate variability can indicate the predominance of either the parasympathetic or sympathetic system. Heart rate variability measures values such as low frequency divided by high-frequency ratios (LF/HF) ratios, root mean square of successive RR interval differences (RSMDD), standard deviation of normal-to-normal RR intervals (SDNN), and stress index (SI) (37). The parasympathetic system can be measured through mean RR, RMSSD, and HF power, while the sympathetic nervous system is measured through mean HR, and the stress index (37).

The LF/HF ratio is used to estimate the ratio between sympathetic and parasympathetic nervous system activity (37,38). The HF component is affected solely by the parasympathetic nervous system activity and has a range from 0.15 to 0.4 Hz (37). The LF component is affected by the sympathetic nervous system and has a range from 0.04 to 0.15 Hz (37,38). A low LF/HF ratio reflects parasympathetic dominance while a high ratio reflects sympathetic nervous system dominance (38). This measure is a valid and reliable measure of which component of the autonomic nervous system is more active (38). Salahuddin, Cho, Jeong et al. (2007) obtained five minutes of resting ECG data from twenty-four healthy students and noted that there was valid and reliable LF/HF ratio data to measure the autonomic nervous system response (39).

Root mean square of successive RR interval differences (RSMDD) is also used to measure heart rate variability (38). The RMSSD value is influenced more by the parasympathetic nervous system activity (38). Munoz, Van Roon, Riese et al. (2015) measured SDNN and RMSSD in 3387 adults and analysed data using Pearson's correlation coefficients. At 120 second recordings, there were good correlations between 240–300 s for RMSSD (40). This shows that it is a valid and reliable measure for parasympathetic nervous system activity (40).

Standard deviation of normal-to-normal RR intervals (SDNN) is a fundamental measure that indicates standard deviation of normal to normal R-R Intervals. SDNN is influenced by both nervous systems, however, it is influenced more by the sympathetic nervous system. The higher the individual's SDNN, the greater the measure on the sympathetic nervous system response (41). In studies over a longer duration, SDNN is more valid and reliable (41). Bonnemeier et al. (2003) recorded a twenty-four-hour ECG for 166 healthy volunteers aged twenty to seventy years of age (41). They obtained hourly RMSSD and SDNN (41). During the study, there was a significant decline of 24-hour values of all HRV parameters ($P < 0.001$) and a strong negative correlation ($P < 0.001$) with increasing age (41).

The parasympathetic response is reflected by the RMSSD measurement. The RMSSD reflects the beat-to-beat variance in HR and is the primary time-domain measure (38). The RMSSD is also correlated to HF power and the influences of respiratory rate (38). The RMSSD and HF power are controlled by the parasympathetic nervous system. The RMSSD and HF power increase when the parasympathetic nervous system is active.

Stress index (SI) is a measure of the sympathetic nervous system (37). A higher SI indicates a greater influence from the sympathetic nervous system (37). A low SI indicates a parasympathetic nervous system response (37). The measure of SI is a valid and reliable measure of sympathetic nervous system response (37). Rajendra, Joseph, Kannathalchoo et al. (2006) examined patients' blood pressure, acute myocardial infarction (MI), nervous system disorders, diabetes, and sleep (42). In all these cases SI was measured with SDNN and RMSSD (42). In the researcher's study, the SI value reflected an increase in the sympathetic response, making SI a valid and reliable measuring tool (42).

A predominantly parasympathetic system response slows the heart rate, with more variability between the individual heartbeats. With the sympathetic system predominance, the heart rate is elevated, reducing the difference in duration between heartbeats (23). Under stress conditions, the HRV will lower as heart rate increases due to sympathetic response. Karinen, Usitalo, Ypyä et al. (2012) investigated acute mountain sickness, using HRV to determine if heart rate variability is an indicator of acute mountain sickness (24). The researchers reported that HRV was related to acute mountain sickness. As the participants heart rate increases due to hypoxia, this lowers HRV. The researchers also concluded that HRV measures the autonomic response of participants (24). Many studies have used HRV to establish the dominant nervous system in participants (23-28).
Adina and Taylor (2016) studied heart rate variability as an indicator of an individual cardiovascular condition (43). They examined the two primary rhythmic oscillations to determine how heart rate variability was affected (43). One of the oscillations is the respiratory sinus arrhythmia, which is a rhythmic heart rate oscillation at the respiratory frequency (43). Heart rate variability can be an indicator of oscillation as heart rate quickens and slows down with inhalation and exhalation respectively (43).

Researchers confirmed that heart rate variability is an effective measuring tool for the autonomic nervous system and how stress and changes in an individual's environment can bring about these autonomic nervous system adaptations (42). The researchers examined patients with high blood pressure, acute myocardial infarction (MI), nervous system disorders, diabetes, cardiac arrhythmias, renal failure, as well as patients that smoked, used drugs and, alcohol or had sleep deprivation (42). In all these cases where HRV was measured, significant changes in the autonomic nervous system were found. Increases in sympathetic nervous system responses and a decrease in parasympathetic nervous system responses were noted by the researchers (42). In conclusion, they stated that heart rate variability is an important tool in cardiology because it is a non-invasive measurement (of chronic conditions) and is easy to perform (42).

Cold water immersion and heart rate variability

Cold water immersion causes certain physiological responses as mentioned previously. The activation of the parasympathetic and sympathetic nervous system is an observed response. In the initial immersion into cold water, the sympathetic nervous system (SNS) is activated causing a rapid increase in heart rate and blood pressure. Over time the parasympathetic nervous system (PNS) becomes more dominant, slowing the heart rate down and reducing blood pressure (23).

One of the best measures of this process is using heart rate variability to measure the variations between consecutive inter-beat-intervals in a heart rate (23). This is the most accurate measure for the SNS and the PNS and therefore can be used to assess which system is active during the immersion process.

Bastos, Vanderlei, Nakamura (2012), et al. studied the effects of cold water immersion and active recovery on post-exercise heart rate variability (25). Twenty male subjects performed an incremental exercise test on the first day to determine maximal oxygen consumption and the associated speed of maximum heart rate (25). During the remaining three visits athletes performed constant velocity tests on a treadmill until exhaustion (25). Recovery methods were randomised between passive recovery (sitting down), cold water immersion, and active recovery (stretching and short runs) (25).

Standard deviation of normal-to-normal RR intervals (SDNN), RMSSD and the LF/HF ratio were analysed in this study (25). Standard deviation of normal-to-normal RR intervals (SDNN) and LF/HF ratio were found to be significantly higher response in cold water immersion compared to passive recovery, which did not have any activation of the CNS (25). This suggests that the CWI therapy generated a larger re-activation of the parasympathetic system compared to active recovery. A study conducted by Bastos, Vanderlei, Nakamura (2012) et al. indicated that during the initial CWI test, the SNS was active and then as the test continued, the PNS became more dominant. This was indicated by a lower LF/HF ratio when compared to active recovery. The comparatively lower LF/HF value was attributed to a reactivation of the parasympathetic nervous system. The study also showed that cold water immersion was more effective than the rest and active recovery for PNS activation.

Haddad, Laursen, Chollet et al. (2010) measured the effect of cold versus thermo-neutral (34ºC) water immersion on post-exercise heart rate recovery and heart rate variability (26). Twelve males performed an intermittent exercise bout on three separate occasions. These intermittent exercise bouts included a 30 second Wingate test with a five minute seated recovery. This was then followed by a five-minute sub maximal running test. Recovery was then randomly followed by seated recovery in either cold water immersion, thermo-neutral immersion (TWI), or control conditions (no recovery) (26). RMSSD was higher in CWI compared to control conditions. No differences were noted between CWI and TWI (26). The researchers concluded that water immersion is an effective means of immediately triggering post-exercise parasympathetic activity (26).

Haddad, Laursen, Chollet et al. (2010) reported that the RMSSD was higher in CWI compared to controlled conditions. Through heart rate variability the researchers determined that the PNS was more active during CWI and less active in control conditions. This again indicates that RMSSD is an effective measure of the PNS through heart rate variability. Interestingly no differences were noted between CWI (14°C) and TWI (34°C). This indicates that the PNS will activate in water colder than an individual's skin temperature. (26). The researchers' concluded that the PNS in both the CWI and TWI is activated causing a lowering of the participants' heart rate and blood pressure. Parouty, Haddad, Quod et al. (2010) measured the effect of cold water immersion in well-trained swimmers (27). Ten well-trained swimmers performed two 100m swimming sprints interspersed with a 30-minute passive recovery (27). Athletes were randomly assigned to either four minutes of CWI at 14ºC or a control group (out of water condition at 28ºC) (27). Heart rate variability was recorded during tests. Root mean square of successive RR interval after the first sprint had a smaller decrease after the CWI test when compared to the control group, which again shows a larger parasympathetic response to cold water immersion therapy (27).

Parouty, Haddad, Quod, et al. (2010) study also indicated a parasympathetic response due to a higher RMSSD recording during cold water immersion therapy. This shows that the PNS has a larger response than the SNS. The cutaneous cold thermo receptors stimulate a response that causes the PNS reaction. The hypothalamus receives a response/stimulation from the thermo-receptors, and send out a response through the CNS to react to the temperature change. This reaction causes a "cold shock response", which leads to the activation of the PNS. This study also indicated that the PNS is more active in CWI than the control group (no immersion) (27).

From the studies (25,26,27) above and many others (60,61,62,63), the parasympathetic nervous system has a greater effect on the physiological response of the human body when submerged in cold water. Also, heart rate variability is an effective tool for measuring the SNS and PNS using RMSSD and SDNN.

Summary

The use of CWI therapy has proven to be successful in improving recovery in athletes post matches and after high-intensity training sessions (5-15). Cold water immersion causes a reduction in oedemas, it speeds up recovery time and can help reduce DOMS. However, evidence suggests autonomic conflict can be a cause for concern in cold water events (such as full immersion swimming and diving). The stimulation of the vagus nerve, which will cause the PNS to activate through the dive response during submersion, can be detected through heart rate variability. Heart rate variability is an indicator of both sympathetic and parasympathetic nervous systems. There are four main measures used to measure heart rate variability and to determine which nervous system is active. These measures are SDNN, RMSSD, SI, and LH/HF ratio. Cold water immersion is linked to greater activation of the PNS system and this is indicated through a higher RMSSD and a low LH/HF ratio. The activation of the PNS affects blood pressure through vasoconstriction of the blood vessels due to the cold-water shock response. However, there is still an absence of research on the effects of CWI therapy on the electrical activity of the heart. Despite there being very little research on CWI cardiac electrical responses (47,48), it would be prudent to study the effects of cold water immersion on the hearts of healthy male rugby players (17).

CHAPTER 3: METHODS

Study design

A prospective, randomised crossover study design was used to investigate the effects of CWI therapy at two different water temperatures (8ºC and 14ºC) on the cardiac electrical effects and heart rate variability of university rugby players. The participants in this study were randomly placed into two groups. A computer randomise generator was used to randomly choose names and place them in a group. A crossover study design was used as each participant followed a repeated testing procedure for different treatments (CWI).

Site of study

The study was performed at the Centre for Exercise Science and Sports Medicine, Wits Education Campus, University of Witwatersrand.

Study population

Eighty (n=80) male rugby players in University of Witwatersrand rugby club who were registered to play for Wits Rugby Football Club semi-professionally during the 2018 season were recruited. Most of the male rugby players would have been regularly exposed to CWI therapy sessions from around the age of 16 years to present (after training and/or matches). Wits rugby teams use this recovery strategy after each match during the competitive season and after intense training sessions (frequency of up to a maximum of twice per week during the competitive season).

Sample size

Eighty (n=80) rugby players, or 60% of the full population. Sample size calculation: 48 (Population size of 80, confidence level of 95%, margin error of 8%), gives a sample size of 48.

Calculation:

$\underline{Z^2 \text{ X } p(1-p)}$

$\underline{\quad\quad e^2}$

$1+ \underline{(Z^2 \text{ x } p(1-p)}$

$\quad\quad e^2N$

$1.96^2 \text{ X } 80(1-80)$

$\quad\quad 8$

$1+ (1.96^2 \text{ X } 80(1-80)$

$\quad\quad 8^2$

$= 48$

Study participants - Inclusion/exclusion criteria

Inclusion criteria

- Male rugby players playing for the Wits Rugby Football Club.
- Ages between 18-30 years, this is the age range of club rugby players at Wits university

Exclusion criteria

- Any reported cardiac or metabolic medical conditions.
- Any skin disorders, rashes, or infections.
- Health risk(s) detected in their medical screening (pre-screening) ECG and medical questionnaire).
- Health risks include chest pains, shortness of breath, fainting during rugby sessions, high blood pressure, infections of the heart, or any treatment for heart conditions.
- If athletes became sick during the testing procedure, the participants were asked to only come back to testing once they were healthy again.

Invitation to participate

An email was sent to the Head of Wits Sport (appendix I) and the respective team coaches of the University of Witwatersrand (appendix J) requesting permission to collect data for the study. Permission was also required from the registrar of the University of Witwatersrand to test students (Appendix k). Ethics approval was granted after receiving permission from the Wits rugby coach, Wits head of Sport and the University registrar to collect the data for this research. The researcher then approached the coach of the respective rugby teams to get permission to speak to the players about the study (Appendix J). The researcher approached the players through WhatsApp messaging and face-to-face discussions after training sessions once the coach gave permission. The researcher then invited the players to participate in the study.

Procedures and instrumentation

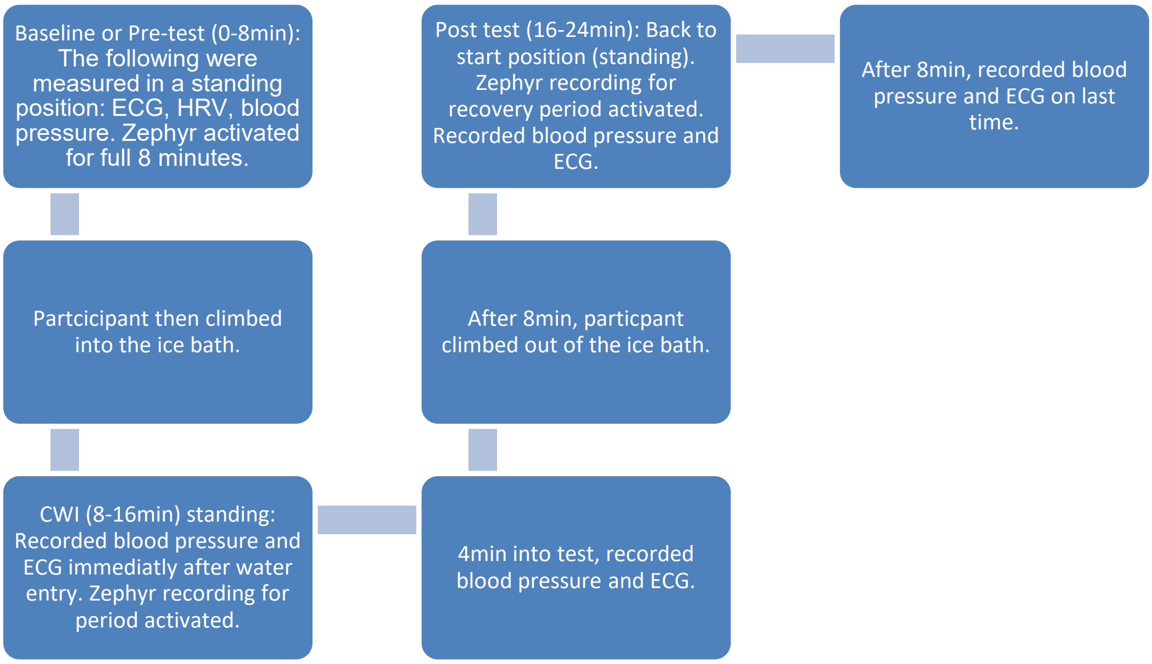


Figure 1: Procedure for testing participants

The figure above shows the procedure followed during the CWI testing process for both testing temperatures.

Participant pre-participation medical screening

A pre-participation health risk questionnaire (appendix C) and resting ECG were performed to exclude participants with any heart conditions, family history of heart problems, high blood pressure and any related health issue that would place participants at risk. This was done on a separate day one week prior to actual testing.

Medical screening questionnaire

On arrival for medical pre-screening testing, players completed an informed consent form (Appendix B) and medical screening form (Appendix C). These are standardised forms and were not modified. The medical screening form allowed the researcher to identify cardiac risks. The researcher also noted blood pressure and family history of cardiac events on the medical screening form. Blood pressure was also recorded with a stethoscope and a blood pressure cuff (Littmann classic III stethoscope, Curaplex blood pressure cuff).

Electrocardiogram testing (ECG)

After completing the medical screening questionnaire the participant was asked to lie in a supine position on his back and the 12 ECG electrodes were placed as follows: V1= Right of the sternum, 4th intercostal space; V2= Left of the sternum, 4th intercostals space; V3= in between V2 and V4; V4= mid-clavicular line, 5th intercostals space; V5= In between V4 and V6; V6= Mid-axillary line, 5th intercostals space; AVF= Left hip; AVR= Right mid clavicle, just below; AVL= Left mid clavicle, just below (Appendix D). International standardised settings for time and amplitudes were applied.

The ECG reliability and validity were tested by Harlan, Graybiel, Mitchell et al. (1967) who examined the ECG durations, amplitudes, and vectoral orientations. These measures were very similar to values from cross-sectional surveys of men of the same ages (22). During the study, minor changes were noted in their research making it reliable and valid (22).

A labelled diagram of an ECG printout

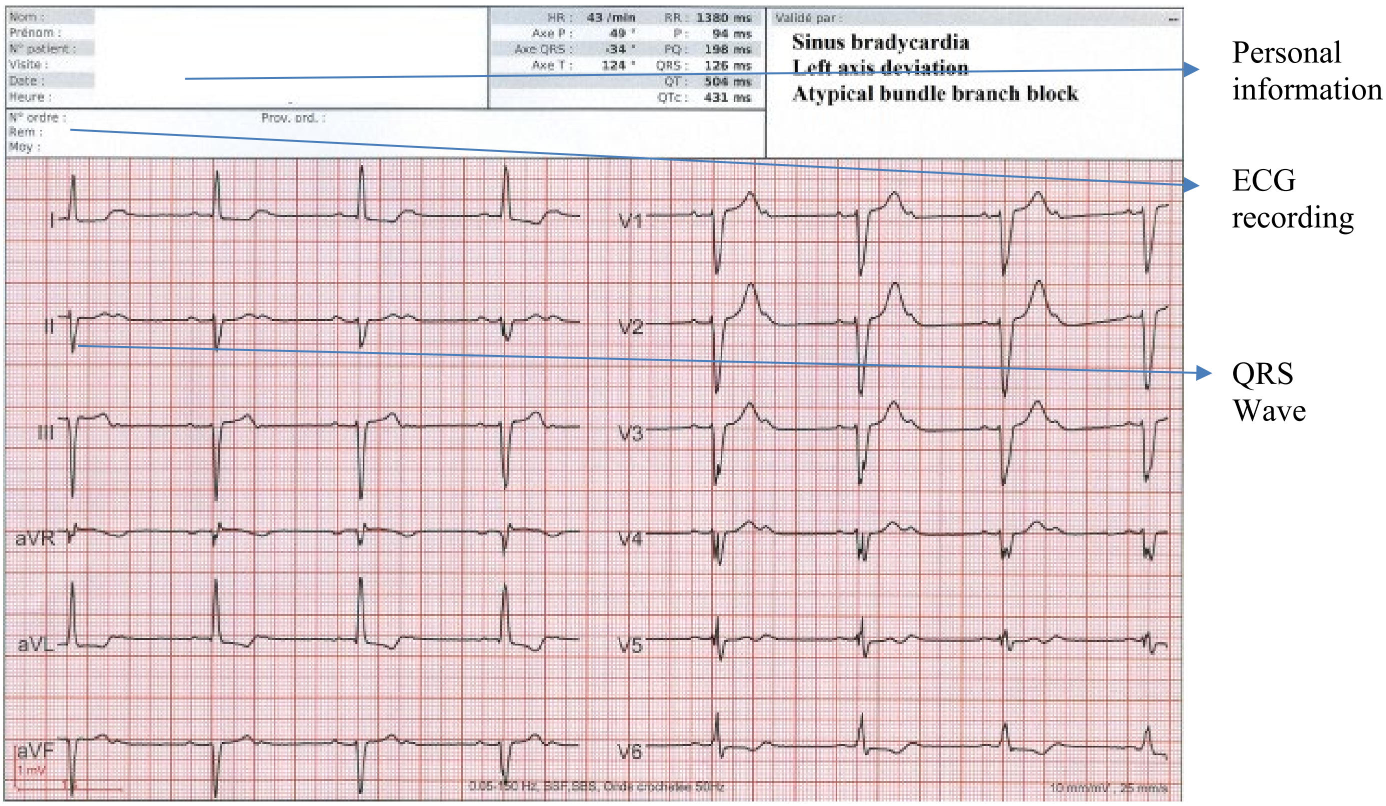


Figure 2: Example of an ECG printout.

This is what the ECG recordings would have looked like and how the data was originally displayed.

Pre-participation cardiac screening

Players underwent a medical pre-screening ECG prior to cold water immersion (see Figure 2). This was done so that the baseline ECG recording was analysed for any cardiac abnormalities. The medical pre-screening ECG test was conducted by the Centre for Exercise Science and Sport Medicine (CESSM) sports physician based at CESSM and the researcher. Participants were standing during the ECG medical pre-screening testing. ECG readings were saved and then examined by the sports physician for any cardiac abnormalities. Players with an abnormal medical pre-screening ECG would have been excluded from the study and referred to a cardiologist if deemed necessary. Once players were cleared to participate, they were notified as to when they could proceed in completing the cold water immersion tests. The tests were conducted within seven days after clearance by the physician had been given.

Procedures and instrumentation

Cold water immersion

All participants were standing during both cold water immersion periods at 8ºC and 14ºC. Participants were tested in random order organised one day prior testing. The same test procedures were carried out for both water temperatures. Participants stayed immersed in the water for eight minutes. Duration for submersion was based on research analysed in successful cold water immersion studies (5-15). Each participant stood waist-deep in the water (i.e. up to their anterior superior iliac spine).

The ECG was recorded over a one-minute period. Blood pressure was also recorded at the same time. Blood pressure was recorded by the researcher using a sphygmomanometer (Welch Allyn DuraShock DS54 Thumbscrew Aneroid Sphygmomanometer). These recordings were performed in a standing position at the beginning of the eight-minute pre-test (first-minute mark). Players were then recorded on water entry (nine-minute mark), and then four minutes into CWI test (13th minute). Both measurements were then recorded in the recovery period (post-test) once the participants climbed out of the water (in a standing position) (17th-minute mark), and after eight minutes in a standing position (25th-minute mark).

To keep the electrodes dry, plastic cling film was placed over the electrodes so that the water did not splash onto the electrodes. All participants were tested and monitored individually with appropriate emergency procedures in place i.e. access to an automated external defibrillator (AED).

Table 2: Time periods of the procedure implemented on the participants

Cold water immersion test	ECG recordings	Blood pressure recordings
Pre-test - Start test	1st minute	1st minute
CWI test - Entry into water	9th minute	9th minute
CWI test - 4 minutes into CWI test	13th minute	13th minute
Post-test - immediately on exit out of the water.	17th minute	17th minute
Post-test - end of the 8-minute test	25th minute	25th minute

Each recording period took approximately one minute to complete.

Heart rate variability

Heart rate and heart rate variability data (R-R intervals measured in ms) were recorded using a Zephyr™ BioHarness™ 3 (Zephyr™ Technology, Auckland, New Zealand) (45). Zephyr™ BioHarness™ 3 system is a valid and reliable system to measure heart rate (45). Kim, J.H et al. (2013) tested the Zephyr™ BioHarness™ system to determine the accuracy of heart rate (HR) and respiratory rate (RR) measurements during two exercise protocols (45). In one protocol, HR and RR were measured using the BioHarness™, in another protocol, HR and RR were measured using the portable metabolic system (45). During the graded exercise, HR but not RR, obtained from the BioHarness™ was higher compared to the Vmax at baseline, however, it showed no significant difference at any other stages with high correlation coefficients for HR (45). In conclusion, the researcher found that the BioHarness™ is comparable to Vmax and the portable metabolic system over a wide range of VO2 during graded exercises (45).

When players arrived, they were allocated a Zephyr™ BioHarness™ code and the date they were recorded. Players were allocated the same Zephyr™ BioHarness™ for each test at 8ºC and 14ºC.

Participants had the Zephyr™ attached to them before the test began. At pre-immersion, the Zephyr™ was activated and recorded for an eight-minute period (one to eight minutes). The Zephyr™ was then switched off and the participant moved across to the cold water immersion test. Once the participant entered in the water, the Zephyr™ was reactivated (start of the ninth minute) and recorded for eight minutes. After eight minutes (end of the 16th minute) the Zephyr™ was switched off and the participant climbed out of the cold water and returned to the start position. The Zephyr™ was activated for the final eight-minute post-test recording.

Table 3: Time periods of the Zephyr™ recordings following the procedure

Cold water immersion test	Time period the Zephyr™ records
Pre-test period	(0-8 minutes): Zephyr™ activated and recorded for 8 minutes, after 8 minutes it was switched off.
CWI-test period	(8-16 minutes): Zephyr™ reactivated and recorded for 8 minutes and then it was switched off.
Post-test period	(16-24 minutes): Zephyr™ was reactivated and recorded for 8 minutes and was then switched off. This was the end of the testing procedure.

Four participants were excluded from the study due to corrupted recorded data or blood pressure baseline recordings that were determined to be too high.

Data analysis

Data collection

Data during the testing procedure were collected on data record templates (see appendices' F, H, I). Zephyr™ BioHarnesses™ were collected at the end of the testing and the R-R(ms) recordings from each test period were downloaded into separate .csv files. These R-R recordings were then entered into a Kubios HRV (ver. 3.1), MATLABr 2016b1 (The MathWorks, Inc.) standard computer programme. The Kubios program determined the following heart rate variability data: RMSSD, SDNN, SI, and LH/HF ratio for the pre-test (baseline), CWI, and post-test periods.

Eleven ECG tracings were recorded and printed out per person, one pre-test and five for each testing interval. These ECG printouts were delivered to a sports physician to examine for any abnormalities. Blood pressure was also recorded for each participant in the same order and procedure as the ECG procedure. This data was recorded on Template I.

Once all the raw data was collected, a Microsoft Excel spreadsheet was created as a master copy and the data for each participant was entered. The spreadsheet contained all the anthropometric, blood pressure, ECG abnormalities, and HRV data for each phase of the testing procedure.

The statistical analysis

The data were analysed using Statistica 12 software (StatSoft, USA) and is presented as means and ±SD. The data were tested for normality using the Shapiro-Wilk test. The data were found to be normally distributed. The cardiac abnormalities were analysed using ANOVA. Statistica 12 was used to analyse blood pressure, heart rate, RMSSD, SDNN, SI and LF/HF ratio. Means, confidence levels, maximum, minimum, and standard deviation for these data were calculated and compared to baseline data (pre-test). Significance was set at $p<0.05$.

Data was captured using a Microsoft Excel spreadsheet. The ECGs were printed out and analysed separately. (refer to table 2 below).

Table 4: Table indicating which variables are being measured and how they are going to be measured

Outcome:	**Definition**	**How to measure it**	**Continuous or Categorical:**
Rate	Heart rate is measured by the number of contractions of the heart in a minute (bpm).	300/ (number regular beats per sequence) = bpm.	Continuous
Rhythm	A sinus rhythm is any cardiac rhythm where depolarisation of the cardiac muscle begins at the sinus node.	The mm spread and differences between the ECG P wave, QRS complex and ST interval.	Continuous
QTc Interval	It is a measure of the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle. QTc will be used as QT lengthens at slower heart rates and shortened at faster hearts rate. This formula corrects the QT to the QT at the heart rate of 60.	The mm spread and differences from the baseline test. The formula used Hodges formula: QTc= QT + 1.75 (heart rate-60).	Continuous
ECG Changes	Were there changes noted in the participant's ECG between baseline and test ECG? The following will be analysed: PR intervals, QTc, ST segments, QRS complexes, T waves.	Yes/NO	Categorical

Tachycardias and bradycardias were determined in terms of beats per minute (bpm) as >100 and <60 respectively. Categorical data are presented as frequencies and proportions. Differences between categorical data were assessed using the Chi-squared test. The ECG categorical data was formed on whether ECG changes were detected on a yes or no basis. Any abnormal changes were specifically reported descriptively. The continuous data (heart rate, blood pressure, and Zephyr™ values) was tested for normality and presented through graphical tests (bar charts), and where appropriate are presented as means and standard deviation. If not normally distributed the continuous data were presented as median and interquartile range.

Participants' heart rates were compared using a chi-squared test between the water temperatures. All analyses were performed at a 95% confidence interval and a significance level $P < 0.05$.
Blood pressure was analysed for differences in systolic and diastolic pressures between baseline and mid-test measures. Differences were calculated (if any) and were represented graphically (bar charts).

Ethics

The researcher approached the rugby players in person and provided them with an information sheet (Appendix A) and explained the purpose of the study after being given permission to do so by the Head of Wits Sport and their coaches. Participants were given an opportunity to ask questions and the researcher provided answers. To keep participants' information confidential, codes were provided for each participant on a Microsoft Excel spreadsheet. One sheet had the player's name and code, while the other had the code and data. The name and code data (master) sheet was kept in hard copy form and placed in a secure location where only the researcher knew where it is was. All participants initially completed and signed an informed consent form (Appendix B) and a medical screening form (Appendix C). All participants had baseline ECG performed and interpreted by a registered sports physician with experience in the cardiac screening of athletes. Ethical clearance was applied for and granted from the University of the Witwatersrand Human Research Ethics Committee (Medical) (clearance certificate M180268) (Appendix L).

CHAPTER FOUR: RESULTS

Anthropometric results

Participants had a mean age of 21.48 ±2.25 years and an average mass of 93.98 ±19.69kg. Additionally, their mean height and body mass index (BMI) recorded were 1.78 ±0.15m and 32.61 ±29.99kg/m², respectively (Table 5).

Table 5: Anthropometric data of the study population (n=50)

	Mean ±SD	Min	Max
Age (years)	21.48 ±2.25	18	28
Mass (kg)	93.98 ±19.69	60	185
Height (m)	1.78 ±0.15	0.88	1.97
BMI (kg/m²)	32.61 ±29.99	22.04	238.89

BMI = Body Mass Index

Blood pressure

Systolic blood pressure

Mean systolic blood pressure readings at pre-test were 124 ±7.24mmHg at 8ºC, and 125 ±8.66mm/Hg at 14°C. On entry to the cold water, systolic blood pressure significantly increased to 164 ±17.37mmHg (8°C) and 151 ±11.66mmHg (14°C). At 4min into the cold water period, SBP at 8ºC and 14ºC, decreased to 145 ±12.22 mmHg and 140 ±11.85 mmHg, respectively from the initial entry. For both water temperatures the post-test result decreased again back to similar baseline recordings 126 ±9.11 mmHg for 8ºC, and 124 ±9.45 mmHg for 14ºC.

Table 6: Systolic blood pressure changes during the testing procedure for both water temperatures

	Pre-test	CWI (on entry)	CWI (at 4 min)	Post-test	P-value
8˚C	124 ±7.24	164 ±17.37*	145 ±12.22*	126 ±9.11	< 0.00001
14˚C	125 ±8.66	151 ±11.66*	140 ±11.85*	124 ±9.45	< 0.00001

*Significantly higher compared to pre-test values (*P* < 0.05). SBP = systolic blood pressure, CWI = Cold water immersion. Values measured in mmHg.

Diastolic blood pressure

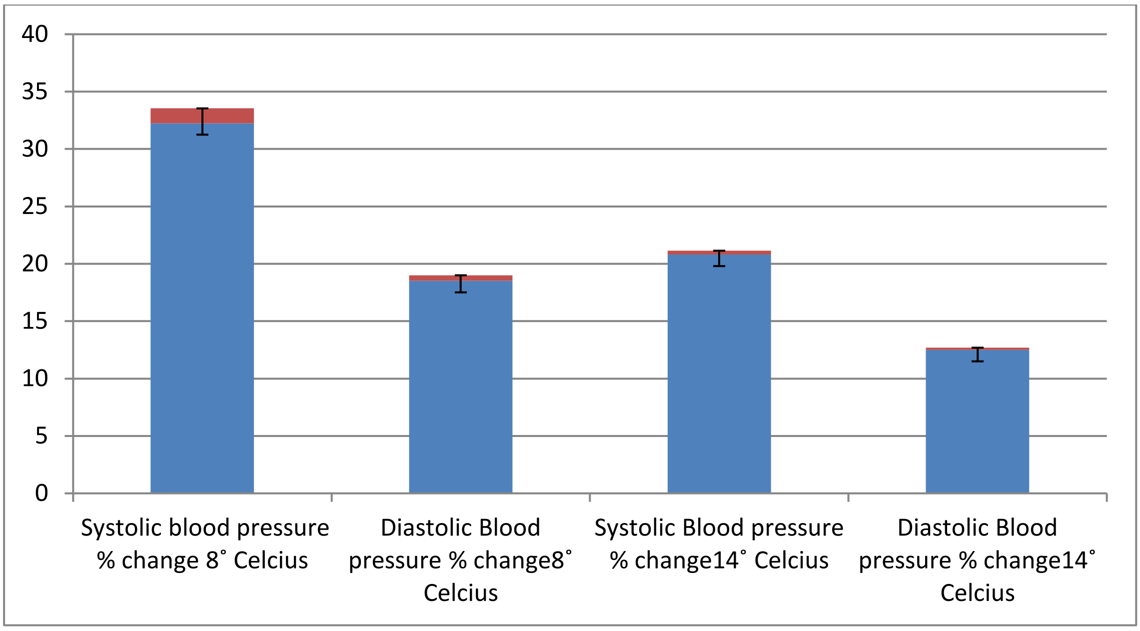
Mean blood pressure at pre-test immersion was 81 ±5.34mmHg diastolic blood pressure at 8ºC, and 80 ±5.73mmHg at 14ºC. On cold water entry, diastolic blood pressure then significantly increased to 96 ±7.94mmHg for 8ºC and 90 ±6.89mmHg for 14ºC. Four minutes into the test both water temperatures (8ºC and 14ºC) decreased compared to initial entry to 88 ±5.59 mmHg and 84 ±6.82 mmHg, respectively. For post-test measures, both temperatures returned to similar baseline measures, 82 ±5.09 mmHg for 8ºC and 81 ±6.38 mmHg for 14ºC.

Table 7: Diastolic blood pressure changes during the testing procedure for both water temperatures

	Pre-test	CWI (on entry)	CWI (at 4 min)	Post-test	P-value
8 ˚C	81 ±5.34	96 ±7.94*	88 ±5.59	82 ±5.09	< 0.00001
14 ˚C	80 ±5.73	90 ±6.89*	84 ±6.82	81 ±6.38	< 0.00001

*Significantly higher compared to pre-test values (*P* < 0.05). DBP = diastolic blood pressure. Values measured in mmHg.

Figure 3: Bar chart showing the percentage change from the pre-test to initial immersion



Bar chart showing the percentage change of both systolic and diastolic blood pressure from the pre-test to the first minute in the cold water.

The systolic pressure increased by 35% at 8ºC compared to the pre-test and diastolic blood pressure increased by 18% at 8ºC compared to the pre-test. At 14ºC systolic blood pressure increased by 21% and diastolic blood pressure increased by 13.5%.

Average resting heart rate

The average resting heart rates (pre-test) for both temperatures (8˚C and 14˚C) were 82.08 ±13.4bpm and 83.92 ±12.98bpm. However, during cold water immersion, the mean heart rate was significantly lower compared to the pre-test, with values of 70.54 ±10.5bpm and 67.1 ±8.96bpm at 8ºC and 14ºC, respectively. The post-test results for both water temperatures record heart rate still being lower than the pre-test recordings with values at 75.8 ±12.5 bpm for 8ºC and 77.16 ±11.1 bpm for 14ºC, respectively (Table 8).

Table 8: The average heart rate for both water temperatures at pre-test, CWI, and post-test

Temperature	Pre-test heart rate (bpm)	CWI test heart rate (bpm)	Post-test heart rate (bpm)	P-value
8 ˚C	82.08 ±13.4	70.54 ±10.5*	75.8 ±12.5	<0.016705
14˚C	83.92 ±12.98	67.1 ±8.96*	77.16 ±11.1	<0.00001

*Significantly lower compared to pre-test values ($P < 0.05$). RHR = resting heart rate, HR = heart rate, bpm = beats per minute.

Stress index

At 8ºC, the SI decreased significantly from 11.716 ±3.8 to 8.19 ±3.6. At 14ºC, the SI significantly decreased from 12.48 ±5.0 to 7.53 ±2.8 (Table 9).

Table 9: The stress index (SI) changes between the two different temperatures for pre-test, CWI, and post-test

Temperature	Pre-test	CWI-test	Post-test	P-value
8ºC	11.716 ±3.8	8.19 ±3.6*	10.85 ±3.90	<0.000754
14ºC	12.48 ±5.0	7.53 ±2.8*	10.11 ± 3.66	<0.00001

*Significantly lower compared to pre-test values ($P < 0.05$).

Standard deviation of normal to normal (SDNN)

At 8ºC, the SDNN significantly increased from 41.0 ±15.8 to 59.7 ±21.9. While at 14ºC, SDNN significantly increased from 40.6 ±18.4 to 62.2 ±21.4 (Table 10).

Table 10: The participants' SDNN values between the two different temperatures for pre-test, CWI, and post-test

Temperature	Pre-test	CWI-test	Post-test	P-value
8ºC	41.0 ±15.8	59.7 ±21.9*	45.06 ±19.36	<0.000027
14ºC	40.6 ±18.4	62.2 ±21.4*	46.52 ±18.16	<0.00001

*Significantly higher compared to pre-test values ($P < 0.05$).

Root mean of the successive differences (RMSSD)

At 8ºC, the RMSSD significantly increased from 29.8 ±15.58 to 61.41 ±30.84. While at 14ºC, RMSSD significantly increased from 29.20 ±17.62 to 64.44 ±30.23 (Table 11).

Table 11: The RMSSD differences between pre-test, during test, and post-test measures at both 8 and 14 degrees Celsius

Temperature	Pre-test	CWI-test	Post-test	P-value
8ºC	29.8 ±15.58	61.41 ±30.84*	37.07 ±20.3	< 0 .00001
14ºC	29.20 ±17.62	64.44 ±30.23*	37.30 ±20.5	< 0.000165

*Significantly higher compared to pre-test values ($P < 0.05$).

LF/HF ratio

The LF/HF ratio was significantly lower in the CWI test (1.95 ±1.85) compared to the pre-test (5.32 ±4.46) for the 8ºC temperature. Similarly, at 14ºC it was significantly lower in the CWI test (2.1 ±2.35) compared to the pre-test 5.63 ±4.5 (Table 12).

Table 12: The LF/HF ratio differences between pre-test, during test, and post-test measures at both 8 and 14 degrees Celsius

Temperature	Pre-test	CWI-test	Post-test	P-value
8ºC	5.32 ±4.46	1.95 ±1.85*	4.45 ±4.50	< 0.00001
14ºC	5.63 ±4.5	2.1 ±2.35*	4.55 ±4.1	<0 .00001

*Significantly lower compared to pre-test values ($P < 0.05$).

Electrocardiogram recordings

Six cardiac abnormalities were detected at pre-immersion during the testing procedure; however, they were non-pathological changes according to Drezner, Sharma, Baggish, et al. (2017) (48) and none were lethal. No abnormalities were detected during cold water immersion test at both water temperatures. (Table 9). Abnormalities recorded were arrhythmias.

Table 13: Participants' electrocardiogram results for both 8˚C and 14˚C

Temperature:	Abnormalities	Type
Pre-test	6	
8ºC	0	0
14ºC	0	0

*Significantly different to baseline measure ($P < 0.05$).

CHAPTER 5: DISCUSSION

Introduction

Cold water immersion therapy is an extensively used recovery strategy that improves recovery in athletes after intensive training sessions or matches (1,2,3). Coaches, however, are implementing this recovery strategy without knowing the full extent of its potential effects on the heart. This dissertation examined the various measures of heart rate variability, heart rate, blood pressure, and ECG tracings of rugby players during cold water immersion at two different temperatures. No ECG abnormalities were detected during CWI testing, thereby showing that cold water immersion therapy does not cause cardiac abnormalities in this sample group. Blood pressure significantly increased on entry into the cold water compared to baseline readings. The change in heart rate variability values (RMSSD, SDNN, SI, and LF/HF ratio) indicated that there was a larger parasympathetic nervous system response during CWI therapy.

Electrocardiogram recordings

In this study, no cardiac abnormalities were recorded at both water temperatures. In previous research, some athletes with underlying cardiac problems have experienced issues when exposing the face receptors to cold water (10).

There are very few recorded cardiac incidences in a controlled therapy environment (47). The majority of reported cardiac incidences have been reported during open water swimming. Open water swimmers with underlying cardiac conditions can be exposed to autonomic conflict or Long QT Syndrome. This can lead to cardiac-related conditions such as arrhythmias and even myocardial infarction (10).

Autonomic conflict refers to the activation of both sympathetic and parasympathetic systems which can compromise each other and may cause arrhythmias in the heart (11). During rapid submersion in cold water where the individual's whole body is fully immersed underwater i.e. diving, two powerful autonomic responses are activated (11). The response originates from the initial submersion of a person's head into the water. This causes the facial receptors to send out the responses to the central nervous system. These responses are cold-water shock and diving responses.

The cold shock response is driven by the cutaneous cold thermo-receptors and activates the sympathetic nervous system causing a respiratory gasp, tachycardia, hyperventilation, and peripheral constriction (11). The diving response is driven by the excitation of cardiac vagal motoneurons activating the parasympathetic nervous system. This is to conserve oxygen and causes bradycardia and the redistribution of blood flow to vital organs. These two responses can cause an autonomic conflict which can damage the heart. This type of response is specifically seen only in open-water swimming or deep-sea diving. Long QT Syndrome (LQTS) refers to an abnormal pattern seen on an ECG (10). A person with LQTS will have a much longer QT interval on an ECG, and this extended interval causes disruptions in the timing of the rest of the heart and can cause harmful dysrhythmias. In this study, participants were screened for LQTS. If LQTS was suspected or detected participants were excluded from the study eliminating the risk. Tipton and Datta (2006) researched the respiratory responses to cold water immersion and concluded that on immersion into water, participants would gasp and hold their breath. Similar to this study, it was observed that participants who were only waist-deep in cold water also gasped and held their breath involuntarily as a response to the cold water. This links to the mentioned study as similar cold-water responses could cause the same effects, causing LQTS.

Tipton and Datta (2006) found their participants' responses would last for approximately 23 seconds on average. During this stage tachycardia, hyperventilation, and blood pressure would increase (32). This was similarly noted during this research when players entered the water, they gasped, and an immediate increase in heart rate and blood pressure were observed during testing. Similarities in cold water responses can be found between Tipton and Datta (2006) research on respiratory response to cold water immersion through a rapid increase in heart rate and blood pressure.

Heart rate response to cold water immersion

In this study, heart rate during CWI therapy was significantly lower when compared to pre-test immersion for both water temperatures. The heart rate returned to normal post-test at both water temperatures.

The initial immersion into cold water rapidly increases heart rate. This is caused from the shock response to the water. A response to cold water is hyperventilation. Hyperventilation is a contributing factor to an increased heart rate initially (32). This is the sympathetic nervous system response to the cold water shock (32). This shock can be related to Tipton and Datta's (2006) study on respiratory response to cold water immersion (32). Tipton and Datta (2006) found that the heart rate increased rapidly on entry into cold water. The researcher found a correlation between an initial increase in heart rate and hyperventilation. The researcher explained that this was a sympathetic nervous system response. In another study, Cooper et al. (1976) found that heart rate increased on average by 20bpm at 27ºC water temperature on water entry (30). The researcher also found that in colder water immersion temperatures, males and females had a 49% increase in average heart rate on entry (30). Cardiac output in this study was also found to increase (30). In this dissertation, similar results were obtained, and therefore one can deduce that cardiac output would also increase.

Heart rate variability

Heart rate variability (HRV) is linked to the autonomic nervous system (38). This study looked closely at HRV values that reflect the parasympathetic and sympathetic nervous system. There were higher HRV and lower HR recorded during CWI-test when compared to the pre-test. This could be attributed to a larger parasympathetic response during cold water immersion therapy. The parasympathetic system helps control the various body functions at rest (23). It is used to slow heart rate, decrease blood pressure, and digest food (23). This larger PNS response was contrary to the researcher's initial belief that the sympathetic system would have a larger response due to the cold shock response.

In cold water immersion, the parasympathetic system (PNS) is activated (23,36). The PNS constricts the blood vessels and decelerates the heart rate (36). The PNS interprets the skin receptor signals and acts accordingly. The PNS limits blood flow to the extremities and redirects blood flow around the thoracic cavity, therefore keeping the heart and other vital organs functioning.

This study also shows that the sympathetic nervous system is first activated by the cold shock response. This is seen through the increase in blood pressure and heart rate on entry into the cold water. The sympathetic nervous system activates the fight or flight system, in which all functional activities are activated through this system (25). The initial partial immersion of the body causes a shock response which increases blood pressure and heart rate (25).

Haddad, Laursen, Chollet et al. (2010) researched the effects of cold (15ºC) or thermoneutral water immersion (34ºC) on post-exercise heart rate recovery and heart rate variability indices. The researcher's results examined RMSSD values and found that CWI therapy in seated recovery was faster than recovery with thermoneutral water seated recovery. There were RMSSD differences between water temperatures (26). The researchers concluded that colder water temperatures are likely to be more effective at increasing the parasympathetic nervous response. As the results show in the current study, significantly higher values were observed in the participants' RMSSD values at both water temperatures.

Bastos, Vanderlei, Nakamura, et al. (2012) investigated the effects of cold water immersion and active recovery on post-exercise heart rate variability. The researchers examined the SDNN value and found a significant decrease in SDNN value compared to passive recovery. In addition, the greater SDNN values were found in CWI compared to active recovery interventions. The researchers concluded that CWI therapy was not only more effective than passive recovery but also active recovery. This means that CWI therapy is a more effective option for accelerating parasympathetic reactivation (25).

This study aligns with the findings of Bastos, Vanderlei, Nakamura et al. (2012) where both studies were conducted using two water temperatures. Both studies found increased levels in RMSDD. This indicates that there is a larger parasympathetic response when someone is subjected to CWI therapy (38-39).

This parasympathetic response is a physiological response that allows the body to manage the shock of the cold water (28-33). The parasympathetic response is considered a calming strategy (28-33). As discussed, it reduces heart rate and cardiac output (28-33). It reduces circulation to the body's extremities, and thermo regulates the body temperature (28-33). It keeps the blood circulation around the vital organs of the body (28-33). This effect is the result of the PNS activation to help adjust to the cold water and to keep the vital organs functioning (35).

A study conducted by Haddad, Hintsala, Kenttä, et al. (2019) investigated the effect of daily cold water immersion during a typical training week, on parasympathetic activity and subjective ratings of well-being (46). The researchers studied eight highly-trained swimmers for two weeks (46). Subjective ratings and wellness were recorded in the mornings while recovery was recorded at the end of training sessions for five minutes. Recovery was randomised (46). Data was measured as RMMSD. The researchers found that the CWI therapy improved the parasympathetic nervous system reactivation in participants.

The results of the current study are similar to the findings of Haddad, Hintsala, Kenttä et al. (2019). The activation of the parasympathetic nervous system was seen in both studies through increased RMSSD values.

A study conducted by Huang, Chang, Kao (2011) investigated the radial pressure pulse and heart rate variability in heat-stressed and cold-stressed humans. The researchers specifically looked at two different water temperatures, 35ºC and 7ºC. Participants would submerge their hands in these two different water temperatures separately for two minutes each. The researchers analysed power spectral low-frequency (LF) and high-frequency (HF) components of HRV. The researchers found no significant difference in the LF/HF ratio (36). There were slight individual decreases in LF and HF components separately, however, the overall LF/HF ratio had no significant decrease. This is a slight contrast to this study, as a significant decrease in the LF/HF ratio was recorded. In Huang's (2011) study they only immersed the hand, which could be the reason why the studies contrast, as the current study used half body submersion procedures (36).

The current study and that of Huang, Chang, Kao (2011) have similarities in the LF/HF ratio. This LF/HF ratio and SI decreases were reported in both studies. Stress index (SI) is a measure of the sympathetic nervous system (37) and a decrease in the SI value indicates a larger parasympathetic response.

Blood pressure

When systolic and diastolic blood pressures were compared to pre-test recordings, they were significantly higher during the CWI test in the current study. These increases can also be a result of vasoconstriction of blood vessels in the body. Systolic and diastolic blood pressures were noted be to significantly higher in 8ºC and 14ºC water while the participants were in the water when compared to the pre-test. In post-tests both SBP and DBP dropped back to levels similar to the baseline recordings.

In the current study, it was noted that after four minutes in the CWI test, blood pressure significantly decreased compared to initial entry blood pressure (28,36). This is due to the participants adapting to the cold water and responding to the cold shock response (28,36). Compared to the baseline phase, blood pressure increased in the CWI phase. The lower the water temperature the higher the spike in blood pressure (44). Tipton (1989) had similar results when he examined the initial response in cold water immersion. (28). The researcher found that after two to three seconds blood pressure would have a rapid initial increase and would continue to increase for another 30 seconds (29). On average blood pressure would increase from 130/76mmHg to 175/93mmHg (28). Very similar results were observed in this study as blood pressure increased rapidly on immersion into the cold water. This is caused by the constriction of blood vessels as discussed earlier.

Limitations and noticeable issues in the study

A set schedule was put in place to test players at the same times but on different days. However, due to players varying schedules such as attending classes during training sessions, the players could not be tested at the same time. For consistency, the researchers tried to test the athletes at the same time of day, either in the morning or in the afternoon.

The water warmed while the participants were in the cold water. The researcher did notice that the participant's body heat increased the temperature of the water by approximately 1° during the cold-water immersion test. For consistency, the water should be maintained at a constant temperature during immersion. The participants' response to the cold water could change and an incorrect response may have been measured. This could skew the data, but would not change the overall observation.

The athletes' body types, for example forward and backline players, could also affect the data. Muscle tissue has a higher metabolic rate than fat, so a muscular individual will generate more heat while moving (64). In other studies, with obese patients, fat acts as an insulator for the patients and take them longer to get cold (64). This theory can be used with rugby players and the effects the cold water can have on different players based on positions (64). The forwards, typically have more fat than backline players, and therefore may react differently to the cold water (64). The forwards may have had a lower heart rate and blood pressure spike compared to the backline on initial entry into the water (64).

Strengths of the study

Only four participants were excluded from the study, which means the data collected from the remaining participants were valid and that there were no outliers. Most participants arrived on time for testing, and there were no issues with the operation of the machines used to read the ECG and to use the Zephyr™.

Recommendations

During the testing procedure, the use of an automated blood pressure device would make data more valid and reliable. It would also help to save time if only one practitioner available to conduct the testing.

The study only examined male rugby players. To further the study, research should be done on other sports such as hockey, athletics (track and field events), football, and other physically demanding sports. Male rugby players were selected because they were the main users of CWI therapy at Wits university. Females in their respective sports should also be researched because the female response to cold water may be different. Females have a higher fat percentage which could affect their HRV recordings. The same cold water immersion procedure should be followed.

The testing procedure required the researcher to record results quickly. This was due to the nature of the testing procedure, where at the time interval slots, the researchers had to note down blood pressure and heart rate. A second practitioner on hand would be beneficial to the efficient data collection at each time check-points.

Clinical applications

This research provides a new perspective into CWI therapy. A perspective of safety generally not thought of previously when using CWI therapy. Using cold water immersion at 8ºC would be more beneficial for athletes. The colder water has a greater physiological effect in terms of removal of metabolic waste, reduces cardiac output, and reactivates the parasympathetic nervous system (38). The cold shock response lasts one to two minutes, which produces a sympathetic response that is natural. Thereafter the parasympathetic system becomes the dominant system. This activation of the PNS allows for faster recovery and helps reduce muscle soreness after sessions.

Summary

From the results obtained and from analysing the data, CWI therapy is not harmful to rugby players who do not have underlying cardiac abnormalities. The parasympathetic nervous system has a larger response, as observed through the RMSSD, SI, LF/HF ratio, and SDNN, compared to the sympathetic nervous system. Therefore, the null hypothesis is accepted.

Conclusion

There are very few studies that have observed the cardiac electrical effects of the heart during cold water immersion. As detailed above, there were no abnormalities found in the ECG recordings during the cold water immersion test. This was because the participants were screened for any cardiac abnormalities before testing, only allowing those who had no cardiac issues to test and that CWI therapy does not cause cardiac abnormalities, based on extensive research shown in this study. Additionally, the parasympathetic response to cold water immersion was greater than the sympathetic response. The temperature of the cold water stimulates the vagus nerve which initiates a parasympathetic response which causes the vasoconstriction of the blood vessels in the trunk and limbs of a person, reducing heart rate and blood pressure. The researcher can therefore conclude that CWI therapy is a safe procedure for healthy university rugby players to use. Further research will be required in other sporting fields to measure the safety of their athletes to assist the validity and reliability of the findings in the study.

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Appendix A: Information Sheet

Title: The effect of cold water immersion on electrocardiogram recordings in healthy male rugby union players

Dear Player:

Good day. I, Brandon Palmer, am doing a research on the cardiac electrical effects of cold water immersion on university rugby union players using electrocardiogram (ECG) recordings. Research is just the process to learn the answer to a question. In this study, I want to learn if cold water immersion does have a cardiac electrical effect in university rugby players.

I would like to invite you to participate in this study. Below is a brief explanation on why the research is being conducted and why your participation will be greatly appreciated. Please read through the information thoroughly. If you have any questions please do not hesitate to ask for clarity.

Cold water immersion is recovery strategy used in rugby to minimize delayed set muscle soreness, reduce inflammation and restore muscle function. This strategy involves the athletes submerging the affected limbs and body into ice water for a short period of time. Cold water immersion therapy is an important part of recovery in sport today and it is therefore important to gain a better understanding on the effect that the temperature of the water may have on the certain physiological systems/organs, such as the heart. To get a better understanding of the effect that cold water immersion has on the electrical activity of an athlete's heart, an ECG device will need to be attached to you

The reason you have been invited to perform this study is because you are:

- A rugby player in the Wits Rugby Football Club.
- Aged between 18-25 years of age
- Have no known cardiac or, metabolic diseases.

How the testing procedure will be done:

Firstly you will need to sign the informed consent form as well as complete the health screening form and will then have a baseline ECG run on you. If you are cleared to participate, you will contacted at a later stage and requested to come in at a designated time so that we may perform the cold water immersion test with ECG monitoring.

You will be placed into one of two groups. These groups will be selected randomly. Group One will be tested at a water temperature of 8 degrees Celsius and they will remain in the water for 8 minutes. Group two will be tested at a water temperature of 12 degrees Celsius for 8 minutes. These different temperatures will examine if the colder the water has more of an effect on the players than the warmer water.

The ECG electrodes will be placed on your chest. You will then enter the water of your designated group for the specified time. You will only be waist deep in the water (i.e. your legs will only be in the water up to the anterior superior iliac spine (or hip level. Once you get out, the ECG will be left on until your heart rate returns to normal.

Risks:

- There are no known adverse risks to cold water immersion therapy
- Your team has undergone this type of therapy before and therefore you know what to expect from the procedure.

The testing procedure will take less than one hour to complete

You may withdraw from the study at any time if you feel any discomfort or pain or are uneasy with the apparatus being used on you.

Results will be kept safe and secure and will be private. You will remain anonymous.

Results of this study may be published and put into an academic write up, however individuals will not be identified.

If you feel that your rights have been violated in any way, you may contact Wits HREC (medical) chairperson: HREC Contact details: Mr Lebohang Moeng, lebo.moeng@wits.ac.za

Your participation in this study will be greatly appreciated and is strictly voluntary. Once you have decided to participate, please sign the informed consent form, and fill in the health questionnaire screening form. Please organise with the researcher a date to perform your baseline ECG test.

If you have any please contact me on 083 704 3622 or palmie3@gmail.com

Kind Regards

Brandon Palmer

Appendix B: Informed consent

Title: The effect of cold water immersion on electrocardiogram recordings in healthy male rugby union players

Dear player:

You are currently playing rugby union for ___________________. Research needs to be conducted to further society understanding of cold water immersion therapy. The cardiac electrical effect has never been measured before and is an important aspect to examine. The use of your information will be subject to:

- Approval from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand.
- The players information and data will be kept confidential, and at no time will a player be linked to his or her details on ECG records.
- Players will also need to fill in the health questionnaire and have a base line ECG test completed.

As soon as Ethical clearance has been given, you will be informed as to when you can come in and perform your cold water immersion therapy ECG. It should take no longer than 15min per person. If at any time you choose to withdraw consent you are free to do so and will not be prejudiced in any way.

Should you wish to contact us at any stage regarding consent, contact Brandon Palmer on 083 704 3622

I hereby request that you partake in cold water immersion therapy to further our understanding of this treatment therapy

A. Consent Given

I_____________________________________ hereby give consent for my records to be used as per the above mentioned conditions for the purposes of research:

PATIENT:______________________________DATE: ____________________

B. Consent Not Given

I ____________________________________ do not give consent for my records to be used:

PATIENT:______________________________DATE: ____________________

Appendix C

WORLD RUGBY *Putting players first*

Cardiac Screening

YOUR PERSONAL DETAILS	
Name	
Date of birth	

ID number:.. Mobile phone number:..

You have been asked to fill out this medical questionnaire on your own behalf. This questionnaire has been devised to aid in identification of young people who currently engage in competitive sport, but who could be at risk of suffering a serious cardiac event. This is not a common occurrence. The number of events can however be reduced by careful evaluation of symptoms and family history.

No screening system can guarantee 100% accuracy. If you answer "Yes" to any of these questions, you may be required to undertake further tests or referred to a Physician for further investigation. It is very important that you answer these questions honestly. You must however be aware that it is possible that as a result of this process you could potentially be disqualified from participation in your chosen sport.

In identified situations an ECG may also be mandatory as part of this assessment and the signing of this consent document confirms your agreement to undertake this ECG.

I confirm that I have read and understood the above information, and that the information I have given is accurate to the best of my knowledge.

Signed: . . . .

Dated: . . . .

WORLD RUGBY *Putting players first*

Cardiac Screening

Name	

YOUR PERSONAL HISTORY			
Have you ever experienced any of the following?		**Yes**	**No**
1.	Do you suffer from chest pain, chest heaviness or tightness during or following exercise?		
2.	Do you feel more short of breath or tire more easily during exercise when compared with your team mates?		
3.	Have you ever fainted or blacked out during or after exercise or had an unexplained fainting episode?		
4.	Have you ever experienced dizzy turns during or after exercise?		
5.	Do you have palpitations (racing heart or unexpected fast or irregular heartbeat)?		
6.	Have you ever been told you have:		
a	A heart murmur?		
b.	A heart infection?		
c.	High blood pressure?		

YOUR FAMILY HISTORY (please confirm details with relatives where possible)			
Have either of your parents, brothers or sisters suffered from:		**Yes**	**No**
7.	Heart attack or sudden unexplained death aged 50 years or less?		
8.	Heart rhythm problems requiring pacemaker or other treatment?		
9.	Angina, heart pain under the age of 50 years?		
10.	Any heart condition such as cardiomyopathy, long QT syndrome or been diagnosed with Marfan's syndrome?		

WORLD RUGBY *Putting players first*

Cardiac Screening

YOUR PERSONAL DETAILS	
Name	
Date of birth	

MINIMUM PHYSICAL EXAMINATION		
1.	Pulse rate and rhythm?	
2.	Blood pressure?	
3.	Heart murmur? *	
4.	Femoral pulse delay?	
5.	Marfanoid features? (see below)	

Marfanoid features:

a) Musculo-skeletal - arm span > height, high arched palate, cavus feet, hypermobile, kyphoscoliosis

b) Optic - myopia, lensdislocation

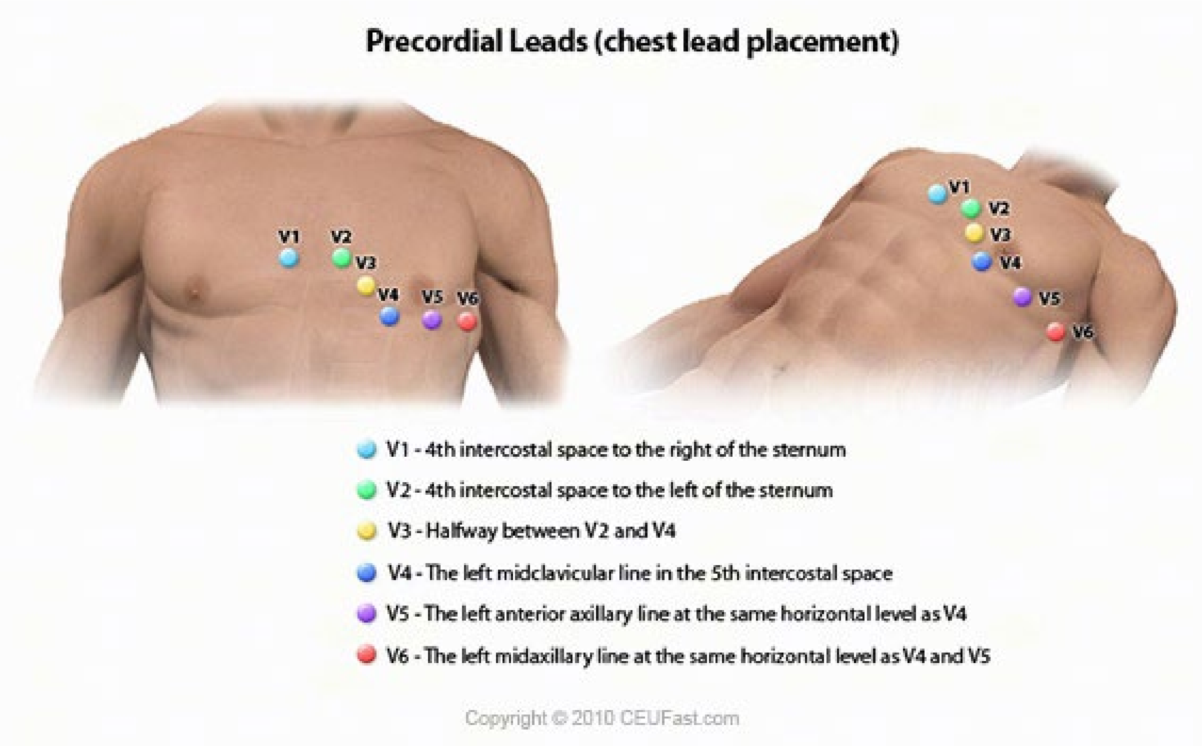
ECG INDICATED: Yes No

* Timing:.........................Location:................Character.........................Intensity....................
Squatting increases venous return to the heart, thereby increasing left ventricular blood volume, chamber size, and stroke volume.

In contrast, standing and the Valsalva manoeuvre decrease venous return, thereby decreasing left ventricular size and stroke volume. Increased stroke volume causes murmurs to become louder with squatting and quieter with standing or the Valsalva manoeuvre.

However, if a murmur is softer when the patient squats or is louder or longer when he or she returns to a standing position or during the Valsalva manoeuvre, hypertrophic cardiomyopathy or mitral valve prolapse should be suspected.

Appendix D


Precordial Leads (chest lead placement)
V1
V2
V3
V4
V5
V6
V1 - 4th intercostal space to the right of the sternum
V2 - 4th intercostal space to the left of the sternum
V3 - Halfway between V2 and V4
V4 - The left midclavicular line in the 5th intercostal space
V5 - The left anterior axillary line at the same horizontal level as V4
V6 - The left midaxillary line at the same horizontal level as V4 and V5
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Appendix E

Data capturing sheet

Player code:	
Age:	
Position	
Age Group:	
Height (cm)	
Weight (KG)	

Appendix F

Name	**Code**	**Zephyr™ number**	**Baseline test date**	**8 degree test date**	**14 degree test date**

Appendix G

Zephyr™ number	**First Name**	**Last name**	**Year born**	**Height (cm)**	**Weight (kg)**	**Test completed**

Appendix H

Name	**Code:**	**Time Zephyr™ on**	**Blood Pressure beginning**	**Heart rate beginning**	**Blood pressure on entry**	**Blood pressure 4min in test**	**Heart rate 4min in test**	**Blood pressure after test (8min)**	**Heart Rate after test (8min)**	**Time Zephyr™ off**

Appendix I

15/05/2018

To Human research ethics committee

Faculty of health sciences

Permission to collect data:

The below student is completing his MSc Sport science and has requested to test the following teams: Varsity cup squad, u/19, U/21. Collection of the data will commence at the centre of exercise science and sport medicine.

- Brandon Palmer 1246025
  - The effect of cold water immersion on electrocardiogram recordings in healthy male rugby union players

This letter serves to confirm that permission has been granted to the student to collect his data from our rugby players, that he will keep their confidentiality and be guided by his supervisors.

Yours sincerely

ADRIAN CARTER
HOD WITS SPORT

Appendix J

01 February 2018

To the Human Research Ethics committee

Permission to use data:

The below student is doing his MSc in Sport science and has requested permission to test the wits rugby players in cold water immersion therapy and use their data garnered from the testing procedures.

Brandon Palmer (1246025)

- The effect of cold water immersion on electrocardiogram recordings in healthy male rugby union players

His letter serves to confirm that permission to use of the athletes in the cold water immersion protocol has been granted, and the confidentiality of the athletes will be respected, and the student will have guidance from a supervisor.

Yours sincerely

Hugo Van As

Head coach Wits rugby

Appendix K

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

OFFICE OF THE DEPUTY REGISTRAR

29 June 2018

Brandon Palmer
Student number 1246025
MSc Candidate
Faculty of Health Sciences

TO WHOM IT MAY CONCERN

"The effect of cold water immersion on electrocardiogram recordings in healthy male rugby union players"

This letter serves to confirm that the above project has received permission to be conducted on University premises, and/or involving staff and/or students of the University as research participants. In undertaking this research, you agree to abide by all University regulations for conducting research on campus and to respect participants' rights to withdraw from participation at any time.

If you are conducting research on certain student cohorts, year groups or courses within specific Schools and within the teaching term, permission must be sought from Heads of School or individual academics.

No research can commence before ethical clearance has been obtained. Kindly forward a copy of the clearance certificate to this office.

Nicoleen Potgieter
University Deputy Registrar

Appendix L

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Mr Brandon Palmer

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M180268

NAME: (Principal Investigator)	Mr Brandon Palmer
DEPARTMENT:	Centre for Exercise and Sport Medicine
PROJECT TITLE:	The effect of cold water immersion on eletrocardiogram recordings in healthy male rugby union players
DATE CONSIDERED:	23/02/2018
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Mr Marc Booysen and Prof Demitri Constantinou
APPROVED BY:	Prof C Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	11/07/2018

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in February and will therefore be due in the month of February each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Appendix M

00300521:Brandon_Palmer_Research_dissertation_FINAL_for_TURNITIN.pdf

by Demitri Constantinou

Submission date: 20-Dec-2019 09:20AM (UTC+0200)
Submission ID: 1237369516
File name: b29_Brandon_Palmer__Research_dissertation_FINAL_for_TURNITIN.pdf (1.09M)
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Character count: 58100

00300521:Brandon_Palmer_Research_dissertation_FINAL_for_T

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