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Post-Exercise Hydrotherapy; Improving Cardiometabolic Health

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Post-Exercise Hydrotherapy; Improving Cardiometabolic Health

Brooke Maree Russell

Supervisors:

Dr Monique Francois
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This thesis is presented as part of the requirement for the conferral of the degree:
Master of Philosophy Research Proposal

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School of Medicine
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Abstract

Purpose: To determine whether the addition of warm or cold hydrotherapy to exercise training can provide similar or greater benefits for cardiometabolic health compared to time-matched exercise training. **Primary Aim:** To compare the effects of short-term exercise training coupled with warm (WWI) or cold water-immersion (CWI), and time-matched exercise alone, on improving exercise capacity and fitness outcomes. It was *hypothesised* that post-exercise WWI would improve $\dot{V}O_2max$ to a similar extent to time-matched exercise, and greater extent than CWI. **Secondary Aim:** To compare the effects of short-term exercise training with post-exercise WWI or CWI, and time-matched exercise alone, on enhancing glucose uptake and improving metabolism. It was *hypothesised* that post-exercise WWI would improve glucose regulation to a similar extent to time-matched exercise, and greater extent than CWI. **Methods:** 24 healthy participants (18-40 years) were randomised to complete two of three training interventions (12 x sessions; 4-week washout between): i) EXS - 60 min of steady state cycling at 70% of max heart rate (HR), ii) WWI – 30 min of steady state cycling at 70% max HR followed by 30 min warm water (~38-40°C) immersion, and/or iii) CWI - 30 min of steady state cycling at 70% max HR followed by 30 min cold water (~10-12°C) immersion. A pre and post Oral Glucose Tolerance Test (OGTT; blood glucose measured at 0, 15, 30, 60, 90 and 120 min) and Respiratory Exchange Ratio (RER via indirect calorimetry), determined post-prandial (PP) glucose and metabolic flexibility, defined as the slope of fasting to PP. A pre and post 20-min time trial (max distance) and $\dot{V}O_2max$ test measured cardiorespiratory fitness. **Results:** Exercise for 60 minutes increased $\dot{V}O_2max$ significantly more than when the second half of exercise was substituted with cold water immersion but not more than warm water immersion. Exercise performance, indicated by work trial distance and power, increased with all interventions. No metabolic indices were improved differentially by the three interventions, although the addition of warm water immersion to shorter duration exercise may attenuate peak postprandial glucose responses to an oral glucose challenge.

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Declaration

I, Brooke Maree Russell, declare that this thesis is original and contains no material accepted for the award of any other degree or diploma at any other university or tertiary institution. I am aware of and understand the universities policy on plagiarism and I certify that this thesis, presented for attainment of a Master of Philosophy at the University of Wollongong, is my own, excepting where explicitly stated otherwise in the text. The data presented in this thesis was obtained from research conducted by the Exercise & Cardiometabolic Lifestyle Research Group at the University of Wollongong. I played a major role in the preparation and execution of research and the data analysis and interpretation are entirely my own. Any contributions from colleagues, in collaboration, have been appropriately referenced in text.

Certification

I, Brooke Maree Russell declare that this thesis submitted in fulfilment of the requirements for the conferral of the degree Master of Philosophy from the University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. This document has not been submitted for qualifications at any other academic institution.

Brooke Maree Russell
17th October 2021

List of Names or Abbreviations

ADLs	Activities of Daily Living
AMPK	Adenosine Monophosphate-Activated Protein Kinase
BMI	Body Mass Index
CHO	Carbohydrates
CO	Cardiac Output
CWI	Cold Water Immersion
CVD	Cardiovascular Disease
GLUT-4	Glucose Transporter Type 4
$HbA1_c$	Glycosylated Haemoglobin
HIIT	High Intensity Interval Training
HR	Heart Rate
HRmax	Maximum Heart Rate
HSPs	Heat Shock Proteins
MET	Metabolic Equivalent
NO	Nitric Oxide
OGTT	Oral Glucose Tolerance Test
PGC1-α	Proliferator-Activated Receptor-Gamma Coactivator-1
PP	Postprandial
PPH	Postprandial Hyperglycemia
RER	Respiratory Exchange Ratio
RPE	Rate of Perceived Exertion
RMR	Resting Metabolic Rate
T2D	Type 2 Diabetes
$\dot{V}CO_2$	Carbon Dioxide Output
$\dot{V}O_2$	Oxygen Consumption
$\dot{V}O_2max$	Maximal Oxygen Uptake

W	Watts
WHM	Wim Hof Method
WI	Water Immersion
WWI	Warm Water Immersion

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Chapter 1

Introduction

Background

Regular physical activity or exercise, including any contractile stimulus in the skeletal muscle (i.e. walking, lifting weights) requiring energy expenditure[1], has been shown to have significant impact on physical fitness/exercise capacity[2] and health outcomes [3] in both those that are healthy[1] and those with or at risk of chronic disease[1,4].

Broadly, exercise improves physical fitness (i.e., Health). Physical fitness encompasses cardiovascular/respiratory fitness, musculoskeletal, body composition and metabolic health and has been shown to be a strong predictor of future morbidity and mortality[2]. The benefits of regular exercise (*Figure 1*) can be attributed to increases in cardiorespiratory fitness ($\dot{V}O_2max$ [maximal oxygen consumption]) and muscle mass[5], improved lipid profile and decreases in fat mass and insulin resistance.

The 'Australian Physical Activity and Sedentary Guidelines' recommend 150-300 min of moderate intensity physical activity per week [6]. Physical inactivity has been reported as a 'global pandemic'[7], is the fourth leading cause of death from chronic disease worldwide and is a significant contributor to preventable morbidity and mortality in Australian adults[6]. Even short periods of sedentary behaviours have been linked to physiological decrements (metabolic homeostasis, reduced muscle mass, increased visceral adiposity) increasing risk of chronic disease[5]. Despite ample evidence supporting the health benefits of regular exercise, alarmingly, only 15% of Australian adults meet the national recommendations. Poor health, injury and lack of time remain consistent barriers to participation in regular exercise amongst Australian Adults aged 18-64 years[8]. Globally, increased urbanisation, rapid economic development and increased screen time have been reported as significant contributors to physical inactivity[7]. Alternate strategies that assist and encourage those who are unable and/or unwilling to engage in sufficient levels of physical activity are urgently needed to address this shortfall. Considering current recommendations, 60 min of daily, moderate intensity (70% HR_{max}) exercise, has been determined as suitable in healthy people aged between 18 and 40 y[9].

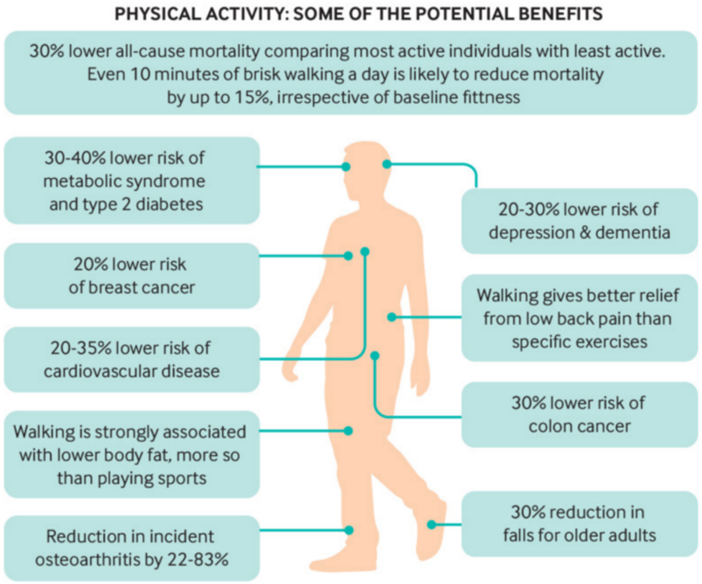

Figure 1. Health Benefits
The Health Benefits of achieving the recommended levels of physical activity[10].

Improved Cardiovascular Fitness/Capacity with Exercise

Maximal oxygen consumption ($\dot{V}O_{2}max$) is the maximum rate at which the body can efficiently use oxygen to sustain maximal exercise and is a primary indicator of both exercise capacity or physical fitness[11] and mortality[12]. Although ones $\dot{V}O_{2}max$ has a large genetic component[13], exercise training is the best way to increase ones $\dot{V}O_{2}max$. A ~3.5 mL/kg/min (one metabolic equivalent [MET]) increase in $\dot{V}O_{2}max$ equates to a 10-25% reduction in all-cause mortality[14] and for those with a low level of cardiorespiratory fitness, even smaller increases can provide large protective benefits [15,16] (Figure 2).

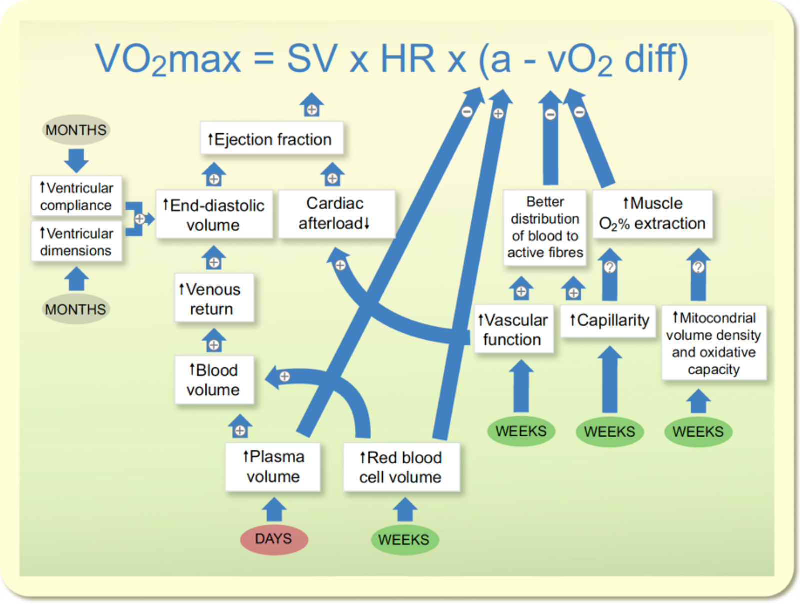

Figure 2. Improvements in $\dot{V}O_2max$
Physiological adaptations underlying improvements in maximal oxygen uptake with exercise training[12].

$\dot{V}O_2max$ can be improved with as little as two weeks of training. Gilbertson et al[17], showed that $\dot{V}O_2max$ was improved after 12 x 60 min of continuous training sessions in people with prediabetes. After comparing continuous exercise with High Intensity Interval Training (HIIT) they concluded that short-term training, independent of dose/intensity or fat loss, has significant benefit for both fitness (p= 0.09) and postprandial fuel use (p= 0.009)[18]. Likewise, De Revere et al[13], found an increase in relative $\dot{V}O_2max$ of ~9%, in healthy, normal weight, inactive women (age 19-35 y) after just nine sessions of exercise training (low-volume HIIT). Conversely, Kirwan et al[19], demonstrates that only seven days of moderate intensity continuous training does not induce significant improvements in $\dot{V}O_2max$ (p= 0.16) or weight loss (p= 0.84) in those who have Type 2 Diabetes (T2D)[19].

Exercise-Induced Uptake of Glucose for Improving Metabolic Health.

Elevated fasting blood glucose levels, impaired glucose tolerance (postprandial hyperglycaemia [PPH]) and reduced metabolic flexibility are key indicators of metabolic health and risk of developing T2D and Cardiovascular Disease (CVD)[20]. T2D is characterised by elevated fasting blood glucose levels and impaired glucose tolerance, whereby pancreatic beta cells are no longer able to produce an adequate amount of insulin and/or the response of cells to that insulin is insufficient[21]. CVD is the leading complication of T2D [22] and is largely due to elevated glucose levels leading to oxidation and inflammation of the artery walls resulting in atherosclerosis or the build-up of fatty deposits overtime[23].

Short-term exercise (independent of weight loss) improves metabolic health by two key mechanisms: glucose uptake and metabolic flexibility. The first, glucose uptake, is important in removing glucose from the blood, where it can cause irreversible damage to blood vessels and exacerbate insulin resistance, increasing risk of T2D and CVD[20]. Exercise increases contraction-mediated uptake of glucose, stimulates muscle glycogen synthesis and improves insulin sensitivity independent of insulin[24]. Muscle contractions increase the uptake of glucose via an increase in GLUT-4 translocation to the cell surface and increased blood flow to the muscle[25]. When glucose is taken up by the working muscles, it is removed from the blood, and stored or used for energy. Skeletal muscle uptake of glucose can increase up to 40-50 times the basal level during exercise[25]. This insulin-independent uptake of glucose remains heightened for several hours post exercise[24] helping to reduce insulin resistance, improving insulin sensitivity and reducing impact of acute and chronic hyperglycemia[19].

In addition to increased glucose uptake, exercise has been shown to improve metabolic flexibility; the ability to switch between fuel sources (carbohydrates [CHO] and fats) in response to changes in substrate utilisation and availability[26] (measured by Respiratory Exchange Ratio [RER]). For example, after having a high sugar beverage, someone who is metabolically flexible will quickly switch from predominately fat oxidation to CHO oxidation; the now readily available fuel source, and then quickly return to using fats; the more energy dense and preferred source of fuel. Gilbertson et al [17], found short-term exercise improved glucose tolerance and increased metabolic flexibility (measured via a 75g Oral Glucose Tolerance Test [OGTT] during which expired gasses were collected to measure RER [via indirect calorimetry]) in subjects with prediabetes (n=31). They compared 12 sessions of work-matched continuous and interval training and found comparable clinically relevant improvements in glucose tolerance and elevations in metabolic rate. Metabolic inflexibility is a key mechanism principal to impaired insulin-stimulated muscle glucose uptake and has deleterious effects on postprandial glucose[17].

Benefits of Water Immersion (Hydrotherapy) on Cardiometabolic Health

Water immersion (WI) has long been used by populations for its benefits for health and performance. Immersion often consists of either just cold water (5-16°C) or warm water (37 to 46°C), or alternating repetitive bouts of both, known as contrast therapy (i.e. from warm to cold and/or cold to warm water)[27]. Immersion in these temperatures may involve the use of spas, baths, showers, plunge pools or whirlpools for periods ranging from 5-25 min[27]. It is common for individuals to be immersed up to their waist or shoulders during water immersion, depending on the musculature that was exercised. The temperatures often used for warm water immersion (WWI) are very similar to that typically used within a spa or hot tub (40°C).

Warm Water Immersion as a Tool for Improving Cardiometabolic Health

WWI is currently being investigated by many researchers for its health and performance benefits. In contrast to CWI, WWI (37-46°C) increases limb blood flow, and muscle heating activates regulators (specifically AMPK and PCG1-α)[28] of mitochondrial biogenesis (important for cell metabolism and regulation of ROS)[29] and glucose sensitivity, thereby offers a potential alternate or adjunct to exercise training[30]. WWI alone has been found to improve fasting glucose, glucose uptake , insulin concentrations [31], vascular function and structure [32,33], and $\dot{V}O_2max$, comparably to exercise training [34].

Two studies have shown improved cardiovascular health with WWI. One study to date has measured $\dot{V}O_2max$. Bailey et al. [34], observed similar increases in cardiorespiratory fitness and cerebrovascular function (cerebral blood flow) following eight weeks of half hourly WWI sessions (immersed to top sternal level in 42°C, 3/wk) when compared to time-matched exercise (cycle ergometer, ~70% HR_{max}). Similarly, Brunt et al[32] investigated 23 subjects over an eight wk intervention (36 sessions). Subjects in the 'heat therapy' were immersed up to their shoulders in water at 40.5°C until rectal temperature reached 38.5°C at which time they remained in waist-level water to maintain a core body temp of 38.5-39.0°C for 60 min. Brunt et al[32] showed that when compared to a thermoneutral water immersion intervention (30min at 36°C shoulder height followed by 60 min at waist level), heat therapy increased vascular function (pulse wave velocity, arterial stiffness and endothelial function) and was a viable treatment for those with reduced exercise tolerance or capacity[32]. Collectively, these studies indicate that WWI might be used as a viable substitute in achieving at least some of the benefits of exercise training (e.g., reduced risk of diabetes), improved heart health, improved cardiovascular fitness particularly for those who cannot or will not exercise sufficiently.

WWI also improves glycaemic control. Hoekstra et al[31] examined the impact of warm water immersion on insulin sensitivity and insulin resistance. Ten overweight (BMI>27kg/m^2) and sedentary men (<2h exercise/wk) participated in a two wk intervention (10 sessions) whereby they were immersed in 39°C water up to their neck for 1h. Hoekstra et al[31] showed that the chronic effects of their intervention included reduced fasting blood glucose and reduced fasting plasma insulin levels. Further to this, Hoekstra et al[35] found that the lowering effect of fasting blood glucose following the intervention period remained at one wk post. In addition, just ten WWI sessions in sedentary, overweight adults or three weeks of WWI in T2D patients was found to increase nitric oxide availability (a key signaling molecule promoting an increase in

GLUT-4 metabolism, amongst other functions) and reduce fasting insulin and glucose concentrations[36], highlighting the potential for WWI to improve cardiometabolic health.

Recently, research has shown that WWI alone improves cardiovascular and metabolic health to a similar extent to exercise training in healthy adults[31,32,37] however no research to date examines the short-term effects of post-exercise WWI on health related indices compared to exercise training alone. Given that exercise also offers specific benefits for increased glucose uptake and carbohydrate utilisation[38], the effects of post-exercise WWI on glucose tolerance after short-term training requires further investigation as a combination of both exercise and WWI may prove superior.

Cold Water Immersion as a Tool for Improving Cardiometabolic Health

Short-duration immersion in cold water is proposed in media and popular press (i.e., cryotherapy) to have profound effects on cardiometabolic health. It has been suggested that cold exposure may increase metabolic rate (non-shivering thermogenesis in brown adipose tissue)[39,40], heart rate (increased catecholamine production and peripheral vasoconstriction) and blood pressure (increased vasoconstriction)[39] and insulin sensitivity (increase in GLUT4 concentration)[41]. Research regarding the benefit of CWI alone, on health, is limited. The effects of cold-water immersion accompanied by controlled breathing (meditation) on health benefits, known as the Wim Hof Method (WHM), has been investigated and suggests augmented inflammatory responses[42,43] and enhanced metabolism[44]. Immersion techniques, however, vary significantly with Wim Hof research and largely focus on immersion in conjunction with WHM breathing, it is therefore not known if CWI alone elicits the same physiological responses. Given the known added benefit of CWI accompanied by the WHM on health (increased brown adipose tissue, fat loss, reduced inflammation) along with current research on the benefit of *'post exercise'* CWI on sports performance, or more specifically, recovery from exercise, it is pertinent to investigate whether post-exercise CWI could also have additive benefit to health-related parameters.

Combined Exercise and Water Immersion on Cardiometabolic Health

Post-exercise CWI has long been used by athletes to improve recovery and to enhance exercise capacity. CWI (5-16°C) has been shown to stimulate metabolic regulators such as mitochondrial biogenesis. For example, Ihsan et al. [45,46] found an increase in skeletal muscle PGC1-α concentration (mechanism for improving $\dot{V}O_2max$ and regulating energy metabolism, *see figure 2.)*, an important regulator of mitochondrial biogenesis and oxidative metabolism, when CWI (10°C for 15 min) occurred post-treadmill running. Conversely, Prior work by Broatch et al., [47] showed post-exercise CWI did not improve $\dot{V}O_2max$ or maximal uncoupled respiration (complexes I and II), compared to six-weeks of exercise training, supporting the work of Yamane et al. [48,49] in showing blunted vascular, endurance and strength effects of CWI. Future research on CWI is needed to determine the mechanisms by which CWI may blunt exercise adaptations (e.g., blood flow, inflammation and cellular metabolism) [50–52]. Literature is inconsistent, with some studies finding improvements in mitochondrial function and endurance performance (reviewed in 47), while others observe no additional benefit with post-exercise CWI [53]. Whilst the inconsistent findings may be due to various factors, including differences in methodologies (i.e. immersion depth, temperature and duration) and participants' body composition, it is plausible that the reduction in limb blood flow (leading to increased vascular dysfunction [reduced shear stress/vasodilation] and a reduction in mitochondrial biogenesis and oxygen delivery to working muscles) with CWI, in part, contributes to impairments in vascular function, metabolic flexibility and performance [49].

Further to this, previous research suggests that 15 min of water immersion is an inadequate duration to elicit a shivering response or lower core body temperature enough to increase PGC1-α mRNA and upregulate cellular energy metabolism, promote mitochondrial biogenesis and allow for changes in mitochondrial function and structure[16]

[47]. Importantly, reduced mitochondrial function has significant cardiovascular consequences including increased ROS, associated endothelial dysfunction, development of atherosclerosis and an increased risk of CVD[54]. Likewise, impaired mitochondrial function has lasting impact on metabolic health with links to reduced insulin sensitivity and increase insulin resistance typical of those with T2D[29,55].

Research on the benefits of post-exercise WWI for health is in its infancy. The combination of passive heat and exercise (rather than complete substitution) may result in additive increases in both metabolic enzyme and protein adaptations in the skeletal muscle, based on prior research in mice [56]. Together, the key elements of heat, coupled with the mechanical tension (tension on muscle provided by exercise load), substrate turnover (use of fuel for energy) and transient oxidative stress (imbalance between production and accumulation of oxygen reaction species) experienced during exercise, drive the wide-ranging and unparalleled health benefits of exercise. Therefore, the combination of WWI and exercise, for those who cannot perform sufficient volumes of exercise, may be more beneficial than WWI alone in achieving positive health outcomes associated with regular exercise (i.e., also including those for bone and muscle). Further, substituting the second half of exercise with WWI may make the recommended amount of exercise more achievable for those who are disinclined or less able.

The potential of post-exercise WWI or CWI to improve health and performance is of significant interest to athletes as well as the general population; particularly if incorporating post-exercise warm or cold hydrotherapy can shorten exercise duration and workload. Currently, no study has comprehensively examined the key cardiovascular and metabolic measures associated with the short-term training effects of both CWI and WWI immersion after exercise. It was hypothesised that post-exercise CWI would augment and further increase metabolic adaptations[57]. Short-term WWI may potentially further enhance and maximise cardiovascular strain and ultimately cardiovascular adaptations. Comparing both the short-term effects of post-exercise CWI and WWI to exercise alone allowed us to establish whether CWI or WWI were potential therapeutic additions to exercise training to enhance adaptations (especially in those individuals that are disinclined or unable to exercise for at least the minimal recommendation of 30 minutes). Therefore, the current study was a proof-of-concept trial that, over a two-wk duration (minimising potential confounding effect of changes in body composition), comprehensively examined the short-term cardiovascular and metabolic health benefits of both post-exercise CWI and WWI and to determine whether CWI or WWI was a practical solution to augment the benefits of exercise.

This is the first study examining the impact of cold-water immersion (CWI), warm water immersion (WWI) and time-matched exercise on cardiometabolic health outcomes. Given the often-reduced capacity of those with chronic disease it is thought that reducing exercise duration could enhance participation and therefore health outcomes for these populations.

Aims and Hypotheses

Overall Objective: To determine whether post-exercise water immersion as a substitute for time-matched exercise improves cardiometabolic health.

Primary Aim: To compare the effects of short-term exercise training coupled with warm or cold water-immersion, and time-matched exercise alone, on improving exercise capacity and fitness outcomes. It was *hypothesised* that post-exercise WWI would improve $\dot{V}O_2max$ to a similar extent to time-matched exercise, and greater extent than CWI. It was thought that post-exercise WWI and exercise alone would elicit a greater cardiovascular stimulus than post-exercise CWI, due to an increased blood flow and volume and therefore enhanced oxygen delivery; a key determinant of $\dot{V}O_2max$.

Secondary Aim: To compare the effects of short-term exercise training with post-exercise

WWI or CWI, and time-matched exercise alone, on enhancing glucose uptake and improving metabolism. It was *hypothesised* that post-exercise WWI would improve glucose regulation to a similar extent to time-matched exercise, and greater extent than CWI. Likewise, metabolic flexibility would improve, with post-exercise CWI improving metabolism (fat oxidation) similar to exercise alone, but more than post-exercise WWI. Based on current literature and known mechanisms it was hypothesised that post-exercise water immersion could augment and further increase metabolic adaptations[57].

Chapter 2

Manuscript

Post-exercise Warm or Cold-Water Immersion to Augment the Cardiometabolic Benefits of Exercise Training: A Proof-of-Concept Trial

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Abbreviated Title

Post-Exercise Water Immersion Vs Time-Matched Exercise

Author Roles

MF, TH and JC designed the study. BR, CC, TH and MF conducted the research. BR, CC & MF analysed the data. BR composed the manuscript. All authors edited the manuscript and approved the final draft.

Key words: Exercise, Metabolic Flexibility, Glucose, Hydrotherapy, Water Immersion

Abstract

We investigated whether substituting the final half within 60-min bouts of exercise with passive warm or cold-water immersion would provide similar or greater benefits for cardiometabolic health. Thirty healthy participants were randomised to two of three short-term training interventions in a partial crossover (12 sessions over 14-16 d, four wk washout): i) EXS: 60 min cycling 70% maximum heart rate (HRmax), ii) WWI: 30 min cycling then 30 min warm water (38-40°C) immersion, and/or iii) CWI: 30 min cycling then 30 min cold water (10-12°C) immersion. Before and after, participants completed a 20 min cycle work trial, $\dot{V}O_2max$ test, and an Oral Glucose Tolerance Test during which indirect calorimetry was used to measure substrate oxidation and metabolic flexibility (slope of fasting to postprandial glucose oxidation). Data from twenty-two participants (25±5 y, BMI 23±3 kg/m^2, Female=11) were analysed using a fixed-effects linear mixed model. $\dot{V}O_2max$ increased more in EXS (interaction p=0.004) than CWI (95% CI: 1.1, 5.3 mL/kg/min, Cohen's d= 1.35), but not WWI (CI: -0.4, 3.9 mL/kg/min, d= 0.72). Work trial distance and power increased 383 ± 223 m and 20 ± 6W, respectively, without differences between interventions (interaction both p>0.68). WWI lowered postprandial glucose ~9% (CI -1.9, -0.5 mmol/L; d= 0.63), with no difference between interventions (interaction p= 0.469). Substituting the second half of exercise with WWI provides similar cardiometabolic health benefits to time matched exercise, however substituting with CWI does not.

Introduction

Physical inactivity has been reported as the fourth leading cause of death from chronic disease worldwide [59] and is a significant contributor to preventable morbidity and mortality in Australian adults [6]. Despite ample evidence supporting the health benefits of physical activity, alarmingly, only 15% of Australian adults meet the 'Australian Physical Activity and Sedentary Guidelines' of 150-300 min of moderate intensity physical activity per week [6,9]. Alternate strategies that assist and encourage those who are unable and/or unwilling to engage in sufficient levels of physical activity are urgently needed.

Post-exercise water immersion has long been used by athletes in an attempt to improve recovery and to enhance exercise capacity [27,53,60–62]. Cold water immersion (CWI: 5-16°C) has been shown to stimulate metabolic regulators such as mitochondrial biogenesis [53]. For example, Ihsan et al. [45,46] found an increase in skeletal muscle PGC1-α mRNA concentration, an important regulator of mitochondrial biogenesis and oxidative metabolism, when CWI (10°C for 15 min) occurred post-treadmill running. Conversely, Prior work by Broatch et al., [47] showed post-exercise CWI did not improve $\dot{V}O_2$ max or maximal uncoupled respiration (complexes I and II), compared to six-weeks of exercise training. Literature is inconsistent, with some studies finding improvements with CWI (reviewed in 5), while others observe no additional benefit in mitochondrial function and endurance performance with post-exercise CWI [53,63]. Whilst the inconsistent findings may be due to various factors, including differences in methodologies and participants' body composition, it is plausible that the reduction in limb blood flow with CWI, in part, contributes to impairments in vascular function, metabolic flexibility and performance [48,49,64].

In contrast to CWI, warm water immersion (WWI: 37-46°C) increases limb blood flow, and muscle heating activates regulators of mitochondrial biogenesis and glucose sensitivity, thereby offers a potential alternate or adjunct to exercise training and is currently being investigated by many researchers for its health and performance benefits. WWI alone has been found to improve fasting glucose, glucose uptake [65,66], insulin concentrations [31], vascular function and structure [32,33], and $\dot{V}O_2max$, comparably to exercise training [34]. The only study to date, Bailey et al. [34], observed similar increases in cardiorespiratory fitness and cerebrovascular function following eight weeks of half hourly WWI sessions when compared to time-matched exercise. In addition, just ten WWI sessions in sedentary, overweight adults or three weeks of WWI in type 2 diabetes patients was found to increase nitric oxide availability and reduce fasting insulin and glucose concentrations[32]. Collectively, these studies indicate that WWI might be used as a viable substitute in achieving at least some of the benefits of exercise training (e.g., reduced risk of diabetes), improved heart health, improved cardiovascular fitness) particularly for those who cannot or will not exercise sufficiently. Furthermore, prior research has shown that the combination of passive heat and exercise (rather than complete substitution) results in additive increases in both metabolic enzyme and protein (*i.e. heat shock proteins:* HSP60 & HSP27) adaptations in the skeletal muscle of mice [56]. Together, the key elements of heat, coupled with the mechanical tension, substrate turnover and transient oxidative stress experienced during exercise, drive the wide-ranging and unparalleled health benefits of exercise. Therefore, the combination of WWI and exercise, for those who cannot perform sufficient volumes of exercise (i.e, people with diabetes or CVD complications), may be more beneficial than WWI alone in achieving positive health outcomes associated with regular exercise (i.e., also including those for bone and muscle). Further, substituting the second half of exercise with WWI may allow those who are disinclined or less able to meet exercise recommendations achieve comparable health benefits. The present study investigates the efficacy of post-exercise warm or cold WI in an apparently health population before aiming to apply this approach to clinical populations.

To date, no research has examined the chronic cardiometabolic health effects of replacing half the exercise duration with warm or cold-water immersion as an alternate strategy to time-matched exercise. Based on previous research [34], we hypothesised that post-exercise WWI would improve $\dot{V}O_2max$ and glucose regulation to a similar extent to time-matched exercise, and greater extent than CWI would.

Research Design and Methods

Experimental Design

A partial-crossover trial was used, employing a balanced, incomplete block design whereby participants were randomised to complete two of three training interventions (12 training sessions over 14-16 d, with four wk washout between, Figure 3): i) EXS - 60 min of steady state cycling at 70% of maximum heart rate (HRmax) ii) WWI - 30 min of steady state cycling at 70% HRmax followed by 30-min warm water (~39°C) immersion of the lower limbs, up to umbilicus, and/or iii) CWI - 30 min of steady state cycling at 70% HRmax followed by 30 min cold water (~11°C) immersion of the lower limbs, up to the umbilicus. Prior to session one, all participants completed a non-blinded familiarisation cycling work trial to familiarise them to the ergometer (Wattbike) and the 20-min duration. To measure changes in performance, each participant completed a blinded 20-min cycling work trial during the first and last training sessions (instead of continuous cycling). The work trial protocol is outlined below. For each intervention, post assessments were completed 24 h (OGTT) and 48 h ($\dot{V}O_2 max$) following the final training session.

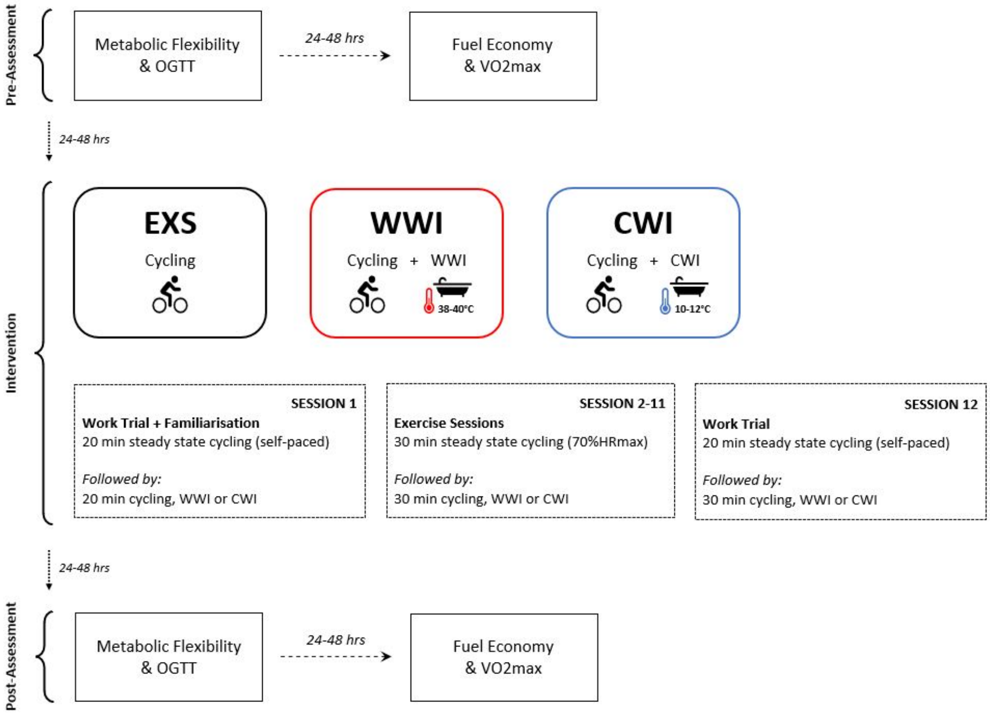

Figure 3. Experimental Design
Pre and post assessments (OGTT & $\dot{V}O_2max$*) were performed with 24-48 h between each and prior to session one, and after session 12. Sessions 2-11 included the full protocols (EXS [60-min moderate intensity cycling], CWI [30-min cold WI + 30-min moderate intensity cycling] or WWI [30-min warm WI + 30-min moderate intensity cycling]). A 20-min work trial was completed in session one and session 12 (50-min) followed by 40-min EXS, 20-min CWI or WWI intervention. Participants completed two out of three interventions with a four-week washout separating each intervention.*

Participants

Thirty healthy adults were recruited to complete two of three interventions (Figure 4). Twenty-two (age=25 ± 5 y, BMI 23 ± 3 kg/m^2, 11 females) completed both interventions (EXS: n=17, CWI: n=15, WWI: n=16) and were included in analyses. The eight participants who did not complete both interventions stated time commitment, relocation, intolerance to exercise/cold, surgery or traveling as reasons for dropping out. Participants were recreationally active (participating in physical activity on ~three days per week but not in any formal exercise training program). Prospective participants were excluded if they had any previously diagnosed health conditions, smoked (in previous three years), were pregnant or had any injury preventing participation. Written informed consent was provided by all participants who were screened for contraindications to exercise using 'The Physical Activity Readiness Questionnaire' at an initial consultation. Research protocols were approved by the UOW Human Research Ethics Committee (Ethics Number: 2018/322. Approval Date: 07/08/2018).

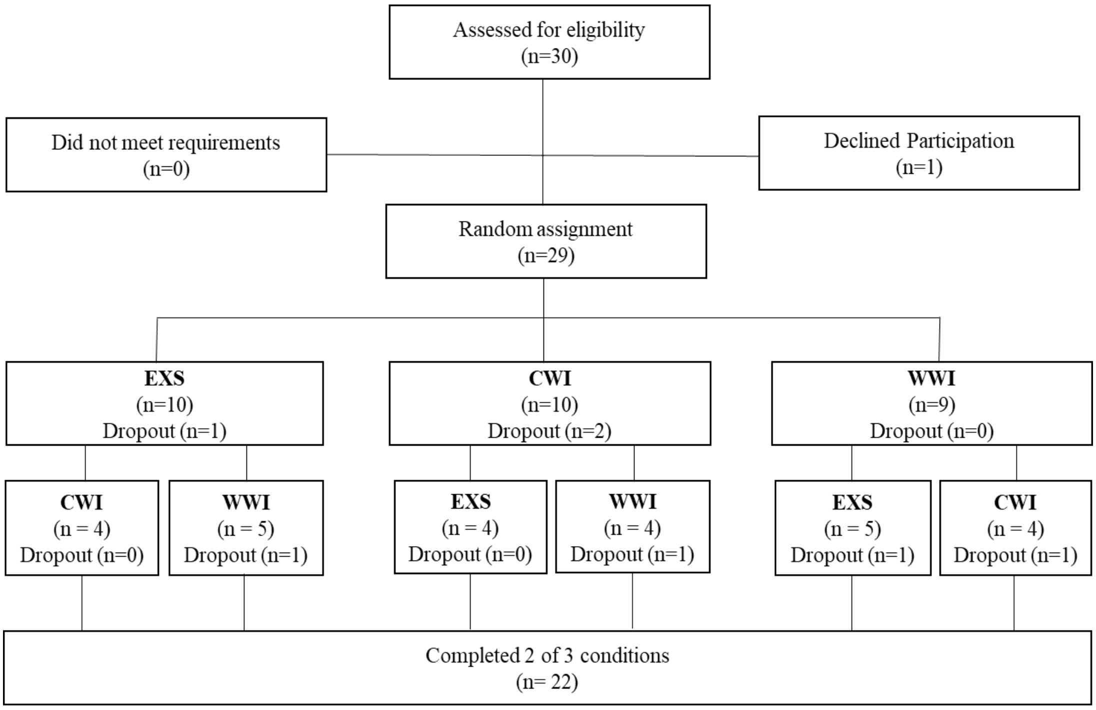

Figure 4. Study Flow Diagram
Partial randomised crossover trial where participants completed two of three training interventions (each 12 training sessions); 60 min of continuous exercise or 30 min of exercise followed by either 30 min of warm water (~38-40℃) or cold water (~10-12℃) immersion to the umbilicus.

Training Protocol

For each intervention, participants completed 12 sessions over 14-16 d, with a four-wk washout between (Figure 1). The two-wk duration was chosen to investigate the effects of short-term training whilst minimising any potential confounding effect of changes in body composition. Several studies have shown benefits in cardiometabolic health after a similar exercise intervention period [36,67]. All twelve training sessions were supervised by an Accredited Exercise Physiologist. Blood pressure was taken before and after each training session, with heart rate measured via telemetry (Polar Electro, Kempele, Finland) and

Rating of Perceived Exertion (RPE) recorded at five-minute intervals throughout each training session.

The first session of the EXS intervention involved 50 min of cycling only, with the first session of WI including 30 minutes of cycling followed by 20 min of immersion in either cold or warm water. Thereafter, each exercise training session involved continuous cycling at an intensity of 70% of HRmax (assessed via initial $\dot{V}O_2max$ test) for either 30 min (CWI and WWI) or 60 min (EXS) with WI interventions including 30 min of immersion so that all protocols were 60 min in total. All exercise training and water immersion interventions occurred in a room with an ambient 23°C temperature. Participants were able to drink water ad libitum during all interventions.

Water Immersion Interventions

The CWI and WWI interventions involved 30 min cycling at 70% HRmax followed by water immersion for 30 min. Participants sat with their legs fully extended, immersed in water up to their umbilicus between 10-12°C (CWI) or 38-40°C (WWI), i.e., temperatures routinely used in water immersion therapy for healthy and clinical populations [27]. Water temperature was monitored (via a calibrated mercury thermometer) before and throughout immersion to ensure correct temperature was maintained. Warm water/ice was added to the bath and an external tap allowed removal of volume when necessary to maintaining correct immersion temperature and depth. Core temperature (auditory canal, using a Braun ThermoScan), thermal sensation and discomfort scales [68] were measured every five minutes during immersion.

Pre and Post Assessments

$\dot{V}O_2max$

Participants fasted for three hours prior to completing two stages of submaximal cycling, at a prescribed absolute and relative intensity, to assess metabolic flexibility [the ability to adapt substrate utilisation to substrate availability [69]], immediately followed by a ramp cycle test to volitional exhaustion for the measurement of $\dot{V}O_2max$. Stage one involved 10 min cycling at 50 W for females and 70 W for males (absolute: elicits a low-intensity response of approximately 30-40% of HRmax). In stage two, participants exercised for 10 min at 70% of predicted HRmax (220-age). Indirect Calorimetry (Gas Exchange via Parvo Medic's TrueOne® 2400) was used to measure participants' energy use (oxygen uptake [$\dot{V}O_2$], expired carbon dioxide [$\dot{V}CO_2$]), and substrate utilisation (carbohydrate/fat oxidation) [70]. During the ramp stage, the load was increased by 35 W at 1-min intervals until volitional exhaustion. During the last 30 s of each stage, heart rate, via telemetry and RPE [Borg 6-20 scale [71]]) were recorded. HRmax was recorded at the completion of the ramp stage.

Work Trial

Participants completed a self-paced 20-min work trial on an air-braked ergometer (Wattbike, Nottingham, UK). Participants were encouraged to complete the maximum distance possible, with only their cadence and time used as feedback during the test. Heart rate, distance (km) and power (watts) were recorded at five-minute intervals. A minimum of 48 h separated the $\dot{V}O_2max$ and work trial sessions and the exercise training interventions.

Resting Metabolic Rate (RMR) and Oral Glucose Tolerance Test (OGTT)

Participants fasted for 10 h before test initiation. RMR was measured using a Parvo Metabolic Cart, with a canopy hood to measure rates of $\dot{V}CO_2$, $\dot{V}O_2$ and RER. Participants were instructed to minimise activity prior to arrival at the laboratory, they rested for 10 minutes prior to test initiation with the final 10 min of a 20-min data collection (in the supine position) was used for analysis (~20 minutes of rest). The same protocol was used for all comparison time points. Fasting capillary glucose concentration was then measured using a standard lancing device and a HemoCue glucose analyser (HemoCue Glucose 201 RT glucose; HemoCue AB, Sweden). A standard 75 g glucose tolerance drink was consumed, then RMR was measured for a further 20 min at 30- and 60-min post consumption. Energy use ($\dot{V}O_2$) and fuel utilisation (CHO and fat oxidation, assuming minimal protein contribution) [70] were calculated at each time point. Capillary glucose concentration was measured 30, 60, 90 & 120 min postprandially (PP).

Data Analysis

The study was powered on effect size for our main outcome the change in $\dot{V}O_2$max [34]. Power (80%) was calculated to analyse 24 participants, and accounting for a potential dropout rate of 20% we recruited 30 participants. Data were first assessed for normality using Q-Q plots, histograms, and the Shapiro-Wilk test. After outliers were removed (all data points for that measurement) using the default value of ±3 standard deviations in SPSS, all assumption of normality conditions were met. A linear mixed model (with time x intervention interaction, and main effects of time) was used to compare outcomes between interventions. To determine differences between interventions, post hoc tukey tests were performed following a significant interaction. Significance was set at $P<0.05$. A statistician from UOW was consulted in the study design, performed the blocked randomisation and assisted with statistical analyses. 95% CI were calculated using SPSS, and Cohen's *d* were calculated for effect size (Cohen's d 0.2-0.5 [small]; 0.5-0.8 [moderate]; 0.8+ [large]).

Results

All participants completed the 12 sessions in 14-16 d, with no more than two consecutive days between training sessions. HR profiles for the three interventions are shown in Figure 5. HR was not different between interventions for the first 30 min of cycling but was significantly higher for the total 60 min during EXS (143 ± 19 bpm) compared to CWI (114 ± 30 bpm) and WWI (121 ± 27 bpm) (interaction $p<0.001$). HR was significantly higher during WWI than CWI (p= 0.002). RPE did not differ between interventions (EXS: 11 ± 1, CWI: 11 ± 1 & WWI: 11 ± 1, interaction p= 0.986). On average auditory canal temperature was not different during the 30 min of WI between WWI (37.4 ± 1.3°C) and CWI (37.5 ± 1.3°C, interaction p= 0.931). As expected, thermal sensation differed between CWI ('cold'; 4.0 ± 0.3) and WWI ('slightly warm'; 8.0 ± 0.3) [interaction p < 0.001].

Thermal discomfort was greater with CWI ('slightly uncomfortable'; 2.0 ± 0.2) compared to WWI ('comfortable'; 1.0 ± 0.1) (interaction p=0.052).

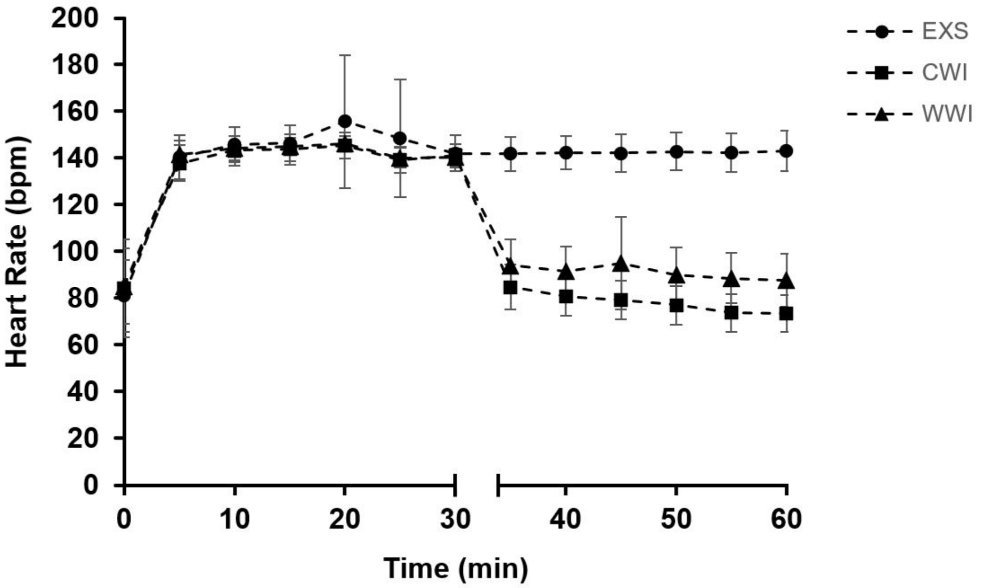

Figure 5. Change in HR
Mean heart rate (HR [bpm]) during time-matched exercise (EXS [n=16]), post- exercise Cold Water Immersion (CWI [n= 14]) and post-exercise Warm Water Immersion (WWI [n= 14]). After 30 min of cycling, CWI and WWI began 30 mins of water immersion (indicated by gap in x axis). HR was not significantly different between interventions from 0-30 mins but was significantly higher with EXS than WI (p<0.001) from 0-60 mins. HR was significantly higher during the 30 mins of WWI when compared to CWI (p= 0.002)

$\dot{V}O_2max$

The change in $\dot{V}O_2max$ differed between interventions (interaction p=0.004, Figure 6; EXS [Pre: 39.9 ± 7.4 mL/kg/min to Post: 43.6 ± 6.4 mL/kg/min, p= 0.001], CWI [Pre: 41.8 ± 7.7 mL/kg/min to Post: 41.3 ± 8.1 mL/kg/min, p= 0.495], WWI [Pre: 40.0 ± 8.9 mL/kg/min to Post: 41.3 ± 9.0 mL/kg/min, p= 0.103]). EXS significantly increased $\dot{V}O_2max$ [large effect] by 3.2 mL/kg/min more than CWI (CI: 1.1, 5.3 mL/kg/min, *d*= 1.36), but not significantly more than WWI (CI: -0.4, 3.9 mL/kg/min, *d*= 0.73).

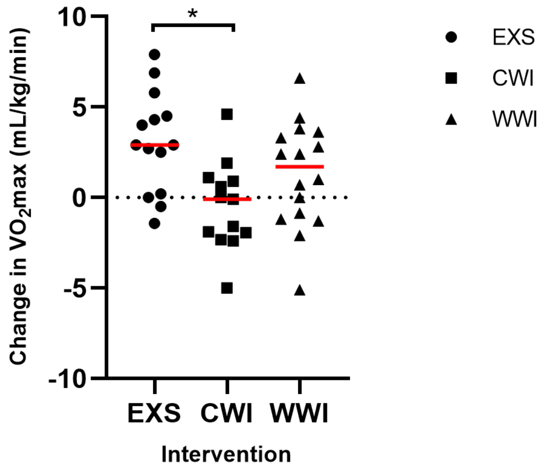

Figure 6. Change in $\dot{V}O_2max$
*Change in cardiorespiratory fitness ($\dot{V}O_2max$ [ml/kg/min]) following 12 sessions of Exercise (EXS [n=14]), post-exercise Cold Water Immersion (CWI [n= 14]) and post-exercise Warm Water Immersion (WWI [n= 16]). *indicates significant difference between EXS and CWI (post hoc, p = 0.002), following significant interaction (p=0.004).*

Work Trial

Distance (time effect p= 0.048) and power (time effect p= 0.008) increased for the 20-min work trial, by an average 380 m and 20 W, respectively, with no difference between interventions (interactions Distance [p= 0.692], Power [p= 0.812], Table 1).

Table 1. Work Trial
*Total distance travelled, average Heart Rate (HR) and Power during the 20-min cycle ergometer work trial. Values are means ± SD. Data are mean of pre and post intervention work trial for each participant (Exercise [EXS n=15], Cold Water Immersion [CWI n= 14], Warm Water Immersion [WWI, n=14]). No significant difference between interventions. *indicates significant main effect of time.*

	Distance (km)			HR (bpm)			Power (watts)		
	Pre	Post	Change	Pre	Post	Change	Pre	Post	Change
EXS	11.4 ± 0.93	12.0 ± 0.97	0.58*± 0.64	168± 23	176± 11	8 ± 18	160± 41	187± 34	27* ± 22
CWI	11.2 ± 0.65	11.6 ± 0.66	0.43*± 0.48	166± 20	172± 13	6 ± 10	152± 24	171± 28	19* ± 20
WWI	11.4 ± 0.96	11.5 ± 1.1	0.14*± 0.46	170 ± 8	170± 10	0.36 ± 9	160± 35	175± 45	14* ± 14

Glucose Tolerance (OGTT)

Fasting (interaction p= 0.769), mean (interaction 0.193) and peak postprandial glucose (PPG) (interaction = 0.149) were not significantly different between interventions. The change in mean PPG is shown in Figure 7a. Mean PPG changed by +0.1 mmol/L following

EXS (CI -0.5, 0.9 mmol/L; *d*= 0.19), -0.2 mmol/L following CWI (CI -0.5, 0.3 mmol/L; *d*= 0) and -0.6 mmol/L following WWI (CI -1.2, -0.03 mmol/L; *d*= 0.62). Peak PPG showed a small change after EXS (CI -0.6, 1.2 mmol/L, *d*= 0.21) and no change after CWI (CI -0.6, 0.6 mmol/L; *d*= 0.00), but WWI was found to moderately decrease peak PPG by -1.0 mmol/L (CI -1.9, -0.5 mmol/L; *d*= 0.63). The change in Peak PPG is shown in Figure 7b.

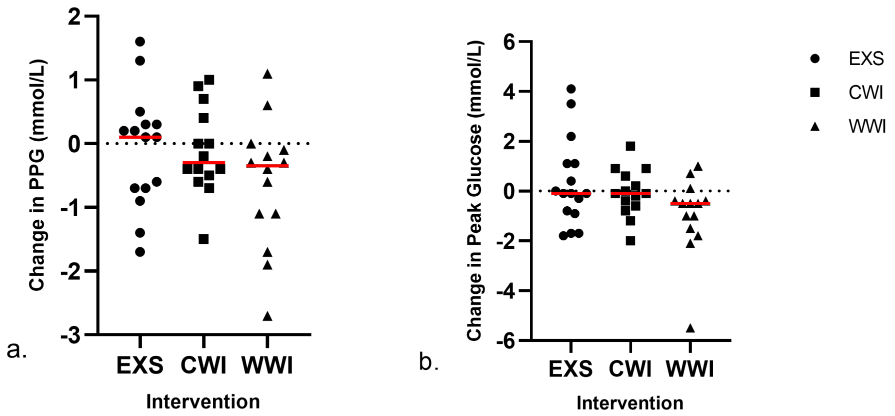

Figure 7. Change in Blood Glucose
***a.** Change in postprandial Glucose (PPG) after 12 sessions of time-matched exercise (EXS [n= 15]), post-exercise Cold Water Immersion (CWI [n= 14]) and post exercise Warm Water Immersion (WWI [n= 14]). No significant interaction effect. **b.** Change in Peak PPG after 12 sessions of EXS (n =16), CWI (n=14) and WWI (n=14). No significant interaction effect.*

Metabolic flexibility

Fuel utilisation glucose challenge OGTT

Fasting (p= 0.67) and postprandial RER (p= 0.71) were not significantly different between interventions, nor was fasting (p= 0.39) or postprandial (p= 0.40) carbohydrate (CHO) utilisation (Table 2).

Fuel utilisation submaximal exercise

Change in RER from rest to exercise was not significantly different between interventions (interaction p= 0.676). Fat utilisation at an absolute (interaction p= 0.270) and relative power (interaction p= 0.515) was not significantly different between interventions, nor across time (time effect p= 0.687). Power output at 70% of HRmax was not significantly different between interventions (interaction p= 0.443) nor across time (p = 0.158) [Table 2].

Table 2. Substrate Utilisation

Fasting and Postprandial (PP) Respiratory Exchange Ratio (RER) and substrate use (Carbohydrate [CHO]), following 12 sessions of time-matched Exercise (EXS [n= 15]), post-exercise Cold Water Immersion (CWI [n= 14]) and post exercise Warm Water Immersion (WWI [n= 14]) during a two h Oral Glucose Tolerance test. Fat utilisation, at an absolute (70 Watts Males/50 Watts Females) and relative watts (70% HRmax) was measured during submaximal exercise. Values are Mean ± SD. No significant difference between interventions.

	Fasting RER		Postprandial RER		Fasting CHO (cal/min)		Postprandial CHO (cal/min)		RER Change Rest to EXS		Fat Usage (cal/min) Absolute Watts		Fat Usage (cal/min) Relative Watts		Watts at 70% HRmax	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
EXS	0.84 ± 0.07	0.86 ± 0.09	0.90 ± 0.06	0.88 ± 0.04	56.55± 29.49	63.22 ± 30.60	84.88 ± 29.13	79.45 ± 20.96	-0.02 ± 0.13	0.01 ± 0.09	246.75 ± 111.24	238.95 ± 65.44	166.95 ± 108.63	169.93 ± 80.45	99 ± 40	113 ± 42
CWI	0.82 ± 0.05	0.82 ± 0.05	0.88 ± 0.06	0.86 ± 0.05	47.60 ± 23.35	47.98 ± 30.39	77.53 ± 35.03	67.60 ± 34.21	0.01 ± 0.06	0.04 ± 0.14	266.97 ± 126.55	214.62 ± 78.30	198.30 ± 159.84	168.25 ± 121.03	106 ± 28	110 ± 31
WWI	0.88 ± 0.11	0.85 ± 0.07	0.91 ± 0.06	0.88 ± 0.06	71.52 ± 39.65	62.03 ± 31.81	99.17 ± 29.62	74.88 ± 28.54	0.01 ± 0.11	-0.01 ± 0.08	202.77 ± 56.60	215.83 ± 85.33	98.64 ± 100.41	158.52 ± 104.61	95 ± 46	113 ± 35

Discussion

This study investigated whether substituting the final thirty minutes of each exercise bout with warm or cold-water immersion, could provide similar or greater benefits for cardiometabolic health and exercise performance. The main finding was that exercise training alone (EXS) results in the greatest increase in cardiovascular fitness ($\dot{V}O_{2}max$), being significantly greater than the change following two-weeks of post-exercise CWI but not post-exercise WWI. All short-term interventions improved performance related indices such as power output and distance attained during a 20 min work trial (albeit this study did not include a non-intervention control). WWI was also perceived as thermally comfortable, which may further facilitate its efficacy as an adjunct to exercise training, allowing for less exercise whilst still achieving cardiometabolic health benefits in people who cannot/will not perform sufficient volumes of exercise. Although the changes in metabolic indices were not significantly different between interventions, future research is needed given the non-significant but moderate decrease in peak postprandial glucose after WWI. WWI used alone has previously been shown to improve glucose control [31,72], and thus warrants further research as an adjunct to exercise for improving glucose tolerance in those at risk. Together, our study adds to the current literature on hydrotherapy and highlights post-exercise WWI as a potential alternative to longer duration exercise. Given similar benefits to longer duration exercise, post-exercise WWI has important implications for those who are either unable or disinclined to exercise for 60 min, such as those with low exercise tolerance, chronic obstructive pulmonary disease, congestive heart failure, cardiomyopathy, peripheral vascular disease, obesity and T2D.

Cardiorespiratory Fitness ($\dot{V}O_{2}max$)

The benefits of ~300 min/wk of exercise training (physical activity guidelines) on fitness are well known. The present study found that, although $\dot{V}O_{2}max$ improved to a greater extent with EXS alone when compared to post-exercise CWI, there were no significant differences between EXS alone and when 30 min of exercise was substituted with 30 min of post-exercise WWI. This suggests WWI can be a partial substitute to exercise for achieving gains in fitness. Bailey et al [34] found comparable improvements in cardiovascular fitness after 30 min of WWI alone versus time-matched moderate-intensity cycling, whereas we have shown here that substituting the second half of an exercise session with WWI also provides comparable benefits. In contrast, post-exercise CWI was less effective than EXS alone for improving cardiorespiratory fitness. Prior work by Broach et al., [47] also showed CWI did not improve $\dot{V}O_2max$ or maximal uncoupled respiration (complexes I and II), compared to six-weeks of exercise training, supporting the work of Yamane et al. [48,49] in showing blunted vascular, endurance and strength effects of CWI. Future research on CWI is needed to determine the mechanisms by which CWI may blunt exercise adaptations (e.g., blood flow, inflammation and cellular metabolism) [50–52].

This is the first study to compare the effects of post-exercise WWI, CWI and EXS. The duration and intensity of our interventions are in line with current physical activity recommendations that suggest 150-300 min of moderate intensity exercise per week for improvements in cardiorespiratory fitness [9]. The larger increase in cardiorespiratory fitness with EXS than CWI presumably reflect at least partly a greater cardiovascular stimulus. Thomas et al. [37] previously showed that WWI and exercise increase muscle temperature, limb blood flow and antegrade shear stress, with the increases following WWI being of a greater extent compared to exercise. Indeed, many key adaptations to exercise training are stimulated by elevations in muscle and body temperature, resulting in increases in blood flow and volume, metabolic rate, and the regulation of gene expression and signaling molecules i.e. for mitochondrial biogenesis [37]. Exercise remains superior for improving cardiovascular fitness, however, for those who are unable to perform larger volumes of exercise, WWI could be used as an alternate option to retain $\dot{V}O_2max$ or achieve some improvement.

Exercise Performance Work Trial

Exercise performance, measured as work trial distance and power, improved after all interventions. In contrast, prior research on exercise performance and WI (often used to promote recovery) has shown little to no effect on exercise training adaptations [53]. For example, Vaile et al. [73] found that CWI following exercise led to modest improvements in time trial performance but did not find any benefits with WWI. Likewise, Malta et al[74] concluded that aerobic exercise performance, specifically, mean power and time-trial performance (duration) were not adversely affected by regular use of CWI. Our findings that post-exercise CWI and WWI had similar (but not reliably identifiable additive or detrimental) effects for performance indicators compared to time-matched exercise alone, suggest WI could be used as an alternate to exercise alone. We acknowledge that without a 30-min exercise alone intervention, it is unclear whether WI is additive in our study. The aim of this proof-of-concept study was to see if WI could provide comparable effects as an exercise substitute for those who cannot perform sufficient volumes of exercise. Previous research comparing 30 to 45 min of post meal exercise has found similar benefits for postprandial glucose, however benefit to fitness and exercise performance needs to be further investigated[75].

Metabolic Health

Continued research on hydrotherapy is needed to determine the impact of WI as an exercise adjunct for enhancing metabolic health. In contrast to Hoekstra et al [31], the current study found no intervention effect on fasting blood glucose, mean PPG or metabolic flexibility; despite similar intervention durations. This may be due to differences in water immersion depth (neck) and duration (60 min), which were used by Hoekstra et al [31] would likely elicited greater thermal stress and thus adaptation. Despite there being no significant difference between interventions, our WWI results are promising given the on average 1.0 mmol/L change in peak PPG following the short-term WWI intervention; particularly in an apparently healthy population where blood glucose improvements would be expected to be minimal. A 1 mmol/L (~10%) has been associated with improved glycaemic control, reduced risk of diabetes related complications and CVD [76,77]. Furthermore, prior studies have shown that WWI interventions improve HbA1c, fasting blood glucose and insulin sensitivity in at risk populations [78–80]. The proposed mechanisms underlying these changes in other studies are an increase in heat shock protein (iHsp72) expression and reduced chronic low-grade inflammation [35]. Further investigation regarding the intensity of exercise preceding WWI, the required temperature of water and the duration of immersion, in metabolically compromised individuals, is required to better understand how WWI could be used as an adjunct therapy.

Future Directions

The aim of the present study was to investigate the feasibility and efficacy of post-exercise WI in an apparently health population before implementing this approach in clinical populations. In keeping with the physical activity recommendations and to allow translation of results to 'free living', insufficiently active adults, moderate intensity physical activity was chosen for the present study. Further to this, WI involved temperatures and depths that individuals could implement at home in the future. Improvements in work trial performance after all interventions suggest that partial substitution of exercise training, with either WWI or CWI, can improve performance indicators despite the reduced exercise volume. Whilst changes in $\dot{V}O_2max$ were similar between EXS and post-exercise WWI, CWI did not lead to improvements in cardiorespiratory fitness. Our findings, together with previous work, suggest WWI can be used in clinical populations to enhance their ability to perform physical activity. Adherence to WWI in Akerman et al [81] was ~four times higher than the exercise only group. In the present study, discomfort experienced during CWI was significantly greater than with WWI. Together with the fitness benefits, this comfort indicates post-exercise WWI may be more efficacious than CWI due to the reduced discomfort experienced by participants, thus potentially improving exercise compliance. Future studies should focus on WWI as an adjunct to exercise training for health benefits.

Strengths and Limitations

A four week washout period was implemented, allowing participants to return to baseline for main outcomes of cardiorespiratory fitness and glucose control before beginning their second intervention and to ensure reversibility of training effect [82]. We also employed 30 min of WI, that together with exercise training is in line with the current physical activity guidelines of 150-300 min per week. Body mass was not a contributing factor and did not change significantly from baseline with any of the interventions; this was by design and thus expected. Ideally, participants would have completed all three trial interventions (complete crossover design), however the associated time commitment and seasonal impact may have deterred participation and resulted in larger dropouts. Further to this, we acknowledge the limitations of prescribing exercise relative to fixed percentages of HRmax [83]. As mentioned above, a limitation of the present study, was that it did not include a 30-min exercise only intervention, therefore it is unknown whether WI is indeed additive. In addition, because this proof-of-concept study involved young, healthy subjects, this may have contributed to an increased ceiling effect for significant differences in other metabolic outcomes, particularly for glucose tolerance. As such, duration (12 sessions) and intensity (moderate) of intervention may not have been enough to elicit significant differences.

Conclusions

This study adds to current literature highlighting the therapeutic potential of post-exercise WWI. Findings from this study show that two-weeks of post-exercise WWI can provide similar benefits for cardiovascular fitness and time trial performance compared to 60-min of EXS alone, whereas EXS was superior to post-exercise CWI for cardiovascular fitness. For people who cannot exercise for the full 60-min, substituting 30-min of the exercise session with WWI provides a promising adjunct to achieve similar positive health outcomes. The combination of WWI and exercise in the present study is noteworthy given

WI is not a full substitute for all exercise adaptations (e.g., bone density, calcium handling, neuromuscular junction maintenance, etc.). Results pertaining to metabolic health remain inconclusive and further investigation is required to determine if this could be used as a viable option for individuals with obesity or type 2 diabetes.

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Conflicts of Interest

No conflict of interest exists, and all results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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Chapter 3

Thesis Conclusion

This chapter will expand on findings from Chapter 2 and discuss the effects of substituting the second half of a 60 min exercise session with water immersion on exercise capacity (change in $\dot{V}O_2max$) and metabolic health (change in postprandial glucose). Final discussions will include research limitations, address significance of findings and provide direction/context for future research.

Effects of WI on Exercise Capacity

$\dot{V}O_2max$ is a key indicator of cardiovascular health and exercise capacity[84], has important clinical outcomes that can determine a person's ability to perform actives of daily living (ADL) and has significant impact on overall quality of life[85]. Evidence-based recommendations provide clear guidelines for increasing exercise capacity and reducing risk of all-cause mortality[11,86,87], specifically following 'standardised' adult physical activity recommendations (i.e. ~150-300 min of moderate intensity exercise per week) can result in modest improvements in physical fitness or exercise capacity ($\dot{V}O_2max$)[88]. Unfortunately it is unrealistic for everyone to perform daily exercise in the manner in which it is generally recommended[31,89]. As such there is need for an alternate option for those who are disinclined or unable to exercise for the 30-60 minutes due to a lack of interest or some chronic respiratory or cardiovascular barrier.

The change in $\dot{V}O_2max$ in the current study was larger with EXS alone when compared to post-exercise WWI, but this difference was not significantly so. Although statistically our results show that substituting the second half of an exercise session with WWI can provide comparable benefits to time-matched exercise, on average, EXS improved $\dot{V}O_2max$ ~4 mL/kg/min more than post-exercise CWI and ~2 mL/kg/min more than post-exercise WWI. Given that an increase of ~3.5 mL/k/min (or 1MET) is a clinically meaningful change, these results indicate that, for healthy populations who can achieve the recommended 60 min of exercise, performing greater volumes of remains most advantageous. Equally, although requiring further investigation, a more modest improvement in $\dot{V}O_2max$ (~2 mmol/L) is promising for post-exercise WWI as a *partial* substitute for exercise training but is only applicable to those who cannot meet exercise recommendations. The mechanisms that underlie the beneficial effect of WWI, relative to exercise, are currently unclear but may be due to the sustained cardiovascular stimulus elicited with exercise and WWI; we hypothesised that cardiac output and therefore increased HR led to superior adaptations for $\dot{V}O_2max$. It is however unknown whether these adaptations were peripheral and/or central adaptations. Brunt et al [32] showed vascular changes after eight weeks of heat therapy, given the current intervention was much shorter (14-16 d) a longer-term intervention is needed to confirm our findings longer-term.

In contrast to findings on research pertaining to WWI[32,81], the current study showed that the addition of post-exercise CWI did not improve $\dot{V}O_2max$. Research on post-exercise CWI is largely contradictory[53] and our findings do little to debunk or affirm suggestions that continued CWI is potentially detrimental to performance/exercise capacity outcomes. Whilst post-exercise CWI is largely thought to be detrimental to resistance training adaptations, but negligible to endurance/aerobic training adaptations[53] the possible mechanisms underlying negative implications of CWI on exercise capacity include an increased vasoconstriction, reduced CO and blood flow and changes in sympathetic nerve activity[27]. It is possible that in the current protocol, immersion temperature (~10-12 degrees) was potentially not cold enough to evoke these same physiological responses as in previous research with cooler immersion temperatures (~5- 10 degrees). Future research is however warranted to measure blood flow during immersion and further investigate these mechanisms.

Improvements in work trial performance in the present study suggest, that partial substitution of exercise training, with either WWI or CWI, translate to exiguous performance benefits, and therefore, to improved exercise tolerance and capacity. The latter, potentially being important for improving quality of life and ability to perform ADLs because of enhanced functional status[90] (ability to perform activities with less fatigue etc) and an improved capacity to cope with the physical demands of daily living as well as increase habitual physical activity levels[91]. Prior research has shown that WWI improves functional capacity (walking distance/ADLs), resting blood pressure and quality of life in patients with peripheral artery disease [81]. Specifically, Akerman et al[81] compared 12 wks of heat therapy to twice weekly supervised exercise sessions, demonstrating a comparable improvement in functional walking ability (6 min walk test + 41/43 m respectively) and systolic blood pressure between interventions. Akerman et al[81] affirms the potential for improved cardiovascular and functional capacity when exercise tolerance is limited, and a more conservative approach is adopted. Collectively, therefore, these findings suggest WI could be used in clinical populations to enhance their ability to perform physical activity or at least achieve similar health benefits to exercising for longer durations.

Effects of WI of Metabolic Health

This study has demonstrated promising improvements in metabolic health with post-exercise WWI. Specifically, a potentially clinically relevant 1mmol/L (~10%) reduction in PPG was attained when the second half of a 60 min exercise session was replaced with WWI. Previous research has shown, over the long-term, that a >0.5 mmol/L reduction in glucose is associated with improved glycemic control[76], reduced risk of diabetes related complications and CVD [76,77]. Possible mechanisms for improved metabolic control may be attributed to a continued increase in systemic blood flow (stimulated by the 30 min exercise session) that potentially increases nitric oxide bioavailability (a key signaling molecule promoting an increase in GLUT-4 metabolism) and AMPK activation; stimulating glucose uptake, fatty acid oxidation, mitochondrial biogenesis, and improved insulin sensitivity[92].

Faulkner et al [93]showed that peak glucose was reduced significantly after heat (thermal) therapy and could be attributed to a stimulation of Hsp70 production, leading to a reduced insulin resistance and enhanced glucose disposal rate. Furthermore, animal literature has shown promising results regarding heat therapy replicating health benefits normal achieved with exercise. Furthermore, Gupte et al.[65], showed that in rats, heat therapy increases HSPs (mitochondrial Hsp60 expression) contributing to reductions in skeletal muscle insulin resistance i.e. enhanced glucose uptake.

Further investigation is necessary to measure and confirm underlying mechanisms for the improvements in glucose uptake with post-exercise WWI in the present study. Comparable improvement seen with post-exercise WWI on metabolic outcomes in those who are healthy suggests that WWI as a partial substitute is an alternate clinical management tool for people with diabetes or those at risk of chronic disease who are unable to engage in larger volumes of exercise.

Findings associated with post-exercise CWI and impact on metabolic health were neither detrimental nor additive. Additionally, current evidence pertaining to adherence to CWI protocols is scant. In the present proof of concept trial, discomfort experienced during CWI was significantly greater than with WWI, indicating post-exercise WWI may be more efficacious than CWI due to the reduced discomfort experienced by participants, thus potentially improving compliance. Further to this, Akerman et al [81] found that adherence to WWI was ~four time greater than exercise interventions. Given the apparent lack of research regarding how the ‘comfort’ of participants impacts longer-term adherence, further investigation is warranted.

Limitations

A limitation of the present study was that it was a partial cross over design, ideally, participants would have completed all three trial interventions (complete crossover design).

Whilst this design has been employed in this type of research previously[94] we believe associated time commitment and seasonal impact to be valid reasons for the partial cross over design. The pool of participants was largely drawn from a university student cohort where sessions are delivered in two 13-wk blocks with a large summer break from December to March. Only completing two out of three interventions meant that students could start and finish in the same session and we were not restricted to the beginning of the year for recruitment of participants. Furthermore, having an intervention that ran for a minimum of ~ 17 wk would have resulted in seasonal sport cross over; involvement in formal exercise training programs or changes to training routines between interventions that formed part of the exclusion criteria. Additionally, Australian summers are typically very hot, meaning allocation of interventions to January-March would have been challenging and potentially could have altered results. A block randomisation for the order of interventions was employed to account for seasons and to ensure that interventions were spread throughout both summer and winter.

The study was powered on effect size for our main outcome the change in $\dot{V}O_2max$ [34]. Power (80%) was calculated to analyse 24 participants, and accounting for a potential dropout rate of 20% we recruited 30 participants. There was, however, a slightly higher dropout rate and only 22 complete data sets were analysed. Significant changes in $\dot{V}O_2max$ indicate this was adequate for cardiorespiratory outcomes, however potentially underpowered for metabolic outcomes. Whilst the small sample size is recognised as a potential study limitation, this was a proof-of-concept trial, with the aim of informing future research design and implementation.

The current study prescribed exercise relative to fixed percentages of HRmax calculated in the $\dot{V}O_2max$ test. It could be argued that given the incomplete design of this study, and the limitations of this method, there may be small inconsistent in the relative intensities across the cohort. However, this method is common when prescribing an exercise training intervention in the general population. Prescription of exercise was at 70% of HRmax to align with the Australian National Physical Activity Guidelines that recommend 'moderate' intensity exercise for 150-300 minutes per week. For future translation, we used this measurement of intensity because it is familiar to clinicians and the general public can easily implement it at home. We feel the variation between groups would have been negligible, as 70% allows for variability within the *'moderate intensity category'* of 65-75% which is commonly used for exercise prescription. Additionally, RPE was recorded simultaneous to HR to ensure moderate intensity was achieved for the duration of each exercise session. RPE was kept between 11-13 on the Borg Scale and participants were instructed on its use prior to intervention initiation.

CWI temperatures and duration used in the current protocol differ from typical CWI studies. Previous research suggests that 15 min of water immersion is an inadequate duration to elicit a shivering response or lower core body temperature enough to increase PGC1-α mRNA and upregulate cellular energy metabolism and promote mitochondrial biogenesis and allow for changes in mitochondrial function and structure[74]. These are important molecular regulators for metabolic health adaptations (i.e., glucose control & cardiorespiratory fitness). We hoped that the 30 min CWI duration would likely be long enough to overcome previous protocol design limitations. Additionally, CWI protocols are typically much cooler (~5-10 degrees) for shorter durations (<15 min). Our protocol includes CWI of 10-12 degrees, slightly warmer but involves a longer duration. Whilst warm water immersion typically occurs for longer durations (>30 min) we needed to 'time-match' our interventions and as such 30 minutes of immersion was deemed most appropriate. Furthermore, the chosen water immersion temperatures were selected so that individuals could implement easily at home in the future.

The present study did not include a 30-min exercise only intervention, therefore it is unknown whether WI is indeed additive. The aim of the study was to inform future investigation regarding the use of WI as a substitute for 'part' of an exercise session in those who are disinclined or cannot exercise for longer durations. This study was two-fold a) to determine the feasibility and efficacy of the approach, and b) to gain pilot data, both to apply for funding to address this in a clinical population and measure mechanisms and outcomes. In addition, we do not anticipate that a two-wk control (no exercise/WI) condition would have altered our findings i.e., unlike clinical trials in diabetes, participants health is not expected to get worse over such a short time. A familiarisation work trial was also included to limit any learning effect of the performance test. Participants completed a four-wk washout between each condition with pre-assessments performed before each trial. There were no pre-trial differences in any outcome (including $\dot{V}O_2max$ and work trial).

Metabolic health, in the present study, refers to glucose control and metabolic flexibility only. It is understood that the term metabolic health encompasses a greater range of physiological and biological processes that were not measured.

Finally, because this proof-of-concept study involved young, healthy subjects, this may have contributed to an increased ceiling effect for significant differences in other metabolic outcomes, particularly for glucose tolerance. As such, duration (12 sessions) and intensity (moderate) of intervention may not have been enough to elicit significant differences. This gives rise to the need to further test our design (particularly post-exercise WWI vs exercise) in a clinical population before moving to a 'free living' setting.

Significance

The global prevalence of physical inactivity has been reported as a 'pandemic'[7]. Associated chronic disease and burden on an already overwhelmed health care system is increasing with physical activity now reported as the 'fourth leading cause of death worldwide'[7].

Exercise is widely prescribed to improve blood glucose control and reduce risk of cardiovascular disease. Lack of time or inability to complete the recommended amount of exercise are often given as reasons for poor exercise adherence[93]. Here we have presented a novel strategy that allows the attainment of comparable health benefits by partially substituting exercise with a warm bath. This intervention strategy could therefore provide an alternate option for those who are unable or disinclined to exercise for 60 mins.

This is the first study to examine whether adding water immersion to shorter-duration exercise can allow for analogous benefits to cardiometabolic health when compared to time-matched exercise. Combining exercise and WI is considered superior to WI alone given that there remain some exercise specific benefits that cannot be achieved with immersion alone (metabolic homeostasis, increased muscle mass, bone density, reduced visceral adiposity)[5]. Post-exercise WI could therefore be considered 'additive' for those who are unable to perform longer-duration exercise. This study supplements an already growing body of literature focusing on water immersion for improvement of exercise capacity/fitness but is unique in its investigation of combing exercise and post-exercise water immersion on health outcomes. Furthermore, CWI findings are largely inconsistent, and it was unknown if CWI or WWI was the superior WI substitute regarding cardiometabolic health outcomes. Whilst the current study results suggest that CWI was potentially less effective, outcome measures were largely specific to WWI. To test the benefit of post-exercise CWI on cardiometabolic outcomes more effectively, a 'fat' or 'mixed-meal' tolerance test along with measurements of fat oxidation may have been more applicable/informative.

Future Implications

This was a proof-of-concept trial that can inform future investigation regarding the use of water immersion as a substitute for 'part' of an exercise session in those who are disinclined or cannot exercise for longer durations. Importantly, the intervention strategy outlined in this study focuses on a transferable alternate method that can be easily prescribed by general practitioners and allied health professionals in the general community. The duration and intensity of our interventions were in line with current physical activity recommendations that suggest 150-300 min of moderate intensity exercise per wk for improvements in cardiorespiratory fitness [9]. Given the positive analogous results of EXS and post-exercise WWI on both exercise capacity and metabolic health this intervention strategy has potential to increase exercise adherence (by reducing volumes) and allow for comparable achievement of health benefits therefore reducing prevalence, burden, and cost of inactivity.

Lastly, the outlined study was two-fold and aimed to determine the feasibility and efficacy of this approach, and to gain pilot data, both for the application for funding to address strategies and measure mechanisms and outcomes in a clinical population. This proof-of-concept trial showed that post-exercise CWI was less effective than both EXS and Post-exercise WWI on cardiometabolic health outcomes. A full cross over trial comparing EXS (60 min AND 30 min), WWI only (30 min) and post-exercise WWI (30 min EXS followed by 30 min WWI) in clinical populations may allow for a greater scope of investigation and the provision of more conclusive data regarding the effectiveness of post-exercise WWI on cardiometabolic health. Given the clinical meaningful changes in both $\dot{V}O_2max$ (~3.5 mL/kg/min or 1 MET equivalent) and peak PPG (~1 mmol/L reduction) achieved after just two wk with EXS and post-exercise WWI in young, healthy participants a longer-term control trial in clinical populations comparing both 30 min and 60 min of exercise to time-matched post-exercise WWI is indicated prior to translation to ‘free living’ investigations. Most importantly, future research should aim to measure, investigate, and detail the mechanisms (e.g., increased: systemic blood flow, NO bioavailability, GLUT4, AMPK and HSPs) underlying changes found in the present study.

Conclusion

Hydrotherapy can provide a therapeutic adjunct to exercise allowing a larger portion of the population to meet daily exercise requirements. The aforementioned protocol could easily be replicated at home and provide a simple alternate prescription option for clinicians when prescribing exercise for those who are not meeting the current exercise recommendations. Whilst meeting exercise guidelines as they are recommended is preferable, results pertaining to clinical improvement in $\dot{V}O_2max$ and PPG indicate that post-exercise WWI could facilitate health benefit attainment for those who cannot perform 60 mins of exercise.

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