THE EFFECTS OF AQUATIC HIGH-INTENSITY INTERVAL TRAINING ON CARDIO-METABOLIC HEALTH, COGNITION AND PERCEPTUAL RESPONSES IN PHYSICALLY INACTIVE AGED WOMEN

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The Effects of Aquatic High-Intensity Interval Training on Cardio-metabolic Health, Cognition and Perceptual Responses in Physically Inactive Aged Women

Kwok Man Ying

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Certificate of Originality

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(Signed)
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Abstract

This thesis begins with a meta-analysis (**Study 1**) of previously-published studies of aquatic high-intensity interval training and its effects on cardiac, metabolic and physical health markers in aged women. The review included thirteen research studies. Given the high prevalence of physical inactivity, obesity and osteoarthritis among aged women, that was the population chosen as a target group. The aim of this study was to investigate the effects of aquatic high-intensity interval training on cardiac-metabolic and physical outcomes. It suggested that aquatic high-intensity interval training has a moderate effect in improving these health markers in such women group. This provided the background for a further review of the effects of deep water running as a modality in improving the cardiorespiratory fitness, physical functioning and quality of life of adults.

Study 2 was a systematic review which identified eleven clinical trials of the effectiveness of deep water running. Improvements in adults' cardiorespiratory fitness, physical functioning and quality of life were compared through land exercises or without any dedicated exercise. The findings suggested that deep water running may offer results comparable to those of land-based training, though with mixed results when compared with taking no dedicated exercise. That motivated the next step of developing an effective intervention program to improve cardiac and metabolic outcomes based on deep water running.

A reliable, valid and water resisted tool that accurately assesses patients' progress would be very helpful. **Study 3** was designed to quantify the validity and reliability of

using an inexpensive 3D-printed portable metabolic analyser PNOĒ to measure the cardio-metabolic outcomes of aquatic exercise in terms of aerobic fitness.

Two cross-sectional pilot studies were performed to develop an aquatic high-intensity interval training protocol in **Study 4**. The first study varied the cadence to establish a matched intensity between aquatic and land-based high-intensity interval training through comparing the cardio-metabolic and perceptual responses. Results indicated that aquatic exercise produced a greater reduction in heart rate and a greater increase in oxygen pulse. The second pilot study explored the benefits of increasing the intensity of the aquatic exercise by adding resistance. The resistance was found to produce no significant change in heart rate or peak oxygen uptake, but it did tend to increase the perceived rate of exertion.

A randomized and controlled trial (**Study** 5) was organized which compared the effects of an 8-week program of aquatic high-intensity interval training based on running in deep water with those of a matched program administered on land. Cardio-metabolic, cognitive and psychological outcomes were compared. The deep-water running program was found to improve oxygen capacity, metabolic equivalents, blood oxygen and ventilation as well as the land-based exercise program. It also produced in this population similar improvements in cognition as assessed by the Mini-Mental Status Examination and the Montreal Cognitive Assessment.

These findings suggest high-intensity aquatic exercise could serve as an alternative to add value to land-based alternatives. It should be considered by healthcare professionals when designing programs targeting specific cardio-metabolic health benefits, metabolic blood markers, cognitive functioning, and perception in physically inactive older women.

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"If I have seen further, it is by standing on the shoulders of giants." Sir Issac Newton

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List of Abbreviations & Units

ACSM	American College of Sports Medicine
AHIIT	Aquatic High- Intensity Interval Training
ASIS	Anterior Superior Inferior Spine
ATP	Adenosine triphosphate
BMD	Bone mineral density
BMI	Body Mass Index
CMD	Cardio-metabolic Disease
CMR	Cardio-metabolic risk
CRF	Cardiorespiratory fitness
CS	Chair to stand
DBP	Diastolic blood pressure
DWR	Deep water running
EE	Energy expenditure
FDM	Fused deposition modelling
FTSST	Five Times sit to stand test
HDL	High density lipoprotein
HIIT	High- Intensity Interval Training
HR	Heart rate
HRR	Heart rate reserve
IPAQ	International Physical Activity Questionnaire
LDL	Light density lipoprotein
L-HIIT	Land based High- Intensity Interval Training
MET	Metabolic equivalent

MMSE	Mini- mental state examination
MOCA	Montreal cognitive assessment
MWT	Minute walk test
PA	Physical Activity
PEDro	Physiotherapy Evidence Database scale
QoL	Quality of Life
REE	Resting energy expenditure
RER	Respiratory exchange ratio
RPE	Rate of perceived exertion
SBP	Systolic blood pressure
TEE	Total energy expenditure
TUGT	Timed Up and Go Test
VE	Minute ventilation
VT	Ventilatory Threshold
VCO_2	Carbon dioxide production
VO_2	Oxygen capacity
VO_2R	Oxygen capacity reserve
VO_2 max	Maximal oxygen/ aerobic capacity
WHO	World Health Organization

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Chapter 1 General Introduction

1.1 Inactivity and Exercise

Physical activity is today understood to have a major role in the prevention and treatment of cardio-metabolic disease. Cardio-metabolic diseases include cardiovascular diseases and metabolic diseases such as obesity and type 2 diabetes (*WHO guidelines on physical activity and sedentary behaviour*, 2020). While land-based exercise has been shown to effectively reduce these risks, there has been growing interest in exercising in water (Nagle et al., 2019). Aquatic settings offer reduced loading, and potentially make exercise more feasible, satisfying and thus successful, particularly for persons unable to exercise effectively on land.

Physical inactivity can be defined in terms of not meeting published physical activity guidelines (Thivel et al., 2018). The World Health Organization (WHO) has published its (*WHO guidelines on physical activity and sedentary behaviour*, 2020) and the American College of Sports Medicine recommends (Liguori & Feito, 2022) that individuals accumulate a minimum of 150 minutes of moderate-intensity physical activity [40–60% of their maximum oxygen capacity (VO_{2max}) and 75 minutes of vigorous-intensity physical activity (60–85% of VO_{2max}), or a combination twice weekly to maintain cardio-metabolic health (Bull et al., 2020). VO_{2max} represents the maximum amount of oxygen utilization by exercising muscles. It is one of the most commonly-used measures to monitor exercise intensity (Kruel et al., 2013a; Schaal et al., 2012).

Inactivity has been identified as one of the four leading contributors to premature mortality (Kohl et al., 2012). And there is epidemiological evidence that it increases the risk of cardiovascular disease and type 2 diabetes (Franklin et al., 2022). Nevertheless, more than 30% of adults worldwide are estimated to be physically inactive (Pedro C. Hallal et al., 2012a). Despite the health benefits of physical activity, about a quarter of adults fail to maintain the lowest recommended level of physical activity, with women over 65 more inactive than men (30.7% inactive compared with 23.7% of men) (Guthold et al., 2018; Pedro C Hallal et al., 2012; Mayo et al., 2019). The physical activity recommendations are for both genders, but women have specific cardio-metabolic, hormonal and physiological features which suggest gender difference in the risk factors and managing cardiac and metabolic disease (CMD). For instance, women have a smaller blood vessel than men and are more vulnerable to developing hypertension. This is particularly true of post-menopausal women with decreased levels of oestrogen. Additionally, lower limb osteoarthritis and obesity are more prevalent in women. Women over 45 years old are reported to be much more obese than the rest of the general population, with a body mass index surpassing 25kg/m^2 in 47% of them and surpassing 30kg/m^2 in 9% (L. Andrade et al., 2020; Ng et al., 2014). Physical inactivity among aged women thus makes them a suitable target population for an exercise study.

Physical activity (PA) is defined as any bodily movement produced by the contraction of skeletal muscles that results in increase in caloric requirements over resting energy expenditure (Caspersen et al., 1985). Exercise is a subcategory of PA that consists of planned, structured, and repetitive bodily movements aimed at improving and/or maintaining one or more components of physical fitness (Caspersen et al., 1985). Physical fitness, although defined in various ways, is generally described as a set of attributes or characteristics that relate to an individual's ability to perform PA and the activities of daily living (Caspersen et al., 1985).The term PA covers a wide range of intensities, with different methods for estimating intensity, including percentage of oxygen uptake reserve, Heart rate reserve (HRR), volume of oxygen consumed per minute (VO_2), heart rate (HR), or metabolic equivalents (METs). Due to age-related declines in VO_{2max} (Nelson et al., 2007), older individuals exercising at a given intensity will be working at a greater relative percentage of their VO_{2max} than their younger counterparts.

1.2 Physical Activity's Benefits

1.2.1 Cardiovascular benefits

There is evidence supporting negative correlations between regular PA and/or exercise and premature mortality, cardiovascular disease, coronary artery disease, hypertension, stroke, osteoporosis, type 2 diabetes mellitus, metabolic syndrome, obesity, cancers, depression, functional health, falls, and cognitive functioning (Anderson & Durstine, 2019; Booth et al., 2012; Leon et al., 1987). Of note, aerobic capacity (cardiorespiratory functioning) has a negative correlation with many adverse health outcomes including the risk of premature death from all causes, but specifically from cardiovascular disease (Asikainen et al., 2002; Blair, 1989). Greater physical activity is associated with better cardiorespiratory fitness, which in turn is associated with many health benefits (Franklin et al., 2022) (McKinney et al., 2016). There may be an additional benefit from cardiovascular conditioning for those with a mood or sleep disorder. In such cases it can have positive cognitive outcomes (Campos et al., 2021; Farinha et al., 2021). Physical inactivity coupled with advanced age is associated with a decline in cardio-metabolic fitness (Harms et al., 2011; Myers et al., 2015) (Figure 1.1)

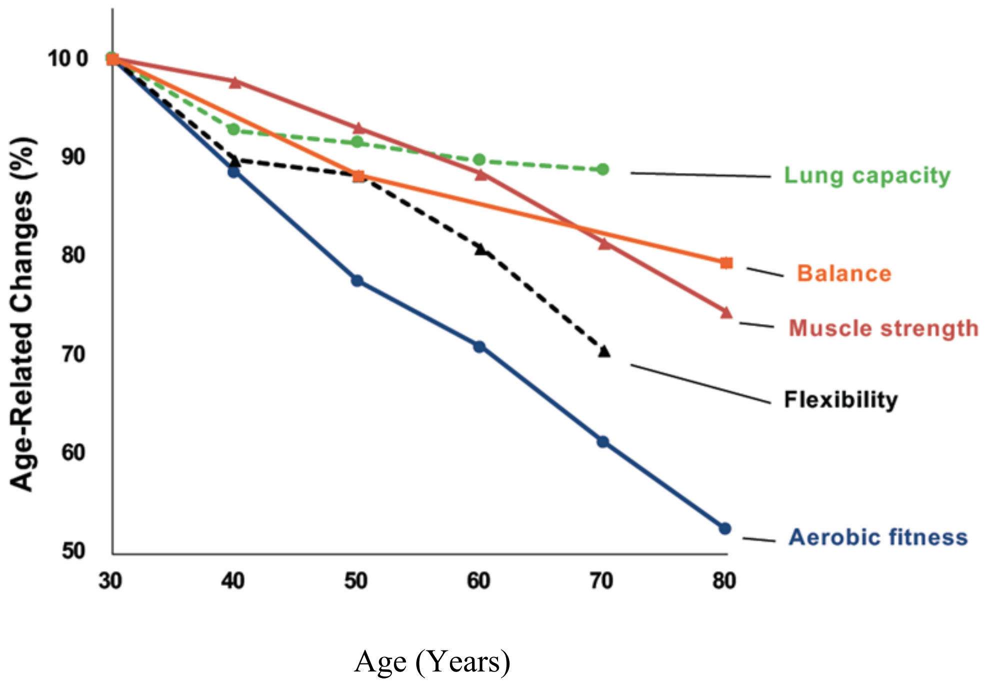


Figure 1.1 Age related Reductions in Physical Fitness (Harms et al., 2011)

CMD is a common cause of premature death and it is linked to physical inactivity (Henson et al., 2013). A meta-analysis has reported beneficial effects of low-intensity physical activity on cardio-metabolic health (Chastin et al., 2019). But low-intensity activities do not provide the same advantages as engaging in moderate or vigorous physical activity (Chastin et al., 2019).

It is also suggested that spending more time on moderate or vigorous physical activity is significantly associated with a healthier level of high-density lipoprotein (though the effect size was small) (Belanger et al., 2022). PA is a key to multiple physiological benefits, better functioning and better quality of life in general, as well as being associated with improved disease control (LaMonte et al., 2017; Pedersen & Saltin, 2015; Rao et al., 2022). Intervention studies have also demonstrated favourable changes in cardio-metabolic risk factors after exercise (Amaro-Gahete et al., 2019; Franczyk et al., 2023; Franklin et al., 2022).

There is evidence to suggest that higher levels of PA, associated with a lower risk of CMD, support favourable changes in cardio-metabolic risk factors (Buckley et al., 1999; Emery et al., 2022; Lu et al., 2022). The favourable health outcomes include better cardiovascular fitness, a better lipid profile, and better control of glycemia (Belanger et al., 2022; Myers et al., 2019; Myers et al., 2015). Exercise can also bring improvements in body composition, insulin sensitivity and blood pressure for individuals with mild or moderate hypertension ("American College of Sports Medicine. Position Stand. Physical activity, physical fitness, and hypertension," 1993). Cardio-metabolic fitness can even be a more powerful indicator of mortality than other well-established risk indicators such as hypertension, smoking and obesity (Myers et al., 2002).

A low VO_{2max} indicates poor cardiovascular fitness. It is probably a consequence of a physically inactive lifestyle and it is a strong predicator of premature mortality (Myers et al., 2017). But studies have demonstrated that even 4 weeks of regular exercise can generate significant improvement in VO_{2max} (of 6.4–26.4%) that are likely to have significant clinical relevance. Every additional metabolic equivalent (MET) is, on average, associated with an approximately 15% reduction in the risk of cardiovascular disease (Kodama et al., 2009). Further, a group led by Ross has reported that an increase in VO_{2max} of >3.5mL/kg/min is a valuable indicator of better health outcomes and longer survival (Ross et al., 2016a).

The minimum clinically importance difference (MCID) in VO_{2max} for healthy adults is generally taken as approximately 3–5mL/kg/min. That would indicate a meaningful change in aerobic capacity that is likely to be beneficial (Bonafiglia et al., 2021). In specific populations, such as those with a chronic disease or older adults, the MCID may differ. For example, in individuals with heart failure or chronic obstructive pulmonary disease, the MCID could be slightly lower, often around 2–3mL/kg/min (Wilkinson et al., 2019). The proposed mechanisms include biogenesis of mitochondria, increased capillary density through increased cellular stress, metabolic signals that lead to increased generation of adenosine triphosphate for muscle contractions, and/or increased stroke volume as a result of enhanced cardiac contractility.

Cardiovascular fitness declines steadily in physical inactive individuals at a rate of approximately 1% per year after the third decade of life (Posner et al., 1995). VO_{2max} might thus be a crucial contributor to cardio-metabolic health. Published cross-sectional studies have shown that VO_{2max} is associated with risk factors for cardiovascular disease (CVD) with moderate to strong correlations (Green et al., 2014; Kodama et al., 2009; Myers et al., 2002). Those with reduced cardiorespiratory fitness have been shown to have a risk of CVD 1.56 times that of comparable others with good cardiorespiratory fitness (Kodama et al., 2009). Cardiorespiratory fitness must therefore be taken into consideration when developing an exercise program for cardio-metabolic health.

1.2.2 Enhanced blood glucose control

Impaired fasting glucose, impaired glucose tolerance or a combination of both indicate a high risk of developing diabetes mellitus (Tabák et al., 2012). Evidence shows that all forms of aerobic, resistance and combined exercise can induce better blood glucose regulation, such as decreasing fasting glucose or decreasing insulin (Snowling & Hopkins, 2006). Exercise enhances the muscles' oxidative capacity and increases levels of skeletal muscle glucose transporter protein. That promotes overall glucose transport (Little et al., 2011). And there is evidence that resistance training is as effective as aerobic training in the management and treatment of Type 2 diabetes mellitus (Boulé et al., 2001; Kobayashi et al., 2023; Wood & O'Neill, 2012; Yang et al., 2014).

1.2.3 Lipid profile

Hyperlipidaemia is a metabolic and vascular condition which can increase oxidative stress, impair vasodilation and promote vascular inflammation, resulting in atherosclerotic disease progression (Racil et al., 2013). Scholarly work examining the effect of aquatic exercise on lipid metabolism has been rare, but one study has demonstrated that water-based aerobic interval training can decrease total blood cholesterol and low- density lipoprotein, and increase high density lipoprotein in pre-menopausal women with dyslipidaemia (Costa et al., 2020). Those results should have great clinical relevance, considering that an increase of 1% to 2% in high density lipoprotein (HDL) levels predicts 2 to 4% less cardiovascular risk (Gordon et al., 1989).

1.2.4 Improved muscular fitness and bone mass

That exercise can improve muscle strength, endurance and power is well established (Garber et al., 2011a; Pate et al., 1995; Zuo et al., 2022). Stronger muscles are associated with a significantly better cardio-metabolic risk profile. But systematic exercise can also bring weight loss of 2–6%, 4 to 8% improvements in cardiorespiratory fitness (Asikainen et al., 2002), increase range of motion by 5 to 20° (Decoster et al., 2005). Additionally, a 25% strength improvement in a trained limb can even improve the strength of the untrained contralateral limb by about 8%. (Carroll et al., 2006) (Garber et al., 2011a). Exercises that enhance muscle strength and mass also tend to increase the mass, mineral density and strength of the specific bones stressed in the exercises. So exercise may serve as a valuable measure to prevent, slow or reverse the loss of bone mass in individuals with osteoporosis (King et al., 2019).

1.2.5 Perceptual benefits

The way individuals perceive exercise is an important determinant of whether they will persist with a particular exercise program, switch to a different program, or cease exercising altogether (Whaley & Schrider, 2005). This is particularly significant in the case of physically inactive older persons. Positive exercise perceptions are a critical determinant of adherence to a program. In fact, it has been observed that perceiving exercise variety is a key factor in promoting adherence (Sylvester et al., 2016). The perception of health benefits and appreciating the competence of facility staff have also been shown to improve the adherence of older adults (Whaley & Schrider, 2005). Work by Trost and his colleagues has confirmed that enjoyment positively predicts exercise adherence, as do self-perceptions of efficacy, while lack of time is the barrier to physical activity most often reported (Trost et al., 2002). This suggests that it is vital to motivate individuals to incorporate exercise into their schedule.

1.2.6 Cognition

The American Psychological Association's definition of cognition takes in all forms of knowing and awareness, covering perceiving, conceiving, remembering, reasoning, judging, imagining and problem solving (Evans et al., 1992). These high-level mental processes have critical implications in synthesizing and integrating one's thoughts and experiences (Williams et al., 2009). There are common patterns of cognitive deterioration associated with normal ageing, though the actual changes vary. Figure 1.2 explains the structural and functional correlates of these cognitive changes, and the prevalence and cognitive effects of age-associated diseases. The most important changes in cognition with normal aging are declines in the ability to perform cognitive tasks that require one to quickly process or transform information in making a decision (Murman, 2015). Speed of processing, working memory, and executive cognitive function all decline (Saikia & Tripathi, 2024). Despite that, cumulative knowledge and skills based on experience are usually well maintained into old age (Lubitz et al., 2017).

There is emerging evidence that PA may decrease the rate of cognitive decline seen with aging and help delay the onset of the cognitive symptoms of age-associated diseases (Bosnes et al., 2022). Regular physical activity can favourably modulate development and aging processes, so it can have important health benefits (Harms et al., 2011). Conversely, physical inactivity can markedly impair normal development and lead to numerous diseases (Murman, 2015). Life-long physical activity is known to delay the onset of functional disability and chronic cardiovascular and metabolic diseases (Harms et al., 2011).

Controlled experiments have demonstrated positive improvements in cognitive functioning (in terms of MMSE scores) as a result of aquatic exercise (Farinha et al., 2021). Aquatic exercise appears to benefit elderly people´s cognition.

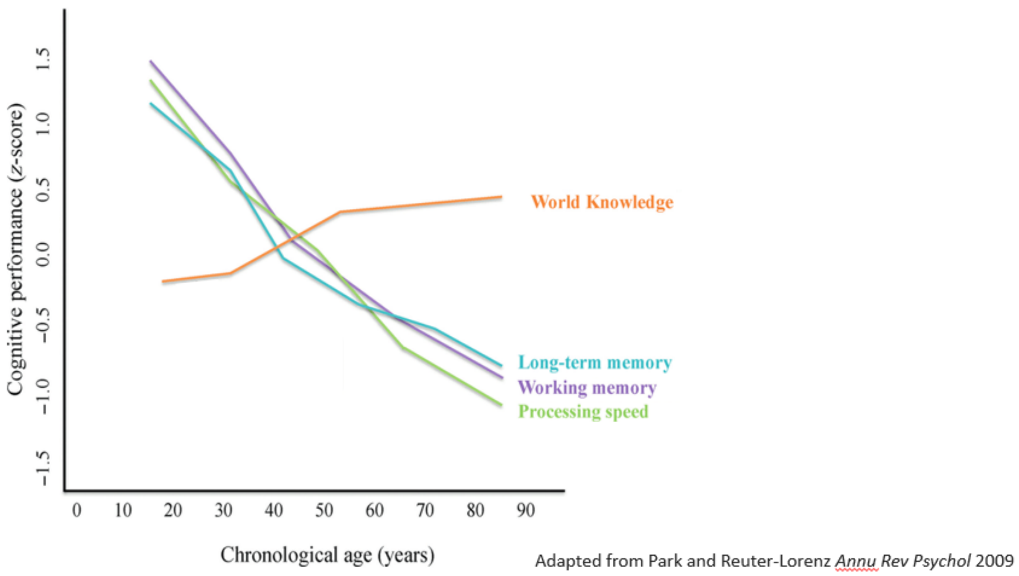


Figure 1.2 The cognitive changes that occur with normal aging (Park & Reuter-Lorenz, 2009)

1.3 Aerobic Interval Training and its Benefits

1.3.1 Interval Training

Interval training is an exercise mode which typically involves bouts of aerobic exercise at moderate to high intensity for a short duration of time (20–240s) separated by 60–360s intervals of rest or active recovery at a lower intensity (Gibala & Jones, 2013). The advantage of this type of exercise is that one is able to complete an exercise regimen in a shorter period of time than with continuous exercise while gaining the same physiological benefits (Poon et al., 2021). However, interval training is not limited to only aerobic exercises like cycling and running. There is also resistance-based interval training which can combine body weight exercises with plyometrics and resistance training using equipment. Either can powerfully stimulate cardiorespiratory fitness (CRF) depending on the lengths of the intervals and the subsequent recovery periods (Gibala et al., 2018).

The American College of Sports Medicine reports (Thompson, 2019) that high-intensity interval training (HIIT) (also referred to as high-intensity interval exercise), has been gaining in popularity among the general public (at least in the US). HIIT involves repeated bouts of high-intensity exercise interspersed with active or inactive recovery periods. The intensity requires a substantially reduced training volume, and thus less time commitment (Martin J Gibala et al., 2012). For example, one of the commonly-adopted HIIT programs involves 10 intervals of 60 seconds conducted at or near maximum aerobic capacity interspersed with 60 second recoveries. Apart from the warmup and cool down, the entire session takes only 20 minutes, but it has been shown to be both effective and feasible in improving the cardiac and metabolic health of formerly sedentary adults (Martin J Gibala et al., 2012).

The exercise intervals in HIIT are performed at or near an individual's current VO_{2max}. That elicits a heart rate within 80 to 100% of one's peak HR even with no exercise between the intervals (Weston et al., 2014). That improves aerobic capacity and endurance by stimulating cardio-metabolic changes (Gibala & Jones, 2013) and thus the health benefits of cardiorespiratory fitness. In terms of VO_{2max} gain such intense exercise is more effective than moderate-intensity continuous training for middle-aged and older adults (Poon et al., 2021).

One of the reasons to exercise most often cited is "lack of time" (Stutts, 2002). HIIT has been shown to increase endurance with as little as two weeks of training (Burgomaster et al., 2005). It is time-efficient. Gibala estimates that 2.5 hours of high intensity interval training is comparable in its effects to 10.5 hours of conventional continuous training (Gibala et al., 2006).

Another advantage of interval training is that the rest periods provide relief from unpleasant feelings of fatigue, pain or even exhaustion (Martinez et al., 2014). As a result, HIIT is considered to be an effective, time-efficient and more enjoyable exercise approach with great potential for mitigating the detrimental health consequences of physical inactivity among the elderly. But in studies investigating clinical populations with metabolic conditions, those pursuing HIIT have recorded higher dropout rates and more adverse effects than among a control exercise group (Poon et al., 2020; Michael Wewege et al., 2017). This of course raises serious concerns about implementing HIIT programs, but Lunt's group has shown (Lunt et al., 2014) that it is possible to apply HIIT in real world settings in ways which improve the cardiorespiratory fitness of overweight, inactive adults with limited exercise duration. Even Lunt's study, however, had poor adherence compared to most studies held in an exercise facility or a clinic. People struggle to adhere to HIIT programs on their own, and without adherence, no program will elicit the improvements it is designed to produce. That suggests a need to modify the workout environment to enhance adherence. Aquatic interval training is one possibility in this regard.

1.3.2 Intensity, frequency and time in interval training

There is a positive dose response in terms of the health and fitness benefits that result from increasing exercise intensity (Garber et al., 2011a). The overload principle of training states that exercising below a minimum intensity threshold will not challenge the body sufficiently to result in changes in physiological parameters (Garber et al., 2011a). The minimum threshold of intensity for benefit varies depending on an individual's current CRF level and other factors such as age, health status, physiological characteristics, genetic endowment, habitual PA, and social and psychological factors. Both moderate and vigorous intensity exercise can be used to meet PA recommendations, but in order to improve CRF, vigorous exercise (>6 metabolic equivalents; 60%–84% HRR) is more effective than exercising at moderate intensity (3.0–5.9 METs; 40%–59% HRR) for increasing VO_{2max} (Swain & Franklin, 2002).

According to the WHO's 2022 Physical Activity Guidelines, aerobic exercise should be performed on at least 3 days each week (Bull et al., 2020) . For most adults, spreading the exercise sessions across 3 to 5 days weekly may be best for reaching the recommended amount of PA, but many combinations of frequency, duration and intensity can be combined to meet the recommendations (Tucker et al., 2011) . Still, even performing aerobic exercise once or twice per week at moderate-to-vigorous intensity can bring substantial health and fitness benefits (Wisløff et al., 2006). Indeed, there is evidence that such an exercise pattern may be sufficient to reduce risks for all-cause mortality, CVD, and cancer mortality, despite not meeting the prevailing PA guidelines (O'Donovan et al., 2017). Most adults should probably be spending 30–60 minutes a day on moderate intensity exercise, 20–60 minutes/day on vigorous exercise, or a combination of the two (Garber et al., 2011a). The fact that any amount of PA can bring health benefits should be particularly encouraging for individuals who are currently sedentary or minimally active. Moving from a state of physical inactivity to any level of activity can result in significant mortality risk reductions (Warburton & Bredin, 2017a).

1.3.3 The effects of high-intensity interval training

Interval training at high intensity improves blood sugar levels, the lipid profile and blood pressure as well as cardiovascular fitness (Batacan et al., 2017). Six to fifteen weeks of HIIT had definitely been shown to generate significant improvements in VO_{2max} with various populations (HELGERUD, HØYDAL, et al., 2007; Racil et al., 2013). And the magnitude of improvements (at least 6% and up to 26%) is likely to have significant clinical relevance, as for each metabolic equivalent (corresponding to oxygen consumption of 3.5mL/kg/min), higher VO_{2max} is associated with a roughly 15% reduction in cardiovascular disease (Kodama et al., 2009).

In terms of glycemia, a number of studies have shown that various HIIT protocols can induce favourable glycaemic responses such as decreasing fasting/postprandial glucose and insulin levels, as well as improving insulin sensitivity (Abderrahman et al., 2018; Connolly et al., 2016; Kessler et al., 2012). It has been suggested that the improved glycaemic responses induced by HIIT are mainly a result of enhanced muscle oxidative capacity and increased skeletal muscle glucose transporter protein content (Little et al., 2011) which promotes glucose transport.

Studies of the effect of HIIT on lipid metabolism have shown inconclusive results (Babraj et al., 2009; Racil et al., 2013). The work by Racil's group demonstrated that 12 weeks of running-based HIIT running decreased total blood cholesterol, low-density lipoprotein (LDL) and triglyceride levels and that it increased HDL levels. The work reported by Babraj, however, did not demonstrate significant changes in these markers (Babraj et al., 2009; Ciolac et al., 2010; Madsen et al., 2015). It appears that a minimum of 8 to 12 weeks of HIIT may be need to generate significant improvements in lipid metabolism (Kessler et al., 2012). Furthermore, significant fat loss or other changes in body composition may also be necessary to achieve such improvements (Kessler et al., 2012).

Finally, HIIT may also have some effect in reducing the systolic blood pressure of persons with hypertension (Madsen et al., 2015). Diastolic pressure is also affected, as is arterial stiffness in general, but some exceptions have been observed (Sijie et al., 2012). One contributing mechanism could be the increased shear stress induced in blood by HIIT. That may enhance nitric oxide bioavailability and promote vasodilation (Ciolac et al., 2010).

1.4 The beneficial effects of water

Many researchers have shifted focus to aquatic exercise for health promotion, and the number of publications related to aquatic exercise has grown rapidly over the past three decades. (Zhou et al., 2021). Much of the benefit of water exercise arises from the physical properties of water.

1.4.1 Hydrostatic pressure

Hydrostatic pressure on the blood vessels leads to central hypervolemia and a cascade of physiological changes including increased venous return, increased stroke volume and cardiac output and reduced lung volumes (Hall et al., 1998). The depth of immersion is crucial, and directly related to the extent of central hypervolemia and physiological change. Pressure on interstitial tissue leads to a fluid shift from the interstitial compartment to the plasma compartment of extracellular fluid (Boussuges et al., 2009).

Pressure at the feet when standing in water is approximately 10% greater than on the upper body (Becker et al., 2009). This is clinically relevant, as immersion is more effective in reducing lower limb swelling when compared with upper limb swelling Figure 1.3.

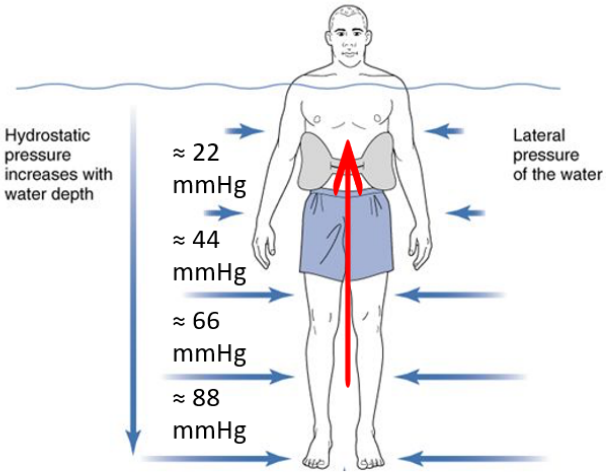


Figure 1.3 Hydrostatic pressure increases with water depth (Becker, 2009)

1.4.2 Buoyancy

Buoyancy can provide both resistance to and assistance with movements. The body's density is very nearly the same as that of water, so the apparent weight in water is nearly zero. With lungs full of air, it becomes negative and the body floats. Weight bearing is directly proportional to the depth of immersion although will vary a little from person to person depending on their body shape and density (Hall et al., 1990). It depends primarily on the depth of submersion. When immersed up to spinal process C7, about 8% of the body's weight remains supported through feet. Submerged to the xiphoid process the corresponding percentage is about 28% for females and 36% for males. Immersed to the to the anterior superior inferior spine the percentages are about 47% for females and 54 % for males (Hall et al., 1990). The remarkable decrease in perceived limb weight when the body is submerged in water is effectively utilized in aquatic physiotherapy. Individuals such as the elderly who are unable to engage in exercises against gravity outside of water, either due to weakness, pain, or the inability to support their body weight, can often perform strengthening exercises by leveraging the buoyancy of their limbs and body during immersion in a warm pool. The buoyancy initially aids movement and later serves as resistance during their rehabilitation process.

Such a reduction of weight bearing stress on the body may even improve the ability to perform some joint movements (Miyoshi et al., 2005). For the elderly and some persons with chronic health conditions, this can allow improvements in motor control, flexibility, range of motion, strength, and walking (Johnson et al., 1998). Improvements in strength, ventilation capacity and aerobic fitness inevitably improve one's quality of life and ability in the activities of daily living (Ayme et al., 2015; Bento & Rodacki, 2015b).

1.4.3 Drag

Water's drag force is influenced by both its viscosity and its turbulence. When limbs move through the water faster, turbulence becomes more important because it causes additional drag that becomes significantly greater as the velocity increases. Consequently, changes in movement speed have a substantial effect on the resistance or exercise load encountered in aquatic physiotherapy. As speed doubles, the resistance caused by turbulence quadruples, meaning even a small increases in speed can lead to a considerable rise in turbulence-induced resistance. (S. Heywood et al., 2016). That rise depends on the surface area of the moving part as well as its speed. The physiological properties of water in aquatic physiotherapy enable a more precise quantification of the assistance or resistance associated with the specific mode of aquatic exercise.

Water's resistance to movement demands greater physical exertion, resulting in a greater recruitment of muscular fibres (Yuen et al., 2019). That increases the energy expended during exercise and also glucose uptake due to the translocation of glucose transporters in muscle cells. These effects are particularly beneficial for individuals with metabolic disorders (Little et al., 2011).

Water's resistance to movement calls for greater physical effort than for the same activity on land. That means a greater recruitment of muscular fibres, greater energy expenditure and increased metabolism. This is particularly helpful in dealing with excess weight, obesity, insulin resistance and type 1 and 2 diabetes mellitus (Nagle et al., 2019).

Aquatic resistance training has been widely examined in recent years. The research has confirmed increased maximum dynamic strength in the lower limbs (Colado & Triplett, 2009) (Barbosa et al., 2009). Resistance exercise has been shown to increase power and, as a consequence, improve physical fitness, health, and functional autonomy (Bayles & Swank, 2018). The density of water generates increased muscle strength because water generates resistance 900 times greater than in air (McGinnis, 2020).

Resistive devices further improve muscular tension when they are moved against water due to the drag force (Figure 1.4). The drag opposes an object's direction of movement, and it can work both in front of and behind the object being moved (Pöyhönen et al., 2000). The magnitude of the drag force depends primarily depends on the object's surface area and shape, but it is also influenced by the velocity of movement, known as exercise cadence. An increase in velocity exponentially amplifies the drag force. The drag force exerted by water engages more numerous motor units with higher excitation thresholds. This can be advantageous for individuals who have difficulty performing similar exercises on land.

Figure 1.4 Water Resistive Exercise

1.4.4 Water immersion

Studies have highlighted the significance of immersing the body to the approximately the level of the xiphoid process during HIIT workouts. The water's gravity resistance can diminish the joint loading exerted by gravity during land-based exercises by up to 60%.

Beyond that, when the water reaches the thorax, its pressure can increase ventilatory effort, particularly during the inspiration phase, eventually resulting in enhanced ventilatory capacity (Nagle et al., 2019; Nagle, Sanders, & Franklin, 2017).

The hydrostatic pressure gradients from water immersion also redistribute blood towards the heart. Approximately 700mL of blood is redistributed from the limbs to the heart. That can cause as much as a 30% increase in stroke volume and cardiac output, a 10% increase in blood flow to the cerebrum and a reduced heart rate (Hall et al., 1990). There is evidence that exercise while immersed in water augments cerebrovascular perfusion compared to intensity-matched land-based exercise (Pugh et al., 2015). That may explain the limited evidence that water immersion can improve the cognitive performance of older adults. Bressel and his colleagues have shown that older adults made fewer cognitive errors on an auditory vigilance task while chest-deep in water compared to on land. and listening errors were 111% greater on land. This was true for the single task of just listening while sitting, and also for the dual task of maintaining standing balance while listening (Bressel et al., 2019).

1.4.5 Water temperature

Water temperature may influence the physiological responses to AHIIT (Hall et al., 1990). Water slightly below body temperature improves heat dissipation, preventing overheating during exercise. Warm water improves joint warming, which can reduce the perception of pain in injury rehabilitation (Nagle et al., 2019). On the other hand, cold water can elicit a reduction in heart rate and cardiac output. Lower muscle temperature decreases enzyme activity in the muscles (Adamczyk et al., 2024). Additionally, cold water immersion can result in increased blood pressure and peripheral resistance by redistributing blood from peripheral tissues to the body's core to maintain body temperature (Reilly et al., 2003; Wilcock et al., 2006). Hence, water temperature should be considered in AHIIT as it affects exercise performance and can limit the beneficial effects of a training program. Some studies recommended water temperatures between 25°C and 28°C for water activities of moderate to vigorous intensity (Nagle, Sanders, & Franklin, 2017).

1.5 Aquatic Exercise and its Benefits

Many find high intensity exercise unpleasant (Biddle & Batterham, 2015), but performing HIIT in the water may provide them a more enjoyable alternative (Nagle et al., 2019). If so, they may be more likely incorporate AHIIT into their weekly routine (Nagle, Sanders, & Franklin, 2017). It could also be a useful alternative for persons whose movements on land are limited by pain or other difficulties.

AHIIT can be tailored to accommodate factors such as diet, age, degree of training, and genetic factors. Volume and intensity are a program's primary tailoring variables, so they are researchers' and physical trainers' focus when designing training programs (Venables et al., 2005).

Water immersion reduces joint loading, the buoyancy helps people with limited mobility and/or weakness to move (Becker, 2009). After an injury or in some other conditions, pain may require reducing joint loading. An aquatic exercise program can then be effective for maintaining or even improving aerobic conditioning (S. M. Heywood et al., 2016). If the reduced loading makes exercising more feasible, successful and satisfying, that is likely to lead to better exercise compliance (Barker et al., 2014b). The aquatic environment can also allow those with limited mobility to train at higher intensity than they could on land (Waller et al., 2014). Anywhere where training in water will reduce some of these barriers to exercising, it will encourage better exercise compliance and physical activity in general.

1.5.1 Forms of aquatic exercise

Walking is a form of exercise widely practiced on land because it is so accessible. However, older adults may face challenges in completing walking exercises due to the impact forces involved, which can lead to pain and other difficulties. But work by Haynes and his colleagues has confirmed that aerobic fitness can improve regardless of whether walking is performed in water or on land (Haynes et al., 2020b). Aquatic treadmill exercise is capable of eliciting submaximal and maximum cardiovascular responses similar to those from traditional treadmill exercise on land (Greene et al., 2011b). When considering exercise options for clinical populations with musculoskeletal problems, it is worth considering arranging reduced weight-bearing. In this regard, the buoyant unloading provided by treadmill exercise in water could serve as a viable alternative to traditional treadmill exercise on land (Figure 1.5).

Figure 1.5 Aquatic Treadmill Exercise

Deep water running (DWR) is another beneficial mode of increased physical activity. DWR can be defined as running with 70% of the body immersed, submerged to shoulder level, with or without a floatation device (Reilly et al., 2003). It simulates land running movement patterns without weight bearing and vertical ground reaction forces. That reduces joint loading and the potential for musculoskeletal injury (Killgore, 2012a; Silva, Dias, Dela Bela, et al., 2020) (Figure 1.6 and Figure 1.7).

Figure 1.6 Deep water Running Form (Pictorial)

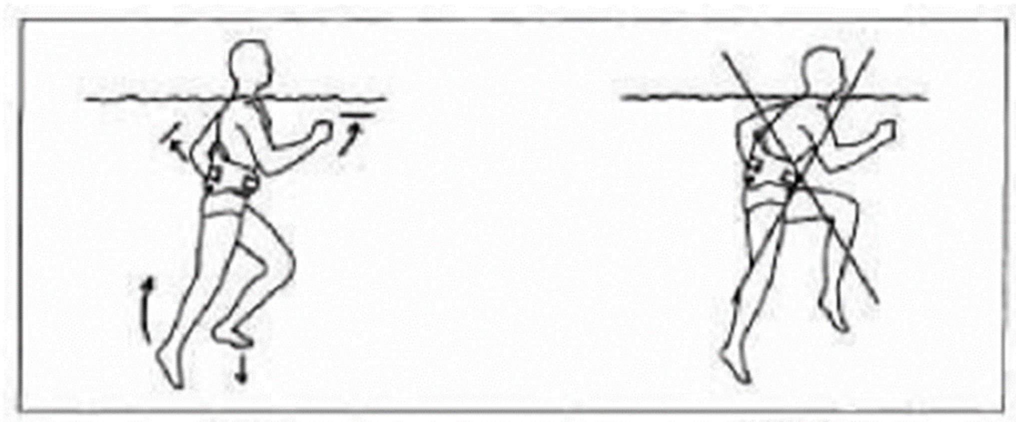


Figure 1.7 Deep water Running Form (Real)

Running in deep water reduces the heart rate, increases energy expenditure, and dissipates body heat when compared to land based exercises (Pendergast & Lundgren, 2009a). The speed of movement influences the drag force, so the level of resistance overload depends on the speed of movement (Becker, 2009). That is the key to exercise program progression in DWR. At a given speed, limbs with a larger surface area experience greater loading (Colado & Triplett, 2009).

Water's resistance to movement demands greater physical effort, which means a greater recruitment of muscular fibres, increased energy expenditure and accelerated metabolism. This is particularly favourable for people with metabolic disorders such as overweight, obesity, insulin resistance or diabetes (Nagle et al., 2019). Aquatic resistance training has been widely examined in recent years, and those studies have documented increases in the maximum dynamic strength of the lower limbs (Colado & Triplett, 2009) (Barbosa et al., 2009). Resistive exercises increase power and, as a consequence, improve physical fitness, health, and functional autonomy (Bayles & Swank, 2018). The density of water generates increased muscle strength because water generates resistance 900 times greater than in air (McGinnis, 2020). And when resistive devices are used, they create muscular tension when they are moved against the water's drag force. The drag opposes an object's direction of movement, and it can act both in front of and behind the object being moved (Pöyhönen et al., 2000). The magnitude of the drag force depends primarily on a device's surface area and shape, but it is also influenced by the speed of movement, known as the exercise cadence. Drag increases exponentially with velocity. The drag force exerted by water engages greater number of motor units with higher excitation thresholds. This can be advantageous for individuals who have difficulty in performing similar exercises on land.

1.5.2 Types of AHIIT exercise

Cycle ergometers and treadmills are the most commonly used equipment when HIIT is performed on land, because they allow quite precise evaluation of the training stimuli during the sessions (Kilpatrick et al., 2014). However, these devices are relatively little used in AHIIT due to the requirement for equipment specifically designed to withstand the corrosive effects of water. Such equipment is more expensive and less available.

Instead, one application of AHIIT is deep water running. DWR involves the use of a floatation vest or belt to eliminate contact with the bottom of the pool floor. The foot strike impact experienced when running on land can be up to 10 times a person's body weight, depending on the surface. That is the cause of a significant number of running injuries. DWR therefore offers a lower risk of injury compared to land-based running and is also more economically accessible for the general population (Dale, 2007).

Some researchers seeking to administer maximum and/or submaximal loads have reported using short periods of high intensity work generally shorter than 30s (Aboarrage Junior et al., 2018a; Gi Broman et al., 2006; Sosner et al., 2019; Sosner et al., 2016), while others have used1 to 4min bouts, still at high intensity (L. S. Andrade, S. S. Pinto, et al., 2020a; Costa et al., 2020; Costa et al., 2018; Handa et al., 2016; Reichert et al., 2016). Only a limited number of studies have examined various HIIT programs conducted on a hard surface. Those studies suggest that the primary distinction between implementing short or long periods of intermittent workload lies in the fact that short protocols require less training time per session. However, it has been found that long protocols yield greater improvements in VO_{2max}, body mass, visceral fat mass, and insulin resistance (Sun et al., 2019; Tong et al., 2018). And it has been shown that active recovery during an HIIT program may have some additional benefits compared to passive recovery (Zouhal et al., 2024).

1.5.3 Prior Research on Aquatic High-intensity Interval Training

Multiple studies have documented differences in the characteristics of low-intensity bouts within high-intensity interval training looking at various combinations of active or passive recovery periods. The research indicates that incorporating active rest periods during HIIT enhances cardiorespiratory capacity better than using passive recovery periods (Zouhal et al., 2024). Protocols with active rest periods demonstrate more efficient glycolytic metabolism due to an increase in the production of hormones associated with glucose metabolism (Abderrahman et al., 2018). No difference between the recovery protocols has been observed in terms of the elimination of blood lactate during HIIT (Germano et al., 2022). Prolonged bouts of active recovery do, however, more effectively eliminate lactate after an HIIT session.

In clinical practice, VO_{2max} is widely regarded as a reliable indicator of the intensity of a training session, and it is widely used. It serves as a measure of the capacity of both the cardiovascular and respiratory systems and their response to physical activity. Assessing VO_{2max} enables the determination of the oxygen transport capacity of the heart, lungs and circulatory system, and their ability to transport other compounds to the muscles during exercise as well (American College of Sports & Medicine, 2018). The assessment is, however, complex, costly, and not very accessible to the general population. AHIIT often does not rely on VO_{2max} for monitoring and controlling workloads. Heart rate and perceived exertion are often used instead (Alexandre et al., 2012; Prinsloo et al., 2014; Schneider et al., 2018).

1.5.4 AHIIT's health effects

Research has shown that AHIIT can improve agility, balance and flexibility as well as strength (Depiazzi et al., 2018). It can also improve BP, lipid profile, bone mineral mass, ventilatory capacity, VO_{2max} and HR during exercise, and all at a lower volume than other exercises (Depiazzi et al., 2018; Moreno et al., 2022). Even a single session of AHIIT can normalize the BP of a person with high a baseline value (Junior et al., 2018). Although the information is limited, there are findings suggesting that AHIIT may be an effective nonpharmacological tool for controlling several health disorders in adults (Aboarrage Junior et al., 2018a; L. S. Andrade, S. S. Pinto, et al., 2020a; Costa et al., 2020; Costa et al., 2018; Handa et al., 2016; Reichert et al., 2016; Sosner et al., 2019; Sosner et al., 2016).

The American Heart Association has reviewed 23 studies involving 1177 participants and concluded that HIIT has demonstrated a relatively low risk of major cardiovascular events in the rehabilitation of heart disease and heart failure patients (Wewege et al., 2018). Regarding AHIIT, there is no evidence that AHIIT is unsafe for persons who are not athletic (Depiazzi et al., 2018). This is consistent with the results of other systematic reviews which have found very few adverse events associated with high-intensity interval training exercise and aquatic exercise (Weston et al., 2014).

Injury is of course an adverse event of concern. The data are limited, but overall there have been fewer adverse events reported in connection with AHIIT compared to L-HIIT. This suggests that the aquatic environment may provide a safer setting for high-intensity interval training, but further research is needed to confirm this.

AHIIT can be a beneficial alternative to L-HIIT, especially for older adults who may struggle to reach higher levels of function or exercise intensity on land. (Moreno et al., 2022). This is especially so given AHIIT's better adherence (84 to 100% in research studies) (Costa et al., 2018; Munukka et al., 2020).

While adherence rates among participants in aquatic high-intensity interval training (AHIIT) are promising, a broader evaluation of AHIIT's effectiveness in improving cardio-metabolic outcomes, cognitive function, and perceptual responses in aged women compared to L-HIIT is necessary. Therefore, AHIIT may serve as a valuable alternative for aged women who cannot perform land-based exercises due to physical limitations or barriers related to loading. A more comprehensive discussion of the research gaps in AHIIT (addressed in Chapter 2) is also warranted.

Chapter 2 An Outline of this Thesis

2.1 Research gaps

As explained in Chapter 1, AHIIT can be a valuable alternative for HIIT for physically inactive aged women, especially those dealing with barriers to exercise performance such as pain or weakness. The aquatic environment may also offer people with limited mobility the ability to train at higher intensities than on land (Waller et al., 2014). There may be a potential for reducing some of the barriers to land-based exercise in the water, which would encourage better longer-term adherence to training programs. But these advantages have not yet be demonstrated in the specific case of aged women.

A second gap in the previous research is that despite the growing interest in DWR in cardiovascular training programs for various clinical populations (Dale, 2007; Bojan Jorgic et al., 2012) and although several clinical trials have evaluated the effects of DWR on numerous quality-of-life (QoL) domains (B. Jorgic et al., 2012; Planna et al., 2019) , there has not yet been a systematic examination of the effects of DWR on cardiorespiratory fitness, physical functioning and QoL.

In terms of methodology, to ensure accurate measurement of cardio-metabolic outcomes, previous studies have assessed them using laboratory (or sometimes portable) metabolic analysers. The findings have often been inconclusive, making it difficult to reliably establish valid cardio-metabolic outcomes which can be generalized. There is therefore a need to assess whether other portable metabolic analysers can be used interchangeably with the COSMED K5, currently regarded as the “golden standard” portable (Perez-Suarez et al., 2018). A portable unit suitable for use with water resistant property is badly needed.

Matching intensity prescriptions on land and in the water also remains a work in progress (Bunæs-Næss et al., 2023). The lack of clarity in ensuring matched intensity between these modalities constitutes a significant research gap, with limited evidence quantifying the intensity responses. Additionally, designing a specific AHIIT program that optimizes cardio-metabolic outcomes requires the application of resistance, which can be achieved by increasing cadence and drag on the limbs (Pöyhönen et al., 2002). No study has yet systematically investigated the cardio-metabolic benefits of adding resistance in an AHIIT program. Ensuring matched exercise intensity prescriptions definitely needs further research.

And finally, a review of the prior research shows that the cognitive responses to AHIIT and L-HIIT have never been properly compared. There is well-established evidence about the effects of L-HIIT on cardio-metabolism, physical health or perception, but similar evidence regarding AHIIT, and specifically DWR, is lacking (Batacan et al., 2017).

2.2 Null hypothesis

The null hypothesis in this work was that the effectiveness of AHIIT in improving cardio-metabolic health, cognition and other psychological outcomes does not differ significantly from that of L-HIIT when the forms and resistive components of AHIIT are properly considered.

2.3 Objectives

This study's aim was to determine whether and to what extent AHIIT can augment the cardiac functioning, metabolism, cognition and other psychological responses of previously physically inactive aged women. More specifically, DWR was tested as an intervention targeting cardiorespiratory fitness, quality of life and physical functioning. Along the way, that involved work evaluating the validity and reliability of a portable metabolic analyser for use with AHIIT.

Achieving those objectives formed the basis for determined an AHIIT protocol that matched the intensity of land-based HIIT for improving cardio-metabolic outcomes. The matching involved adding resistance to AHIIT. The effect of DWR on the cardio-metabolic health, cognition and psychological responses of physically inactive aged women were then compared with those of matched L-HIIT, aimed at testing the null hypothesis.

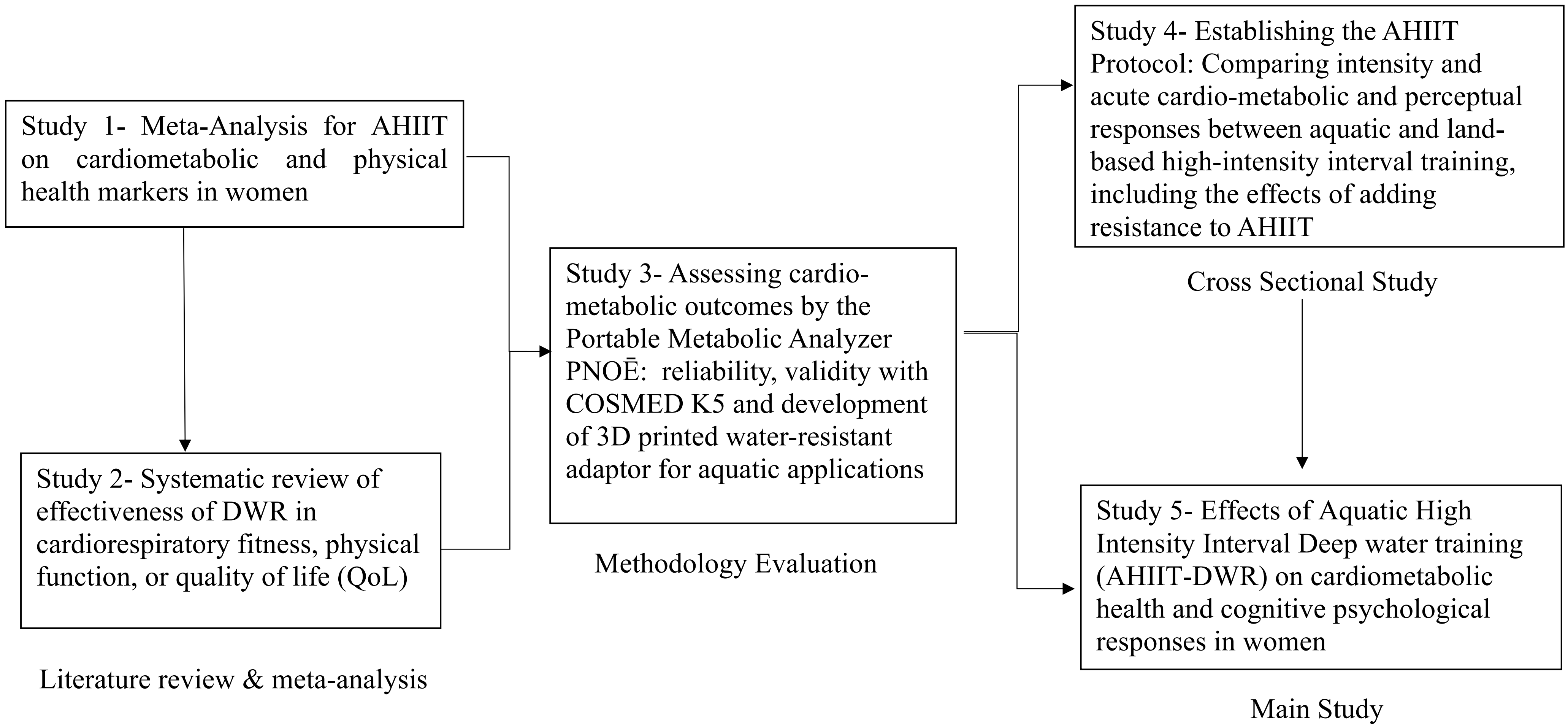
2.4 An outline of the dissertation

This work will be reported as five inter-related studies. The first (presented in Chapter 3) will be a systematic review and meta-analysis of previous scholarly work on the effects of AHIIT on cardio-metabolic and physical health markers in women (termed Study 1). (That chapter has previously been published in the *Journal of Exercise Science and Fitness* (https://doi.org/10.1016/j.jesf.2022.02.001)
and presented as a poster at the 7th HKASMSS Student Conference on Sports Medicine, Rehabilitation and Exercise Science 2021 cum AFSM Scientific Symposium 2021.) Study 2 was a review of the effects of DWR on cardiorespiratory fitness, physical functioning and

quality of life compared with other interventions or control groups without exercise (see Chapter 4, which has previously been published in the *International Journal of Environmental Research and Public Health* (https://doi.org/10.3390/ijerph19159434) And presented at the 2021 annual conference of the Hong Kong Physiotherapy Association). In Study 3 (Chapter 5) the utility of a water-resistant portable metabolic analyser was compared with that of the COSMED K5. The instrument was used in Study 4 (described in Chapter 6) to establish an AHIIT protocol matched in exercise intensity with an L-HIIT routine. The effects of L-HIIT and AHIIT with resistance were then compared in terms of improving cardio-metabolism. In the fifth study the effects of 8 weeks of DWR training were compared with those of a matched L-HIIT program in terms of cardio-metabolic health outcomes, cognition and psychological responses. The subjects were elderly Chinese women who previously had been physically inactive. That work is described in detail in Chapter 7.

This outline is summarized in Figure 2.1.

Figure 2.1 Thesis study design



Chapter 3 The Effect of Aquatic High-intensity Interval Training on Cardio-metabolic and Physical Health Markers in Women: A Systematic Review and Meta-analysis

This chapter has been published in peer-reviewed journal.

Kwok, M. M. Y., Ng, S. S. M., Man, S. S., & So, B. C. L. (2022). The effect of aquatic high intensity interval training on cardio-metabolic and physical health markers in women: A systematic review and meta-analysis. *Journal of exercise science and fitness*, *20*(2), 113-127. https://doi.org/10.1016/j.jesf.2022.02.001

This chapter has been presented in the below conference.

Kwok MMY, Ng SSM, Man SS, So BCL (2021, December). “The effect of aquatic high intensity interval training on cardio-metabolic and physical health markers in women: A systematic review and meta-analysis.” In 7th HKASMSS Student Conference on Sports Medicine, Rehabilitation and Exercise Science 2021 cum AFSM Scientific Symposium 2021: Innovations in Sports Technology: Advancing Sports Medicine and Sports Science (Poster Presentation)

3.1 Abstract

Performing high-intensity interval training in an aquatic environment can be an alternative to land-based HIIT. AHIIT offers a great option for cardio-metabolic and physical training. Those who have participated in AHIIT studies were predominantly women, so this review focused on females, who are recognized to have a higher risk of cardio-metabolic disorders.

AHIIT is considered to be effective because many physiological benefits are related to the physical properties of water. There is well-established evidence about the effects of HIIT on cardio-metabolic or physical health, while similar evidence regarding AHIIT is lacking. This study therefore aimed at a systematic review and meta-analysis of randomized and controlled trials assessing the effect of aquatic high-intensity interval training on cardio-metabolic and physical health markers in women.

The systematic search for randomized and controlled trials that related to AHIIT and cardio-metabolic health and physical functions used seven databases (MEDLINE, PubMed, SPORTDiscus, Cochrane, Embase, CINAL complete, and PsycINFO). The Physiotherapy Evidence Database (PEDro) score was used to evaluate the methodological quality of the studies. Clinical trials compared AHIIT with a control group that received no exercise training. Only randomized and controlled trials published in English in which the participants were women aged >18 years were included. The outcome of interest was the change in cardio-metabolic and physical health markers.

Among the 242 articles screened, 18 articles (13 trials) were included in this meta-analysis comparing AHIIT (n = 261) with a control group (n = 215). The median PEDro score was 5.5 out of 10 (range, 4–8). AHIIT significantly improved peak oxygen uptake (Hedges' g =0.610; 95% CI 0.277–0.943; $p \leq 0.001$), reduced resting heart rate (Hedges' g =0.495; 95% CI -0.866 to 0.124; $p \leq 0.05$), as well as sit-to-stand test times (Hedges' g =0.548; 95% CI 0.019 to 1.077; $p \leq 0.05$).

From these findings, we conclude that AHIIT has a moderate effect in improving cardio-metabolic and physical health markers in women.

3.2 Introduction

Cardio-metabolic health is an important issue to address. Identifying cardio-metabolic risk factors for mortality, morbidity and disability can reduce 73% of all non-pandemic related deaths and 60% of the global health burden (*WHO guidelines on physical activity and sedentary behaviour*, 2020). Globally, more than a quarter of the world's adult population (1.4billion adults) is insufficiently active (Pedro C. Hallal et al., 2012a). The association between physical inactivity and increased mortality appears with a reduction in cardio-metabolic health (R. S. Paffenbarger et al., 1986). Thus, identifying and reducing modifiable CMR factors, namely physical inactivity, will have an essential effect on diseases that would otherwise raise enormous public health concerns.

Women are relatively less physically active than men. Among adults aged 18 years or older, 31.7% of women and 23.4% of men fail to achieve physical activities up to or exceeding the WHO recommended level of at least 600 METs weekly (Guthold et al., 2018). In addition, women have a higher prevalence of lower limb osteoarthritis and obesity; women over 45 years old have been found to be much more obese than the rest of the population, with a body mass index surpassing 25kg/m^2 in 47% of them and surpassing 30kg/m^2 in 9% (L. Andrade et al., 2020; Ng et al., 2014).

Public health promotions have highlighted interval training as an alternative exercise strategy that has gained popularity among the general population (Thompson, 2019). Interval training typically involves bouts of aerobic exercise at moderate to high intensity for short durations (20–240 seconds), with intervals of rest or active recovery at a lower intensity between bouts (60–360 seconds) (Gibala & Jones, 2013). High-intensity interval training, which is performed at alternating intensities near an individual's maximum oxygen capacity, is often conducted at an intensity close to that which elicits ≥80% of a person's peak heart rate. This approach may involve light recovery exercise or no exercise between intervals (Weston et al., 2014), and it offers numerous significant health benefits, including improvements in cardiorespiratory fitness, aerobic capacity, and endurance by stimulating cardio-metabolic changes (Gibala & Jones, 2013; Karlsen et al., 2017). Aquatic high-intensity interval training has emerged as a viable option for individuals who may be more likely to incorporate HIIT into their lifestyles while immersed in an aquatic environment (Nagle, Sanders, & Franklin, 2017). It offers a great option for cardio-metabolic and physical training.

AHIIT is considered to be effective because many physiological benefits are related to the physical properties of water (Becker et al., 2009). Water's buoyancy reduces discomfort and stress placed on the joints. In addition, water's hydrodynamics resist movement, increasing the development of muscle strength (Harrison et al., 1992; Rana et al., 2007). With water's cushioning effect, AHIIT can be a safe option for many, as it minimises some of the hindrances to exercise associated with land-based training, such as pain, fear of movement, and poor balance (Barker et al., 2014b).

There is well-established evidence about the effects of HIIT on cardio-metabolic or physical health, while similar evidence regarding AHIIT is lacking (Batacan et al., 2017). Only one systematic review of the effects of AHIIT has been published (as of 2022). Its outcome measures concerned aerobic capacity, muscle strength and body composition (Depiazzi et al., 2018). Evidence regarding other cardio-metabolic health markers, including lipid profile, blood pressure, and bone mineral density, is lacking. Given this knowledge gap and the lack of evidence on cardio-metabolic health, alongside the growing application of AHIIT, the objective of this study was to investigate the effects of AHIIT on the improvement of cardio-metabolic and physical health markers in women via reviewing the existing literature.

3.3 Methods

A complete search of randomized and controlled trials performed up to January 2021 was conducted. The reporting in this study adheres to the preferred reporting items for systematic reviews and meta-analysis guidelines (Moher, Liberati, Tetzlaff, & Altman, 2009). This review protocol was registered in the PROSPERO database of systematic reviews (CRD42021229631).

3.3.1 Study selection

Two reviewers screened the titles and abstracts of the articles. The reviewers independently reviewed the full texts of the articles and agreed on their eligibility for inclusion in the review. If disagreement occurred, a third reviewer (SN) resolved any discrepancies between the two reviewers (MK, BS) or the article was removed from the review.

3.3.2 Search strategies

An exhaustive search of the literature was conducted to identify publications related to the effectiveness of AHIIT. Articles were retrieved from seven electronic bibliographic databases: MEDLINE, CINAHL, PubMed, SPORTDiscus, PsycINFO, Embase, and the Cochrane Library. The search strategy covered the following medical subject headings or key words: (1) aquatic or water exercise, hydrotherapy, water immersion, or head-out aquatics; (2) high-intensity interval training, intermittent exercise, or interval training; (3) cardio-metabolic health, cardiorespiratory or metabolic markers, physical, or physical health; and (4) women or female only.

3.3.3 Inclusion criteria

Articles were included based on the following criteria: 1) All participants must have been women who were ≥ 18 years old; 2) the study was published in full and in English; 3) the study must have been a randomised and controlled trial; 4) the outcome measures must include cardio-metabolic or physical health markers; and 5) head-out aquatic exercise was assessed. For the intervention to be considered high intensity, the work rate prescribed needed to elicit: i) > 75% of maximum oxygen capacity (VO_{2max}); ii) HR max > 80–95%; and iii) rate of perceived exertion of 15 or more on the BORG exertion scale.

The definitions used are based on a general classification scheme for interval training suggested by Weston and his colleagues (Weston et al., 2014). The control groups included were defined as not receiving exercise training.

3.3.4 Exclusion criteria

Studies were excluded if: 1) the participants were mixed with men; 2) the intervention was conducted for less than two weeks (the minimum time requirement to observe meaningful physiological changes in interval training); or 3) the intervention was not interval training.

3.3.5 Outcome measures

The outcome measures for this study were cardio-metabolic and physical health markers. The cardio-metabolic health markers included peak oxygen uptake (VO_2 peak), resting heart rate, systolic blood pressure (SBP), diastolic blood pressure (DBP), body fat percentage, high-density lipoprotein, and low-density lipoprotein. In addition, bone mineral density, knee flexion and extension strength and the sit-to-stand (CS) test times were the physical health markers measured.

3.3.6 Quality assessment

Two researchers independently graded the quality of the included studies using the Physiotherapy Evidence Database scale (Harrison et al., 1992). Items related to internal validity and quality of reporting were scored across 10 criteria; “yes” was given a score of one and “no” a score of zero. When data were unavailable or unclear, the item was assigned a score of zero. Reliability and validity for the PEDro scale have been established (Barker et al., 2014a).

3.3.7 Data extraction

The same two researchers independently extracted data regarding the methodology and outcome measures using a standardised data extraction sheet.

3.3.8 Meta-analysis

The meta-analysis was carried out using version 3.0 of the Biostat software suite (Biostat, Englewood, USA). The mean value and standard deviation of each study were input for the software. If the values were not available, the authors were contacted by email to obtain the raw data. If no raw data were available, the study was excluded from the meta-analysis. A random-effects model was used to estimate the distribution of observed effect sizes.

3.3.9 Effect size

The effect size in terms of Hedges' g and its 95% confidence interval were computed in all of the meta-analyses, as the trials used various methods to assess cardio-metabolic and physical health markers. Hedges' g is a variation of Cohen's d, which corrects for a possible bias in small sample sizes (Higgins, 2019). Effect size was quantified as large (> 0.8), medium (0.5–0.8), small (0.2–0.5), or not significant (< 0.2) as a general rule of thumb, as suggested by Cohen (1988).

3.4 Publication bias

Egger's regression asymmetry test was performed to test for any publication bias. A *p*-value < 0.1 (two-tailed test) indicated the presence of publication bias (Egger et al., 1997).

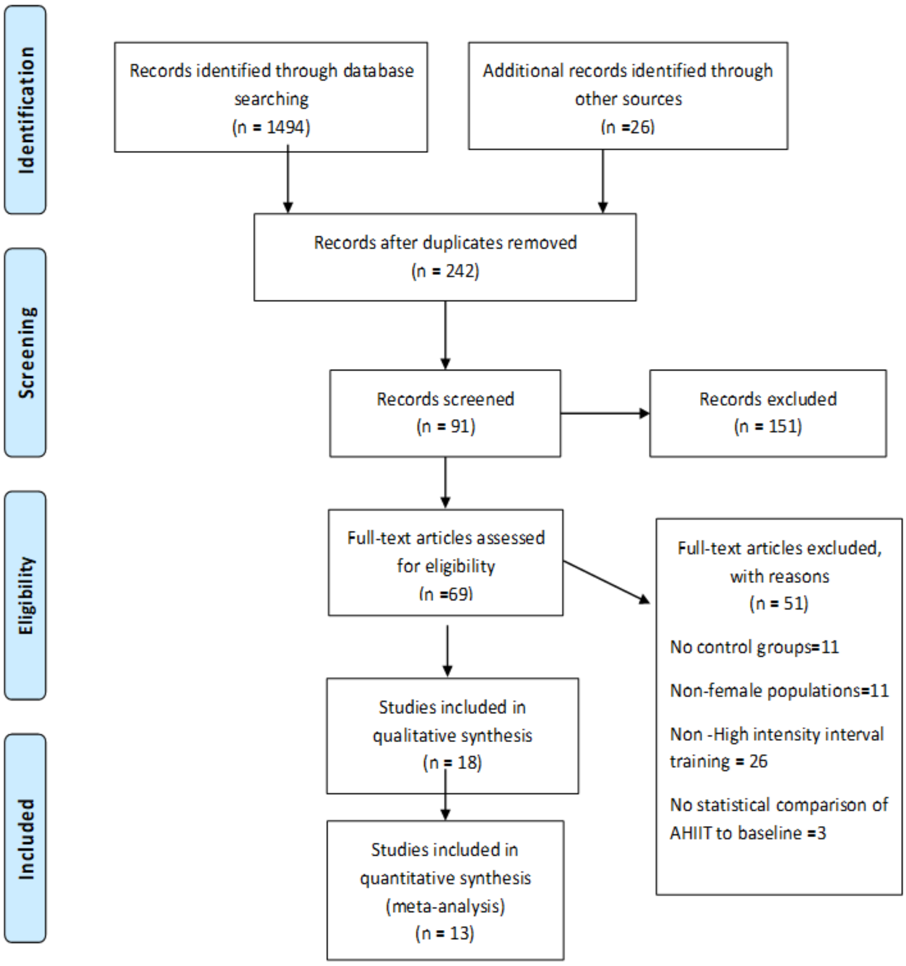
3.5 Results

3.5.1 Study selection

The review search identified 1,520 articles published between 1994 and January 2021, of which 1,278 articles were excluded because they were not relevant to the scope of this review or were duplicates. Out of the remaining 242 articles, 151 were excluded when the abstracts were reviewed, and 51 articles were excluded after reviewing the full texts because they did not measure the outcomes of interest relevant to the review, and/or the targeted populations did not meet the specified age and gender criteria. A total of 18 articles were ultimately identified as being relevant to the study (Aboarrage Junior et al., 2018b; L. Andrade et al., 2020; L. S. Andrade, S. S. Pinto, et al., 2020b; Bento & Rodacki, 2015a; Gi Broman et al., 2006; Connolly et al., 2016; Costa et al., 2018; Denise Fernandes Moreira et al., 2013; Junior et al., 2018; Mohr et al., 2015; Mohr et al., 2014; Moreira et al., 2014; Munukka et al., 2020; Munukka et al., 2016; Nordsborg et al., 2015; Rýzková et al., 2018; Samadi et al., 2019; Waller et al., 2017). Thirteen of these articles were included in the final analysis *(*Figure 3.1).

Figure 3.1Prisma flow diagram

PRISMA 2009 Flow Diagram



3.5.2 Study characteristics

Descriptions of the participants and outcomes of the studies are summarised in Appendix B. All of the included studies employed AHIIT as an intervention. The characteristics of the interventions are summarised in Table 3.1.

Table 3.1 Characteristics of Aquatic High Intensity Interval training

Author	Type of water training	Characteristics of AHIIT intervention			Pool (Type & temperature)	Depth (immersion level)	Description of AHIIT (work-rest ratio)	High intensity measurement	Adverse events recorded
		Times per week	Number of weeks	Time for each session (min)					
L. S. Andrade et al., 2020a	Water based exercise program (stationary running, frontal kick and cross-country skiing)	2	12	44	Pool of a sports club linked to university of water temp 30º C -32º C,	Between xiphoid process and shoulder	2min-2min	RPE 17.3-18.9 corresponds to 80-89% VO2 peak	Nil
L. S. Andrade et al., 2020b	Water based exercise program (stationary running, frontal kick and cross-country skiing)	2	12	44	Pool of a sports club linked to university of water temp 30º C -32º C,	Between xiphoid process and shoulder	2min-2min	RPE 17.3-18.9 corresponds to 80-89% VO2 peak	Nil
Bento et al 2014	1. Upper and lower limbs water aerobic exercise 2. Strengthening lower limb muscles using aquatic resistance devices	3	12	60	Indoor swimming pool of water temp 28º C -30º C	Xyphoid process	1.40s-20s 2.2-3 sets of exercise with 8-12 RM and 2 mins rest	Exercises executed without the feet contacting the bottom of the pool to increase the exercise intensity	NR
Broman et al 2006	Deep water running with vest	2	8	48	27º C	Shoulder level	10min-2min	>75% maximal HR	Nil
Connolly et al, 2016	All out free style swimming (front crawl)	3	15	15-25	NR	Head out swim	30s-2min	Mean HR and peak HR are 86 ±3 and 96±1 % HR max in high intensity interval group	Nil

Author	Type of water training	Characteristics of AHIIT intervention			Pool (Type & temperature)	Depth (immersion level)	Description of AHIIT (work-rest ratio)	High intensity measurement	Adverse events recorded
		Times per week	Number of weeks	Time for each session (min)					
Costa et al 2018	Water aerobic exercises (9 upper limbs and lower limbs exercises)	2	12	45	Swimming pool of the university	Xiphoid process	NR	BORG 15	NR
Aboarrage Junior et al 2018	Jump based aquatic exercise program	3	24	30	Aquatic centre of pool temp 29 º C	Xiphoid process	30s-30s	All-out intensity, self-selected maximal intensity	Nil
Junior et al 2018	Aquatic exercises (a) jumping jacks (b) horizontal adduction and abduction of the shoulder; (c) stationary running with knee	NR	NR	45	Pool temp 29 º C	Xiphoid process	30s-1min	60-89% HRR (Vigorous intensity)	Nil
Mohr et al 2015	All out free style swimming (front crawl)	2.9 (0.1)	15	15-25	NR	Head out swim	30s-2min	Mean HR and peak HR are 86 ±3 and 96±1 % HR max in high intensity interval training group	Nil
Mohr et al 2014	High Intensity Sprint swimming	2.9 (0.5)	15	15-25	NR	Head out swim	30s-120s	Mean HR and peak HR are 86 ±3 and 96±1 % HR max in high intensity interval training group	Nil

Characteristics of

Author	Type of water training	AHIIT intervention Times per week	Number of weeks	Time for each session (min)	Pool (Type & temperature)	Depth (immersion level)	Description of AHIIT (work-rest ratio)	High intensity measurement	Adverse events recorded
Moreira et al 2014	Strength and power exercise and aquatic cardiorespiratory training	3	24	50-60	Covered swimming pool, with water temp 30-31º C	Water depths 1.1-1.3m	1. Two sets of 30s each 2. Three sets of 20 s each, 3. Four sets of 15 s each, 4. 5 sets of 10s each	1. 60% HR max in 16 mins of session 5-9, 2. 70% HR max in 13 mins of session in weeks 10-14, 3. 80% HR max in 9 mins session in weeks 15-19, 4. 90% HR max in 7 mins session in weeks 20-24,	Nil
Moreira et al 2013	Strength and power exercise and aquatic cardiorespiratory training	3	24	50-60	Covered swimming pool, with water temp 30-31º C	Water depths 1.1-1.3m	1. Two sets of 30s each 2. Three sets of 20 s each, 3. Four sets of 15 s each, 4. 5 sets of 10s each	1. 60% HR max in 16 mins of session 5-9, 2. 70% HR max in 13 mins of session in weeks 10-14, 3. 80% HR max in 9 mins session in weeks 15-19, 4. 90% HR max in 7 mins session in weeks 20-24,	Nil
Munukka et al 2019	Lower limbs aquatic resistance training	2.6(0.5)	16	60	Heated pool, 30-32º C	NR	2 sets x 30 reps to 3 sets x 30-45 reps, with rest period 30-45s	-Average Intensity of each session RPE 15 (12-17), HRmax 144 (12) bpm -Intensity of training set at "as hard and fast as possible	-Two medical consultations (bilateral knee pain and dyspnoea) after training
Munukka et al 2016	Lower limbs aquatic resistance training	2.6(0.5)	16	60	Heated pool, 30-32º C	NR	2 sets x 30 reps to 3 sets x 30-45 reps, with rest period 30-45s	-Average Intensity of each session RPE 15 (12-17), Max HR144 (12) bpm -Intensity of training set at "as hard and fast as possible	-Two medical consultations (bilateral knee pain and dyspnoea) after training

Author	Type of water training	Characteristics of AHIIT intervention			Pool (Type & temperature)	Depth (immersion level)	Description of AHIIT (work-rest ratio)	High intensity measurement	Adverse events recorded
		Times per week	Number of weeks	Time for each session (min)					
Nordsborg et al 2015	All out free style swimming (front crawl)	3	15	15-25	NR	Head out swim	30s-2min	Mean HR and peak HR are 86 ±3 and 96±1 % HR max in high intensity interval training group	Nil
Ryzkova et al 2018	Aqua-fitness program: HIIT program	2	10	50	Pool at the university of water-temperature of 28 º C	Central chest area up to shoulder	20s-10s	High intensity training zone >80% HR reserve	NR
Samadi et al 2019	AHIIT training (20 min, quick movements of body)	3	12	30	NR	NR	20s-10s	80-95% HR max	NR
Waller et al 2017	Lower limbs aquatic resistance training	2.6 (0.5)	16	60	Heated pool, 30-32º C	NR	2 sets x 30 reps to 3 sets x 30-45 reps, with rest period 30-45 s in between sets	-Average Intensity of each session RPE 15 (12-17), HR max144 (12) bpm -Intensity of training set at "as hard and fast as possible"	-Two medical consultations (bilateral knee pain and dyspnoea) after training

BORG, Borg scale of perceived exertion; bpm, beats per minute; HIIT, High Intensity Interval Training; HR, heart rate; HRmax, maximal heart rate; Min, minute; NR, not recorded; temp, temperature; RPE, rate of perceived exertion; reps, repetitions; s, second

3.5.3 Quality assessment

Quality assessment of all of the studies resulted in a moderate-quality median PEDro score of 5.5 out of 10 for all of the papers; the highest score was eight (Munukka et al., 2020; Munukka et al., 2016) and the lowest was four (Bento & Rodacki, 2015a; Gi Broman et al., 2006) (Table 3.2). There was no blinding of subjects in any of the studies reviewed due to the nature of intervention studies. Studies with a PEDro scores of seven or above were considered high-quality studies; scores of five and six were considered to indicate moderate quality; scores lower than four were considered poor quality (Moher, Liberati, Tetzlaff, & Altman, 2009). On that basis, four studies were considered high-quality, mainly due to their data analyses (Costa et al., 2018; Moreira et al., 2014; Munukka et al., 2020; Munukka et al., 2016). Twelve studies were of moderate quality. In those the allocations were not concealed and the assessors were not blinded (Aboarrage Junior et al., 2018b; L. Andrade et al., 2020; L. S. Andrade, S. S. Pinto, et al., 2020a; Connolly et al., 2016; Junior et al., 2018; Mohr et al., 2015; Mohr et al., 2014; Moreira et al., 2014; Nordsborg et al., 2015; Rýzková et al., 2018; Samadi et al., 2019; Waller et al., 2017). Two studies were of low quality as a result of a lack of information about the follow-up procedures and blinding of the assessors during the intervention (Bento & Rodacki, 2015a; Gi Broman et al., 2006).

Table 3.2 The PEDro score

PEDro Criterion

Study	A	B	C	D	E	F	G	H	I	J	K	Total Score
L. S. Andrade et al., 2020	1	1	0	1	0	0	1	0	1	1	1	6
L. Andrade et al., 2020	1	1	0	1	0	0	1	0	1	1	1	6
Bento et al 2014	1	1	0	1	0	0	0	0	0	1	1	4
Broman et al 2006	1	1	0	1	0	0	0	0	0	1	1	4
Connolly et al 2016	1	1	0	1	0	0	0	1	0	1	1	5
Costa et al 2018	0	1	1	1	0	0	1	0	1	1	1	7
Aboarrage Junior et al 2018	1	1	0	1	0	0	1	0	0	1	1	5
Junior et al 2018	1	1	1	1	0	0	0	0	0	1	1	5
Mohr et al 2015	1	1	0	1	0	0	0	1	0	1	1	5
Mohr et al 2014	1	1	0	1	0	0	0	1	0	1	1	5
Moreira et al 2014	1	1	1	1	0	0	1	0	0	1	1	6
Moreira et al 2013	1	1	1	1	0	0	1	1	0	1	1	7
Munukka et al 2019	1	1	1	1	0	0	1	1	1	1	1	8
Munukka et al 2016	1	1	1	1	0	0	1	1	1	1	1	8
Nordsborg et al 2015	1	1	1	1	0	0	0	1	0	1	1	6
Ryzkova et al 2018	1	1	0	1	0	0	0	1	0	1	1	5
Samadi et al 2019	1	1	0	1	0	0	0	1	0	1	1	5
Waller et al 2017	1	1	0	1	0	0	0	1	1	1	1	6
												Median PEDro score =5.5 (range, 4-8)

A. Eligible criteria were specified

B. Predictions were randomly allocated groups

C. Allocation was concealed

D. The groups were similar at baseline regarding the most important prognostic indicators

E. There was blinding of all assessors who measured the primary outcome
F. Therapist blinding
G. Assessor blinding
H. Follow-up > 85%
I. Intension-to-treat analysis

J. Between -group statistical comparisons

K. Point measure and measures of variability reported

3.5.4 Effect of AHIIT on cardio-metabolic health markers

The analysis of (L. S. Andrade, S. S. Pinto, et al., 2020b; Gi Broman et al., 2006; Costa et al., 2018; Munukka et al., 2016; Samadi et al., 2019) revealed a significant ($p \leq 0.001$) moderate effect size point estimate of 0.610 in favour of AHIIT compared with the control for peak oxygen uptake (VO_2 peak) (95% CI 0.277 to 0.943) (Figure 3.2). Four studies (L. S. Andrade, S. S. Pinto, et al., 2020b; Gi Broman et al., 2006; Mohr et al., 2014; Rýzková et al., 2018) indicated a significant ($p \leq 0.01$) moderate effect size point estimate of -0.495 (95% CI -0.866 to -0.124) in favour of reducing resting heart rate without significant heterogeneity across the studies (Figure 3.3). However, in the studies of (Gi Broman et al., 2006; Junior et al., 2018; Mohr et al., 2014) the effect size point estimates for DBP and SBP were not significant compared with the controls (Figure 3.4 and Figure 3.5).

For body fat percentage, three studies (Junior et al., 2018; Mohr et al., 2014; Rýzková et al., 2018) produced no significant effect size point estimate in favour of AHIIT compared with the controls, and there was moderate and significant heterogeneity across the studies ($I^2 = 69.197\%$, $p \leq 0.05$) (Figure 3.6).The studies of (Costa et al., 2018; Mohr et al., 2014) both generated point size estimates for HDL and LDL which were not significant with no significant heterogeneity (Figure 3.8) and (Figure 3.7), respectively.

3.5.5 AHIIT and physical outcomes

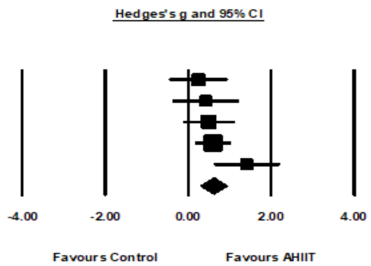
For total body BMD and total femur BMD the studies reported by (Junior et al., 2018; Mohr et al., 2014; Moreira et al., 2014) indicated no significant effect size point estimate in favour of AHIIT compared with the control condition (Figure 3.9) and no significant heterogeneity across studies ($I^2 = 0.000\%$, $P = 0.402$), respectively.

The studies by (L. S. Andrade, S. S. Pinto, et al., 2020b; Bento & Rodacki, 2015a; Munukka et al., 2016) of knee extension strength produced no significant effect size point estimate without significant heterogeneity across the studies (Figure 3.10). The latter two studies also addressed knee flexion strength, and there too there was no significant effect size point estimate in favour of AHIIT compared with the control group. There was again no significant heterogeneity between the studies (Figure 3.12 and Figure 3.11). Two studies (L. Andrade et al., 2020; Junior et al., 2018) used the sit-to-stand test, and they generated a significant ($p \le 0.05$) moderate effect size point estimate of 0.548 (95% CI 0.019 to 1.077) in favour of AHIIT compared to the control group with no significant heterogeneity (Figure 3.13).

Figure 3.2 Meta-analysis and forest plots of AHIIT on VO2 peak

AHIIT overall effect om VO2 peak

Study name	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Andrade 2020 a	Vo2 peak	0.246	0.357	0.128	-0.455	0.946	0.687	0.492
Broman 2006	Vo2 peak	0.424	0.412	0.169	-0.383	1.231	1.030	0.303
Costa 2018	Vo2 peak	0.492	0.315	0.099	-0.125	1.109	1.562	0.118
Manukka 2016	Vo2 peak	0.599	0.221	0.049	0.166	1.033	2.711	0.007
Samadi 2019	Vo2 peak	1.415	0.399	0.160	0.632	2.198	3.542	0.000
		0.610	0.170	0.029	0.277	0.943	3.587	0.000

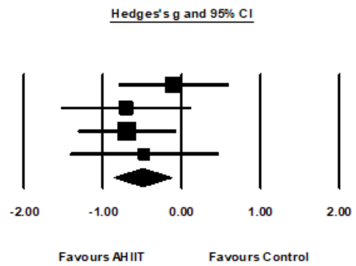


Heterogeneity I^2= 26.56%, p= 0.244
Test for overall effect Z= 3.587 p= 0.000

Figure 3.3 Meta-analysis and forest plots of AHIIT on resting HR

AHIIT overall effect on resting HR

Study name	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Andrade 2020 a	resting HR	-0.101	0.356	0.127	-0.799	0.597	-0.284	0.777
Broman 2006	resting HR	-0.706	0.420	0.176	-1.529	0.116	-1.682	0.092
Mohr 2014	resting HR	-0.694	0.316	0.100	-1.313	-0.075	-2.197	0.028
Ryzkova 2017	resting HR	-0.473	0.480	0.230	-1.414	0.468	-0.985	0.325
		-0.495	0.189	0.036	-0.866	-0.124	-2.614	0.009

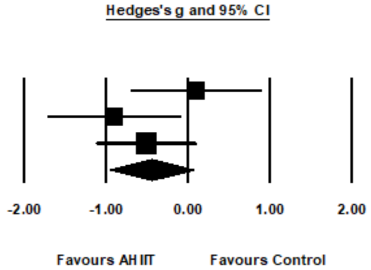


Heterogeneity I^2= 0.000%, p= 0.599
Test for overall effect Z= -2.614 p= 0.009

Figure 3.4 Meta-analysis and forest plots of AHIIT on SBP

AHIIT overall effect on systolic blood pressure

Outcome		Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Broman 2006	SBP	0.096	0.407	0.166	-0.702	0.895	0.237	0.813
Junior 2018 b	SBP	-0.902	0.415	0.172	-1.716	-0.089	-2.174	0.030
Mohr 2014	SBP	-0.511	0.312	0.097	-1.121	0.100	-1.639	0.101
		-0.444	0.266	0.070	-0.964	0.076	-1.672	0.095

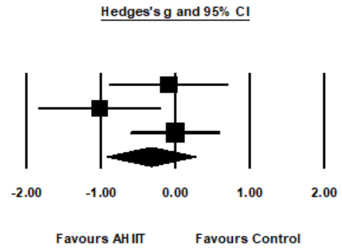


Heterogeneity I^2= 33.90%, p= 0.220
Test for overall effect Z= -1.671 p= 0.095

Figure 3.5 Meta-analysis and forest plots of AHIIT on DBP

AHIIT overall effect on diastolic blood pressure

Outcome		Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Broman 2006	DBP	-0.088	0.407	0.166	-0.887	0.710	-0.217	0.828
Junior 2018 b	DBP	-1.017	0.421	0.177	-1.841	-0.193	-2.418	0.016
Mohr 2014	DBP	0.000	0.306	0.094	-0.601	0.601	0.000	1.000
		-0.328	0.311	0.097	-0.938	0.281	-1.056	0.291

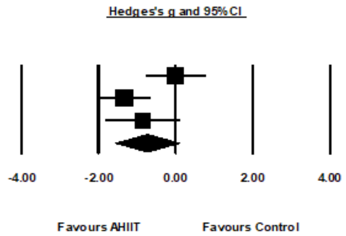


Heterogeneity I^2= 51.54%, p= 0.127
Test for overall effect Z= -1.056 p= 0.291

Figure 3.6 Meta-analysis and forest plots of AHIIT on body fat percentage

AHIIT overall effect on body fat percentage

Study name	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Junior 2018	Body fat percentage	0.000	0.395	0.156	-0.774	0.774	0.000	1.000
Mohr 2014	Body fat percentage	-1.324	0.340	0.115	-1.990	-0.659	-3.901	0.000
Ryzkova 2017	Body fat percentage	-0.847	0.496	0.246	-1.819	0.124	-1.709	0.087
		-0.735	0.422	0.178	-1.562	0.093	-1.741	0.082



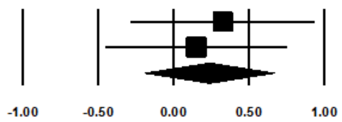
Heterogeneity I^2= 69.20%, p= 0.039
Test for overall effect Z= -1.741. p= 0.082

Figure 3.8 Meta-analysis and forest plots of AHIIT on HDL

AHIIT overall effect on High Density Lipoprotein

Study name	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Costa 2018	HDL	0.329	0.312	0.097	-0.282	0.941	1.055	0.291
Mohr 2014	HDL	0.154	0.307	0.094	-0.448	0.755	0.501	0.617
		0.240	0.219	0.048	-0.189	0.669	1.097	0.273

(Statistics for each study)



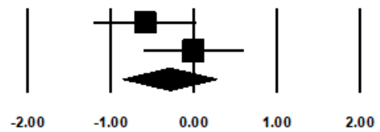
Heterogeneity I^2= 0.000% , p= 0.688
Test for overall effect Z= 1.097 p= 0.273

Figure 3.7 Meta-analysis and forest plots of AHIIT on LDL

AHIIT overall effect on Low Density Lipoprotein

Study name	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Costa 2018	LDL	-0.580	0.317	0.100	-1.201	0.040	-1.833	0.067
Mohr 2014	LDL	0.000	0.306	0.094	-0.601	0.601	0.000	1.000
		-0.285	0.290	0.084	-0.853	0.284	-0.981	0.327

(Statistics for each study)

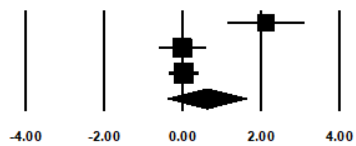


Heterogeneity I^2= 42.376% , p= 0.188
Test for overall effect Z= -0.981 p= 0.327

Figure 3.9 Meta-analysis and forest plots of AHIIT on total body BMD

	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Junior 2018	total BMD	2.141	0.498	0.248	1.166	3.116	4.303	0.000
Mohr 2015	total BMD	0.000	0.306	0.094	-0.601	0.601	0.000	1.000
Moreira 2014	total BMD	0.038	0.194	0.038	-0.343	0.419	0.195	0.845
		0.627	0.516	0.266	-0.384	1.638	1.216	0.224

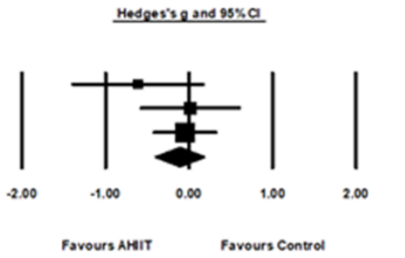
(Statistics for each study)



Heterogeneity I^2= 87.72% , p= 0.000
Test for overall effect Z= 1.216 p= 0.224

Figure 3.10 Meta-analysis and forest plots of AHIIT on total femur BMD

	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Junior 2018	BMD Total femur	-0.612	0.404	0.163	-1.404	0.180	-1.513	0.130
Mohr 2015	BMD Total femur	0.017	0.306	0.094	-0.583	0.618	0.057	0.955
Moreira 2014	BMD Total femur	-0.046	0.194	0.038	-0.427	0.335	-0.236	0.813
		-0.110	0.152	0.023	-0.409	0.188	-0.726	0.468

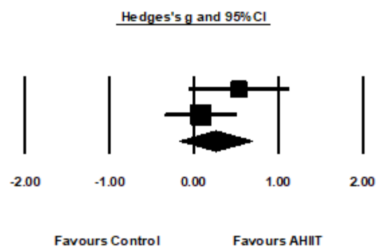


Heterogeneity I^2= 0%, p= 0.402
Test for overall effect: Z= -0.726 p= 0.468

Figure 3.12 Meta-analysis and forest plots of AHIIT on knee flexion strength

AHIIT overall effect on Knee flexion strength

Study name	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Bento 2015	knee flexion strength	0.538	0.304	0.093	-0.059	1.134	1.767	0.077
Manukka 2016	knee flexion strength	0.088	0.216	0.047	-0.336	0.512	0.405	0.685
		0.262	0.219	0.048	-0.168	0.691	1.194	0.232

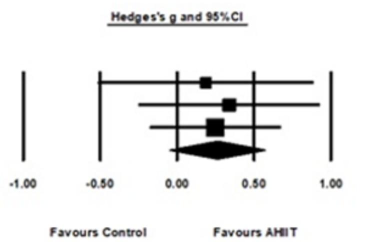


Heterogeneity I^2= 31.18% , p= 0.228
Test for overall effect Z= 1.194 p= 0.232

Figure 3.11 Meta-analysis and forest plots of AHIIT on knee extension strength

AHIIT overall effect on knee extension strength

Study name	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Andrade 2020 a	knee extension strength	0.187	0.357	0.127	-0.512	0.886	0.524	0.600
Bento 2015	knee extension strength	0.339	0.301	0.091	-0.251	0.929	1.125	0.261
Manukka 2016	knee extension strength	0.248	0.217	0.047	-0.177	0.674	1.144	0.253
		0.261	0.158	0.025	-0.048	0.571	1.654	0.098

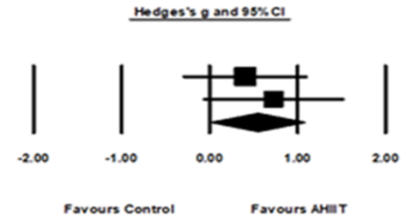


Heterogeneity I^2= 0%, p= 0.945
Test for overall effect: Z= 1.654 p= 0.098

Figure 3.13 Meta-analysis and forest plots of AHIIT on chair to stand test

AHIIT overall effect on chair to stand test

Study name	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Andrade 2020 b	CS	0.407	0.360	0.129	-0.298	1.111	1.131	0.258
Junior 2018	CS	0.730	0.408	0.167	-0.070	1.530	1.789	0.074
		0.548	0.270	0.073	0.019	1.077	2.032	0.042



Heterogeneity I^2= 0%, p= 0.552
Test for overall effect: Z= 2.032 p= 0.042

3.5.6 Publication bias

According to the results of Egger's test, no significant publication bias was observed in the meta-analysis of cardio-metabolic health markers or physical outcomes.

3.6 Discussion

This systematic review with meta-analysis was the first to examine the effects of AHIIT on cardio-metabolic and physical health markers in women. The main findings reveal that AHIIT can result in a moderate improvement in cardio-metabolic markers with regard to VO_2 peak, resting HR, and CS test times.

VO_2 peak is considered to be directly reflective of maximum oxygen processing capacity (VO_{2max}), the highest value of VO_2 attained in a high-intensity exercise test (Whipp et al., 1990). The test of VO_2 peak is designed to bring the subject to the limit of tolerance, a gold standard measure of cardiorespiratory fitness and a solid indicator that can predict mortality and improve prognosis (Keteyian et al., 2008). The findings demonstrate that the VO_2 peak increased moderately but significantly with AHIIT compared to the control groups (L. S. Andrade, S. S. Pinto, et al., 2020b; Gi

Broman et al., 2006; Costa et al., 2018; Munukka et al., 2016; Samadi et al., 2019). With training, the left ventricle of the heart can be stretched to allow for more forceful contractions, which increases stroke volume and results in increased VO_2 peak (McDaniel et al., 2020). HIIT is also able to increase the VO_2 peak rapidly via increasing mitochondrial density, resulting in the generation of more adenosine triphosphate for working muscles (Gibala & Jones, 2013). Another possible explanation is that VO_2 is influenced by a restriction in chest expansion when inspiratory muscle contractions are unable to equal or overcome the force of hydrostatic pressure (McDaniel et al., 2020). It is suggested that central hypervolemia influences lung volumes by reducing lung compliance and promoting gas trapping, which narrows the airways (McNamara et al., 2013). With such unique physiological adaptations during aquatic training, ventilation and breathing frequency are significantly increased when compared with land-based training with an equivalent exercise intensity (Silvers et al., 2007).

These findings also revealed an overall moderate effect in reducing resting HR in women following AHIIT, consistent with the study of Plotnick (Plotnick et al., 1986). As the hydrostatic gradient increases with immersion, AHIIT increases stroke volume, which involves increased cardiac contractions and reduced heart rate (Helgerud, Hoydal, et al., 2007). It has been proposed that the heart rate reduction is due to an increase in parasympathetic activity and a decrease in the sympathetic activity of the heart (Carter et al., 2003). A high resting HR is a risk factor for all-cause mortality, so reducing resting HR through AHIIT minimises mortality risk (Jensen et al., 2013). Such improvements in VO_2 peak and resting HR provided by AHIIT are crucial, since both are independent predictors of cardio-metabolic disease, and particularly cardiovascular disease mortality (Lau et al., 2019). It has been shown that every one MET increase in VO_2 peak is associated with 10–25% improvement in survival rate and an 18% reduction in events related to cardiac disease (Myers et al., 2002). VO_2 peak and resting HR can be easily be improved with the help of AHIIT.

Blood pressure is another common cardio-metabolic measure. A lack of significant reduction in both SBP and DBP was observed after AHIIT. This could be because the studies' participants were all hypertensive aged women, and it is known that SBP increases progressively with age (Strandberg & Pitkala, 2003).

Similarly, the changes in lipid profile were not significant. A possible reason for this result is that lipid metabolism reflects HDL and LDL levels, and depends on the concentration of other enzymes (Costa et al., 2018). Due to the limited number of review papers on lipid profile, the mechanism regarding the lack of AHIIT impact is still unclear.

HIIT appears to promote a significant reduction in body fat percentage in overweight men (Poon et al., 2020), but this meta-analysis did not show any overall significant effect of AHIIT on body fat percentage in female populations. Likewise, there has been a review that showed no overall effects on body fat composition utilising AHIIT among non-athletic populations (Depiazzi et al., 2018). Despite these findings, when trained at the same relative intensity, a higher VO_2 training value could be achieved in water than on land. This could potentially result in a higher caloric cost, and hence, a greater reduction in body fat percentage (Hall et al., 2004b).

When considering physical health markers, the hydrostatic pressure created by water's resistance to movement at high speed promotes muscle contraction (Pöyhönen et al., 2002). High speed body movements promote the recruitment of type II fast-twitch muscle fibres (Cheung et al., 2010). The meta-analysis, however, showed no significant difference in overall knee extension or flexion strength following AHIIT lower limb exercise. That contradicts the results of a meta-analysis conducted by Depiazzi and her colleagues, but it is similar to the findings of Heywood and his colleagues, which showed no significant improvement in upper or lower leg muscle strength following AHIIT focused on leg exercises (Depiazzi et al., 2018; S. M. Heywood et al., 2016). This discrepancy in gaining muscle strength could result from a difference in movement speed and resistance loading exerted on the muscles. The improvement in muscle strength remains controversial at this stage and warrants further investigation.

The results reveal no significant change in total body BMD or total femur BMD after AHIIT. Impact or resistive exercise in an aquatic environment results in different loadings exerted on bone cells, and according to Wolff's law, mechanical loading is considered to be an active osteogenic stimulus (Wolff et al., 1999). The study's finding could be explained by the level of immersion during the AHIIT exercises. Exercises performed immersed to chest level are associated with reduced mechanical loading on the bones (Martyn-St James & Carroll, 2009). A reduced mechanical load on the immersed body would diminish its effect on bone metabolism (Aboarrage Junior et al., 2018a).

The meta-analysis showed a significant effect on CS test times, though only two of the studies used the CS test (Aboarrage Junior et al., 2018b; L. Andrade et al., 2020). The CS test is known to have good test-retest reliability ($R \geq 0.8$) and reasonably good criterion-related validity relative to knee extensor strength (Bohannon, 2009). This review, however, showed a significant improvement in the CS test times without a substantial improvement in knee extensor strength. That may due to be the fact that the CS test is significantly associated with the eccentric contraction of the knees instead of their maximum dynamic strength (Vaidya et al., 2017). Given the improvement in the women's ability to perform the CS test after AHIIT, it seems to be a reliable tool with which to assess lower-body strength and performance (Zanini et al., 2015). It might therefore be considered an alternative tool to determine maximum dynamic strength and potentially simulate daily living tasks, resulting in a functional gain.

Finally, the data generated in this analysis indicate that AHIIT is safe for aged women. This result is consistent with those of another review (Bunæs-Næss et al., 2023), though adverse events associated with HIIT and aquatic training have occasionally been reported (Barker et al., 2014b; Weston et al., 2014).

3.7 Study limitations

Although the evidence synthesized in this review shows the beneficial effects of AHIIT, the study had several limitations. Discrepancies exist in defining "high intensity" across different types of training, using maximum oxygen uptake, and HR or RPE scales. The relatively small number of studies using each outcome resulted in a small sample size, and limiting the sample to women of course affected the generalizability of the results. The literature search was also limited to databases and publications in English peer-reviewed journals. The quality of the majority of the studies included was between low and moderate in terms of reporting. Although no active treatment groups were used for comparison in this study, the data of the studies analysed were carefully and systematically assessed. Overcoming the shortcomings of this research should be considered in the design of future studies.

3.8 Conclusions

This was the first systematic review and meta-analysis of the overall effectiveness of AHIIT interventions in improving cardio-metabolic health markers in regard to VO_2 peak, resting HR, and CS test times among a female population. Although AHIIT showed beneficial effects on various outcome measures, future research is warranted to compare AHIIT with other exercise interventions and to develop optimal AHIIT protocols to produce beneficial effects on cardio-metabolic and physical health.

Chapter 4 The Effectiveness of Deep Water Running in Improving Cardiorespiratory Fitness, Physical Functioning and Quality of Life: A Systematic Review

This chapter has been published as below:

Kwok, M. Y., So, C. L., Heywood, S., Lai, C. Y., & Ng, S. M. S. (2022). Effectiveness of Deep Water Running on Improving Cardiorespiratory Fitness, Physical Function and Quality of Life: A Systematic Review. International Journal of Environmental Research and Public Health. https://doi.org/10.3390/ijerph19159434

This chapter has been presented in the below conference.

Tse DHT, **Kwok MMY,** Ng SSM, Heywood, S., So BCL (2021, December). "Effectiveness of Deep Water Running on Improving Cardiorespiratory Fitness, Physical Function and Quality of Life: A Systematic Review." *In Hong Kong Physiotherapy Association Conference* (Oral Presentation)

4.1 Abstract

Deep Water Running (DWR) is a form of aquatic aerobic exercise simulating the running patterns adopted on dry land. It has gained popularity among many individuals, but little is known about its effectiveness in improving cardiorespiratory fitness, physical functioning and quality of life. This chapter will report the results of a systematic review of the results of recent clinical trials regarding the use of DWR for cardiorespiratory fitness and improving the physical functions and quality of life of healthy individuals.

Seven trials involving 1626 participants were identified in a systematic search of six databases. Four of the trials used group-based interventions. Another eleven clinical trials were identified which evaluated the effectiveness of DWR in improving cardiorespiratory fitness, physical functioning or quality of life compared with no intervention or standard land-based treatment or training. Data relevant to the review questions were extracted by two independent reviewers. When means and standard deviations were reported, standardised mean differences were calculated. Quality assessment was conducted using selected items from the Downs and Black checklist. The 11 clinical trials (seven randomized and controlled) had a total of 287 participants. The review results suggest that compared with land-based training, DWR showed similar effect in terms of cardiorespiratory fitness. Limited studies reported physical functioning or quality of life outcomes compared with those of a no exercise control group.

These findings show that DWR can maintain cardiorespiratory conditioning with some potential advantages as an off-loaded exercise at high intensity in populations that are weak, injured or in pain, but more studies are needed.

4.2 Introduction

DWR can be defined as running with 70% of the body immersed, submerged to shoulder level, with or without use of a floatation device (Reilly et al., 2003). It simulates running movement patterns on land, but it is non-weight bearing. With the unloading from buoyancy, DWR eliminates vertical ground reaction forces and hence reduces joint loadings and reduces the risk of injury to the musculoskeletal system (Killgore, 2012a; Silva, Dias, Bela, et al., 2020).

Deep water running has emerged as an alternative exercise for healthy athletic and untrained populations, as well as for people with musculoskeletal conditions (Nagle et al., 2019). During DWR the buoyancy negates the weight-bearing nature of exercise relative to that conducted on land. The lower impact force experienced in the water allows the exercise to be performed both at higher intensities and for longer durations without an increased risk of injury (M. M. Y. Kwok, S. S. M. Ng, et al., 2022; Nagle, Sanders, & Franklin, 2017). Although DWR may induce lower physiological responses when compared with land running with lower maximum oxygen uptake (VO_{2max}) and HR max (Reilly et al., 2003), the training stimulus is likely to still be sufficient for supplementary training with less orthopaedic trauma compared to running on land (Gi Broman et al., 2006). The reduced loading on the body makes DWR a viable training alternative for athletes who are susceptible to repetitive stress injuries, as well as for elderly individuals who may be vulnerable to, or fearful of falls or joint pain.

Although it is known that DWR provides a form of non-weight bearing exercise, the biomechanics of DWR when compared with land running remain unclear. DWR has two main styles: cross-country style and high knee style (Killgore et al., 2006). Deep water running style prescriptions vary, but the consensus for technique is that (1) the trunk should be slightly forward in upright position, (2) the arms and legs should perform circular motions, (3) the swinging leg should be brought to the horizontal position, (4) the elbows should be maintained at a 90° angle, and (5) the hands should remain either completely open or in a closed fist at all times (K. S. Chu & E. C. Rhodes, 2001). There may not be consensus about whether the DWR styles should be attempting to replicate similar lower limb kinematics or whether larger ranges of movement lead to greater resistance and higher intensity. A review by Silva and his colleagues has suggested that muscle activity during DWR produces greater activation of distal leg muscles than proximal thigh muscles, in comparison with land treadmill running (Silva, Dias, Bela, et al., 2020), but it is not clear what physical functional outcomes this may influence. There is an increasing interest in examining the differences in physical functional outcomes between DWR and land running applying validated measurement tools.

The effect of DWR on health-related quality of life also remains unclear. It is common for people with musculoskeletal conditions to report poorer quality of life, including in domains related to physical functioning, due to fear and pain (Alejandro et al., 2019). The fear of physical activity arising from pain during movement or the risk of reinjury often leads to a downward spiral of negative physical and psychological consequence (Butler, 2013). Deep water running may be helpful for reducing such fear.

Despite the fact that there is growing interest in the including DWR in cardiovascular training programs for various clinical populations, and even though a number of clinical trials have been conducted to evaluate the effects of DWR on various quality of life domains, there has not yet been a systematic examination of the effects of DWR on cardiorespiratory fitness, physical functioning and quality of life. This systematic review was designed to address that gap. Specifically, it sought to evaluate the effects of DWR on cardiorespiratory fitness, physical functioning and quality of life, comparing it with other interventions or control groups without exercise.

4.3 Methods

4.3.1 Search strategy

This systematic review was guided by the Preferred Reporting Items for Systematic Reviews and Meta-analysis guidelines (Moher, Liberati, Tetzlaff, Altman, et al., 2009), and was registered in the PROSPERO database (CRD42020154988) prior to conducting the review. A complete search of the SPORTDiscus, MEDLINE, CINAHL, AMED, Embase and Cochrane Library databases up to December2021 was conducted with combinations of the words listed in Appendix 1. In an effort to unveil complex evidence in obscure locations, manual reference tracking and citation tracking of any relevant articles was performed (Greenhalgh & Peacock, 2005) with the help of citation tracking databases like "Cited by" in Google Scholar.

4.3.2 Inclusion criteria

The articles included were required to be (1) interventional studies, either randomised or non-randomised trials; (2) published as a full paper in English; (3) with participants not limited to only healthy individuals (so including persons with at least one health condition); (4) designed predominantly (≥ 50%) to evaluate DWR, perhaps combined with passive treatments such as education or stretching; and (5) with outcomes including cardiorespiratory measures like VO_{2max}, maximum heart rate, blood pressure, ventilatory threshold), physical function measures related to running or walking (e.g. a timed test, an endurance test, or a function test), or health-related quality of life (using validated questionnaires or assessment tools) (Appendices).

4.3.3 Exclusion criteria

Studies were excluded if (1) the study design was not experimental (e.g. descriptive or exploratory studies) or was cross-sectional, (2) the participants were under 18 years of age, or (3) exercises other than DWR (e.g., general aquatic exercise, shallow water running, underwater treadmill running) were tested (Appendices).

4.3.4 Data extraction

Data were extracted independently by 2 reviewers. They included study design, baseline demographics of the participants (age, sex, health status, body weight, height, body mass index), sample size, intervention details (frequency, intensity, duration, and length of intervention, DWR technique, instructions, supervision of the exercise, equipment used, pool temperature and room temperature), cardiorespiratory outcomes, physical functional outcomes and health-related quality of life outcomes, and measures of variance of the outcomes of interest. If there were missing data or data potentially included in error, the study's authors were contacted by email and asked to provide further information.

4.3.5 Quality and risk of bias assessment

Each included study was critically appraised by the two independent reviewers using selected items from a checklist proposed by Downs and Black (Appendix 3) (Downs & Black, 1998). A preliminary search identified relevant studies with multiple study designs. The Downs and Black quality assessment tool designed for randomized and non-randomized trials was used to assess the quality. It has limitations, but it has been shown to be an effective tool irrespective to the tool development and extensive domains covered (Jarde et al., 2012). Five of its subscales—reporting, external validity, internal validity in bias, confounding, and power— were assessed according to the characteristics of the studies reviewed. Scores using this tool can be interpreted as excellent (26–28); good (20–25); fair (15–19) or poor (≤14) (Hooper et al., 2008).

4.3.6 Data analysis

The outcomes of interest and program parameters in specific populations were pooled for data analysis using version 7.1 of the Cochrane Software Review Manager (The Nordic Cochrane Centre, Denmark). The effects of DWR on the three domains of interest were analysed with standardized mean difference (SMD) calculations if the studies reported means and standard deviations (Cohen, 1988). The SMD was considered significant only if the 95% CI did not include zero (Sedgwick, 2015). Effect size thresholds were classified as small (0.2), medium (0.5) or large effect (0.8) (Cohen, 1988). The SMDs and 95% confidence intervals were reported to demonstrate effects of DWR in comparison with a control group (without exercise or other interventions such as land-based training). Meta-analyses were not completed due to the diversity in the studies' methods and the risk of bias in individual studies. Therefore, the results will be presented as effect sizes only.

4.4 Results

4.4.1 Studies selected

Following an initial search which identified 1416 articles, 11 articles were included in the review for quantitative synthesis (Figure 4.1).

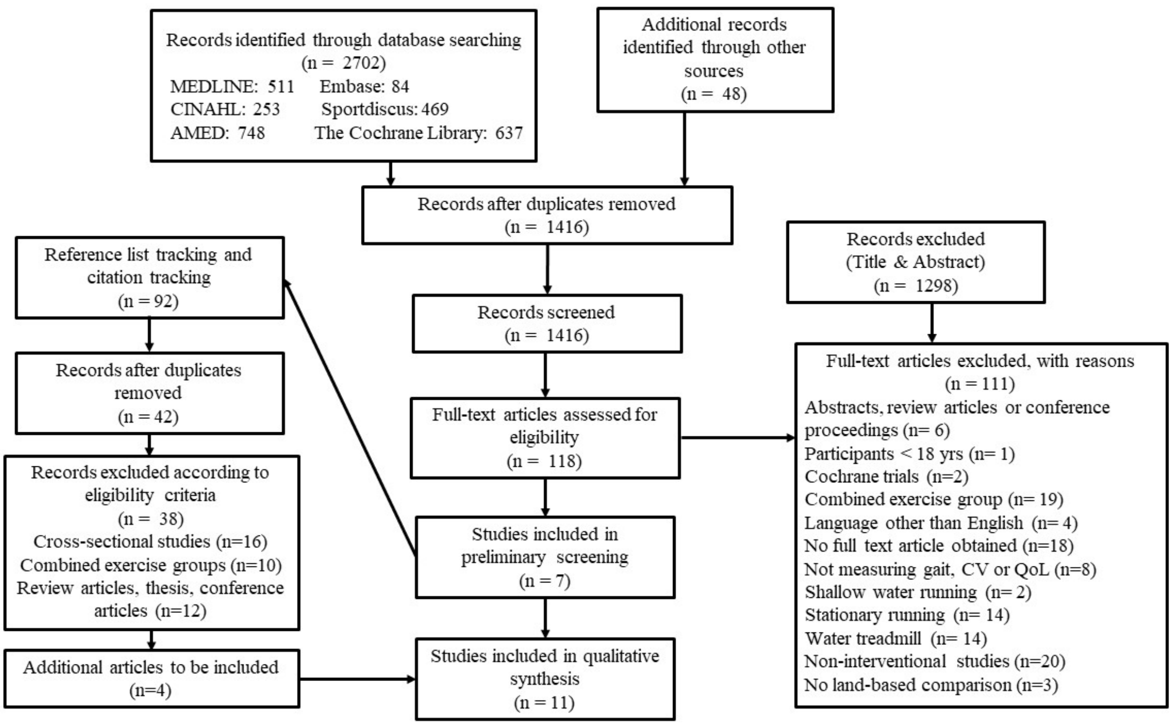


Figure 4.1 PRISMA flow diagram of selection process

4.4.2 Study Quality

Of the 11 studies included, seven were randomized and controlled trials (Alberti et al., 2017; Assis et al., 2006; G. Broman et al., 2006; Colato et al., 2017; Cuesta-Vargas et al., 2012; K. Davidson & L. McNaughton, 2000; Eyestone et al., 1993; Kanitz et al., 2019; McKenzie & McLuckie, 1991; Michaud et al., 1995; Wilber et al., 1996), two were longitudinal studies (Colato et al., 2017; K. Davidson & L. McNaughton, 2000), and two were quasi-experiment studies (Michaud et al., 1995; Wilber et al., 1996). Four studies investigated physically active or trained adults (Eyestone et al., 1993; Kanitz et al., 2019; McKenzie & McLuckie, 1991; Wilber et al., 1996), three investigated physically inactive or obese women (G. Broman et al., 2006; Colato et al., 2017; K. Davidson & L. McNaughton, 2000), two investigated healthy or community-dwelling elderly (Alberti et al., 2017; Gi Broman et al., 2006), and one study investigated populations with low-back pain (A. C. Kanitz et al., 2015). Study participant numbers varied from 10 to 60, with a total of 287 participants in the review (Table 4.1).

As for the comparison group, five studies included a control group that did not participate in any exercise (Alberti et al., 2017; G. Broman et al., 2006; Colato et al., 2017; Cuesta-Vargas et al., 2012; Michaud et al., 1995), and six included a land-based exercise group (Assis et al., 2006; K. Davidson & L. McNaughton, 2000; Eyestone et al., 1993; Kanitz et al., 2019; McKenzie & McLuckie, 1991; Wilber et al., 1996). Two of the studies matched land exercise time to that of the DWR, having a total of 45 matched sessions over three weeks (Assis et al., 2006; Cuesta-Vargas et al., 2012). The rest of the studies varied in exercise intensity and sessions.

4.4.3 The deep-water running's characteristics

The duration of the DWR sessions varied from 30 to 70 minutes, 2 to 5 sessions per week over 3 to 18 weeks. Compliance ranged from 80% to 100% (Table 4.2). Of the 11 studies, all except that reported by McKenzie & McLuckie (1991)10 had their participants performing DWR with the assistance of a flotation device. McKenzie & Mcluckie asked their subjects not to touch the bottom of the swimming pool without wearing flotation device during DWR (McKenzie & McLuckie, 1991).

All of the studies involved running in the water, but there were variations in body positions during the DWR. Four of the studies (Assis et al., 2006; Eyestone et al., 1993; A. C. Kanitz et al., 2015; McKenzie & McLuckie, 1991) required their participants to maintain a vertical body position. Davidson & McNaughton (2000) allowed them to run in a vertical or slightly forward leaning position, while Broman's group and Michaud's group instructed their participants to run bending slightly forward (Gi Broman et al., 2006; Michaud et al., 1995). Four studies did not specify a running posture (Alberti et al., 2017; Colato et al., 2017; Cuesta-Vargas et al., 2012; Wilber et al., 1996).

Table 4.1 Study Design of included studies

	Davidson, K., & McNaughton, L.	Kanitz et al.	Colato et al.	Assis et al.	Broman et al.	Cuesta-Vargas et al.	Alberti et al.	Michaud, T. J. et al.	Mckenzie et al.	Wilbur et al.	Eyestone et al.
Number of subjects (M/F)	10 (0/10)	14 (7/7)	20 (0/20)	60 (0/60)	29 (0/29)	58 (25/33)	19 (0/19)	17 (2/15)	12 (12/0)	16 (16/0)	32 (32/0)
Age (years old) of respective groups	22.6 ± 3.4	DWR: 39 (95% CI: 31-47) LWR: 40 (95% CI: 36-50)	DWR: 48.81 ± 12.87 Control: 49.9±10.5	DWR: 43.96 ± 10.28 LBE: 44.04 ± 8.87	DWR: 69.0 ± 4.0 Control: 69.8 ± 3.5	DWR: 38.6 ± 12.2 Control: 37.8 ± 13.2	DWR: 64.33±4.24 Control: 64.40±4.22	DWR: 32.6 ± 6.8 Control: N/A	23.9	32.5 ± 5.4	18-26
Subjects' characteristics	Untrained women	Physically active patients of both sexes with chronic low back pain	Overweight obese women	Sedentary women with fibromyalgia	Healthy aged women	Non-specific low back pain	Community dwelling elderly	Healthy sedentary	Competitive runners	Aerobically-trained male distance runners	Finished a 1.5 mile run in less than 10'45
Outcome measure of Physical Functions	/	/	/	/	/	/	4MWT, 6MWT, 10MWST FTSST TUGT	/	Time to fatigue	/	2 mile run time
Outcome measure of Cardiovascular	VO_2max	VO_2peak, VO_2Vt2	VO_2max	HR, VO_2max	HR (rest+test) BP (rest+test) Peak VO_2	/	/	VO_2max	VO_2max	VO_2max, Ventilator threshold	VO_2max
Outcome measure of Quality of life	/		/	SF-36	/	SF-12	/	/	/	/	/
Study Design	L	R	L	R	R	R	R	Q	R	Q	R

Note: 4MWT: 4-meter walk test; 6MWT: 6-meter walk test; 10MWST: 10-meter walking speed test; FTSST: 5 times sit to stand test; TUGT: Timed up and go test; L: Longitudinal; Q: Quasi-experimental; R: Randomized Controlled Trial

Table 4.2 Intervention Design of included studies

	Davidson, K., & McNaughton, L. 2000		Kanitz et al. 2019		Colato et al. 2016		Assis et al. 2006		Broman et al. 2006		Cuesta-Vargas et al. 2012		Alberti et al. 2017		Michaud et al. 1995		Mckenzie et al. 1991		Wilbur et al. 1996		Eyestone et al. 1993		
Nature of groups in respective studies	DWR	RR	DWR	LWR	DWR	Control	DWR	LBE	DWR	Control	DWR+GP	GP	DWR	Control	DWR	Control	DWR	LWT	DWR	TR	DWR	C	RR
Number of subjects	5	5	7	7	11	9	26	26	18	11	29	29	16	14	10	7	6	6	8	8	10	11	11
Supervision	✓		N/A		N/A		✓	2 PT	N/A		✓		✓		N/A		N/A		N/A		N/A		
Adverse effects %	N/A		N/A		N/A		10	16	×		N/A		N/A		N/A		N/A		N/A		N/A		
Drop-outs	0	0	3 (30%)	3	N/A		4	4	3	2	3	4	7	4	6		N/A		1	1	N/A		
Pool temperature	22 - 25ºC		N/A		28ºC		28-31°C		27°C		N/A		28-30°C		27-29ºC		N/A		27ºC		N/A		
Water depth	N/A		N/A		1.7m		N/A		N/A		2.15m		1.35m		Diving pool		Deep end of summing pool		N/A		Diving pool		
Floating device	✓		✓		✓		✓		✓		✓		✓		✓		×		✓		✓		
Compliance %	96	94	83	80	N/A		Excellent (no stats)		100		N/A		N/A		100		N/A		96	98	N/A		
Program time (weeks)	4		12		12		15		8		15		18		8		3		6		6		
Session time (mins)	50		45		70		60		48		30		50		40 - 70		30		30 - 60		20 - 30		
Sessions per week	3		2		3		3		2		3		2		3		5		5		Week 1: 3 Week 2: 4 Week 3-6: 5		
Total number of sessions	12		24		36		45		16		45		36		24		15		30		27		
Warm up	N/A		✓		✓		✓		✓		N/A		✓		×		N/A		N/A		N/A		
Cool down	N/A		✓		✓		✓		✓		N/A		✓		×		N/A		N/A		N/A		

Note: C: Cycling; DWR: Deep Water Running; RR: Road Running;; LWR: Land Walking/Running; LBE: Land-based Exercises ; GP: General Practice Consisting of Advice and Education about Exercise ; TR: Treadmill Run; WT: Water Training; ✓: Included; ×: Not Included; N/A: Not Available.

4.4.4 Quality assessment

All of the 11 articles clearly described their study's objectives and main outcomes, which were reliable and valid (Table 4.3). Nine out of 11 studies failed to report concealment of allocation (Alberti et al., 2017; Assis et al., 2006; G. Broman et al., 2006; Colato et al., 2017; K. Davidson & L. McNaughton, 2000; Eyestone et al., 1993; McKenzie & McLuckie, 1991; Michaud et al., 1995; Wilber et al., 1996). Only two studies reported on blinding of outcome assessors or intention-to-treat analysis (Assis et al., 2006; Kanitz et al., 2019).

Table 4.3 Quality Assessment

Subscale	Items	Davidson, K., & McNaughton, L	Kanitz et al.	Colato et al.	Assis et al.	Broman et al.	Cuesta-Vargas et al.	Alberti et al.	Michaud et al.	Mckenzie et al.	Wilbur et al.	Eyestone et al.
Reporting	*1. Hypothesis/aim/objective clearly described*	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	2. Main outcomes clearly described	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	3. Characteristics of the patients clearly described	N	Y	Y	Y	Y	Y	Y	N	N	Y	N
	4. Intervention and comparison group clearly described	Y	Y	N	Y	N	N	N	Y	Y	Y	Y
	5. Distributions of principal confounders in each group of subjects clearly described	N	Y	Y	Y	Y	Y	Y	N	Y	N	N
	6. Main findings clearly described	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y
	7. Estimates of the random variability for the main outcomes provided	N	Y	Y	Y	Y	Y	Y	N	Y	Y	Y
	10. Actual p values reported for main outcomes	Y	N	Y	Y	N	Y	Y	N	N	N	Y
External validity	*11. Subjects asked to participate represented the population*	N	N	Y	Y	Y	Y	N	N	N	N	N
	12. Subjects prepared to participate represented the population	N	N	N	N	N	N	N	N	N	N	N
Internal validity - Bias	*15. Blinded outcome assessment*	N	Y	N	Y	N	N	N	N	N	N	N
	18. Appropriate statistical tests performed	Y	Y	Y	Y	Y	Y	Y	N	N	Y	Y
	20. Accurate outcome measure used (reliable and valid)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Internal validity - Confounding	*23. Subjects randomized to intervention groups*	N	Y	N	Y	Y	Y	N	N	Y	N	Y
	24. Concealed allocation from subjects and investigators	N	Y	N	N	N	Y	N	N	N	N	N
	26. Losses to follow-up taken into account	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N
Power	*27. Power calculation*	N	Y	Y	Y	N	Y	Y	N	N	N	N

4.5 Outcomes

Three of the 11 studies investigated physical functioning outcomes (functional mobility and balance tests) or walking (Alberti et al., 2017; Eyestone et al., 1993; McKenzie & McLuckie, 1991). Nine investigated cardiorespiratory fitness (Assis et al., 2006; Gi Broman et al., 2006; Colato et al., 2017; K. Davidson & L. McNaughton, 2000; Eyestone et al., 1993; A. C. Kanitz et al., 2015; McKenzie & McLuckie, 1991; Michaud et al., 1995; Wilber et al., 1996), and three studies investigated quality of life (Assis et al., 2006; Cuesta-Vargas et al., 2012; Kanitz et al., 2019).

4.6 Cardiorespiratory fitness

4.6.1 Peak Heart rate

Three studies evaluated the changes in HR peak (Gi Broman et al., 2006; Michaud et al., 1995; Wilber et al., 1996), but they did not report means and SDs so SMDs could not be calculated.

4.6.2 Maximum Aerobic Capacity

Broman's group found a significant large effect (SMD= -1.28) of DWR in improving VO_{2max}. In contrast, Michaud and his colleagues observed no significant improvement. Both used controls who did no active exercise (Figure 4.2)

Study or Subgroup	DWR Mean	DWR SD	DWR Total	No active exercise Mean	No active exercise SD	No active exercise Total	Std. Mean Difference IV, Fixed, 95% CI
2.2.1 Untrained healthy population							
Broman 2006 (Cycle ergometer, mL/kg/min)	−27.2	2.1	15	−23.3	4	9	−1.28 [−2.20, −0.37]
Michaud 1995 (Treadmill test, L/min)	−2.49	0.68	10	−1.99	0.36	7	−0.83 [−1.84, 0.19]

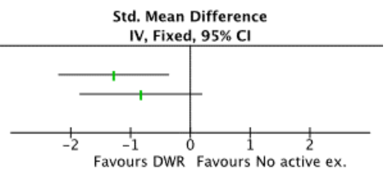


Figure 4.2 Standardized mean difference (95% CI) for the effect of DWR compared with No active exercises on VO2 max

Five studied that compared deep water running with running on land all found similar effects in improving VO_{2max} (K. Davidson & L. McNaughton, 2000; Eyestone et al., 1993; Kanitz et al., 2019; McKenzie & McLuckie, 1991; Wilber et al., 1996) (Figure 4.3).

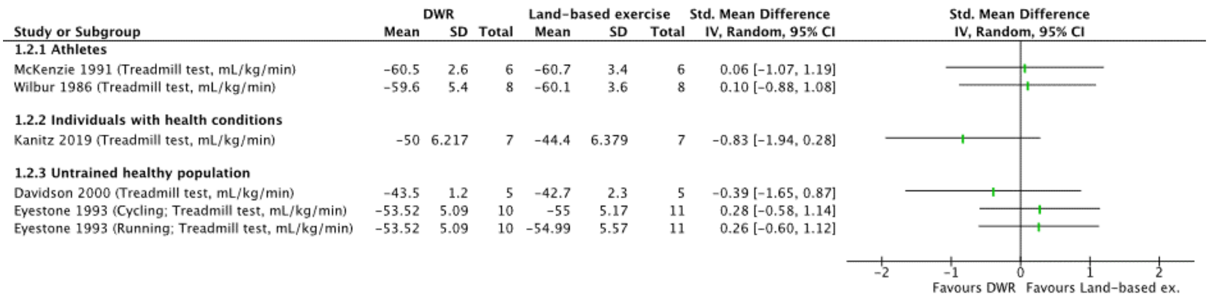


Figure 4.3 Standardized mean difference (95% CI) for the effect of DWR compared with land trainings on VO2 max

4.7 Physical function

One study, led by Alberti compared physical functioning between a DWR group and a control group that did no active exercise using 4-minute, 6-minute and 10-minute walk tests, a functional mobility test (the Five Times Sit to Stand test) and a dynamic balance test (the Timed Up and Go test) (Alberti et al., 2017). Large effect sizes were observed in the three walking tests (SMD = −2.39 to -1.33), but the effect sizes of the other two tests revealed no significant effect of DWR compared to no active exercise. There were, though, mean improvements favouring the DWR group in this study (Figure 4.4).

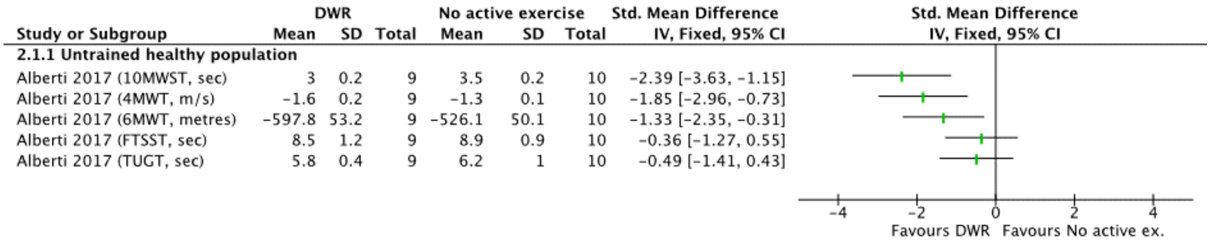


Figure 4.4 Standardized mean difference (95% CI) for the effect of DWR compared with No active exercises on physical function outcomes

When comparing deep water running with running on land, two studies reported similar effects on physical functioning. The group led by Mckenzie found a similar effect in terms of time to fatigue when running on a treadmill (McKenzie & McLuckie, 1991). And Eyestone concluded that DWR had an effect on two-mile run time similar to that of both land-based running and cycling (Eyestone et al., 1993).

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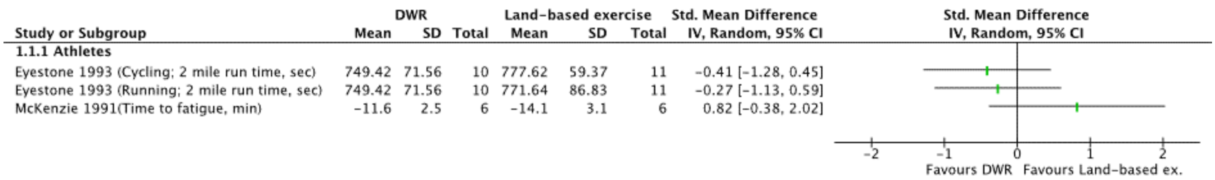


Figure 4.5 Standardized mean difference (95% CI) for the effect of DWR compared with Land trainings on physical function outcomes

4.8 Quality of Life

In their study measuring quality of life, Cuesta-Vargas and his associates found a large effect of DWR (SMD 2.01–3.23) on both the mental and physical indicators of QoL compared with their no-exercise control group (Cuesta-Vargas et al., 2012) (Figure 4.6).

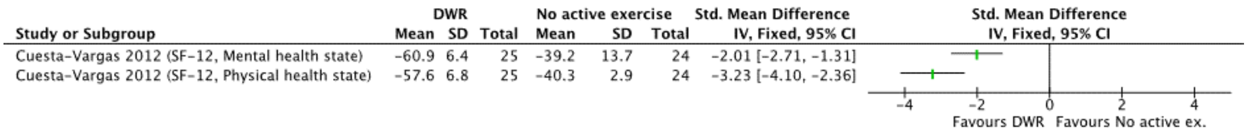


Figure 4.6 Standardized mean difference (95% CI) for the effect of DWR compared with No active exercises on quality of life

But the work by Assis and Kanitz found similar effects between DWR and training on land in terms of improving QoL (Assis et al., 2006; A. C. Kanitz et al., 2015) (Figure 4.7).

Figure 4.7 Standardized mean difference (95% CI) for the effect of DWR compared with land trainings on quality of life

4.9 Discussion

This systematic review found comparable effects of DWR and training on land in improving the VO_{2max} of active individuals, sedentary participants and people with chronic health conditions. Similar effects were also found in terms of physical functioning and QoL, though the conclusions there are weaker due to the smaller number of studies. Only a few studies have compared the effect of DWR with no exercise in terms of all three domains of interest, and their results have been mixed. Heterogeneity in the interventions and their reporting, as well as varied types of participants limits stronger conclusions.

The results of the review do though indicate that DWR can be equally effective in maintaining or improving cardiorespiratory fitness compared to land-based training. Water immersion decreases joint loading and buoyancy supports movements that may be difficult to perform on land (Waller et al., 2016). For individuals experiencing pain who need to keep weight off a joint, aquatic exercise can effectively reduce loading, making exercise more successful, feasible and enjoyable for those unable to train effectively on land, thereby resulting in greater compliance (Barker et al., 2014a). Additionally, the aquatic environment may enable individuals with limited mobility to train at higher intensities than on land (Waller et al., 2014).

This review found that DWR elicits more response from healthy but untrained and sedentary elderly individuals. One possible explanation is the greater scope for improving the aerobic capacity of sedentary elderly people with poor initial fitness (Reilly et al., 2003). Of note, such a magnitude of improvement likely has greater clinical relevance for sedentary yet healthy elderly persons than for those who are trained or at least physical active. Cardiovascular function decreases with age, and cardiorespiratory fitness declines steadily in sedentary individuals at a rate of approximately 1% per year after the third decade of life (Posner et al., 1995). So training like DWR that improves cardiorespiratory fitness can have important clinical implications for the sedentary, particularly those of advanced age.

One of aim of this review was to compare the effects of DWR to those of land-based walking and running. The one study that did so explicitly (Alberti et al., 2017) found a large effect for DWR using 4-, 6- and 10-minute walking tests and a no active exercise control group.

Similarly, little data on the effect of DWR on quality of life was found. When Cuesta-Vargas compared DWR with no active exercise, his group found a significant effect on physical and mental health using the SF-12 instrument (Cuesta-Vargas et al., 2012). But that was the only relevant study. There is potential for QoL to be influenced via changes in physical functioning which improve well-being by promoting relaxation, vasodilation, reduced joint loading, and general analgesic effects (Assis et al., 2006). DWR may release cortisol and adrenaline into the bloodstream, raising the pain threshold (Cuesta-Vargas et al., 2012). The exercise should also promote the removal of metabolic waste and reduce nociceptor activation (Hall et al., 1990). More evidence is required to understand if DWR has enough of an effect to improve land-based functioning and/or quality of life.

4.10 Study limitations

Although the synthesized evidence in this review is encouraging, the study had several limitations. Most of the included studies had small sample sizes and the recruitment of participants was often by convenience sampling. That tends to increase the chance of type II error and affect the results about efficacy because of the subjects' poor representativeness. That then influences the generalizability of the findings.

The participants' heterogeneous characteristics also make it difficult to draw definitive conclusions about clinical outcomes. Indeed, meta-analysis was hindered by the diversity in the studies' interventions, methods, and risks of bias. For instance, there were wide range of DWR protocols and outcome measurement techniques. In particular the methods for measuring VO_{2max} varied dramatically. That limits any attempt to prescribe a recommended DWR dosage. There were few studies with homogeneous groups of subjects available for review.

The lack of a rigorous meta-analysis hinders a precise estimation of effect and a statistical analysis of DWR. More clinical trials in specific populations with larger sample sizes could help yield more solid conclusions about the effectiveness of DWR.

4.11 Future directions

Conducting correlational studies could have been useful in applying biopsychosocial approaches to investigating the relationships between the measured outcomes and DWR, but that would require a larger and more homogenous group of participants. The results could, though, further justify the clinical significance of DWR for the specific population represented.

Additionally, studies normally measure short-term effects immediately after DWR sessions. However, long-term effects should be considered after completion of an intervention program to evaluate any carry-over of the effects.

And there is currently a lack of evidence about participants' experiences in and perceptions towards DWR programs. Qualitative research is therefore suggested to explore participants' attitudes, behaviour, beliefs and satisfaction. That would nicely complement the quantitative data about adherence, functioning and QoL.

It is also difficult to recommend optimal training dosages, so investigations of program intensity are warranted.

4.12 Conclusions

DWR may have effects on exercise capacity similar to those of training on land in terms of improving cardiorespiratory fitness, physical functioning and quality of life. It may be a valuable alternative for people with barriers to performing exercise on land. While there may be differences in physiology and biomechanics in water, DWR appears to provide an adequate stimulus for cardiovascular training.

The small number of studies and the quality of the evidence limit further conclusions. To better understand the potential benefits of DWR, future research is needed in developing an effective prescription for targeted populations.

Chapter 5 Assessing cardio-metabolic outcomes with the PNOĒ portable metabolic analyser

This chapter has been published as below:

Kwok MMY, Ng SSM, Myers J, So BCL. (2025). Assessing the Validity and Reliability of the PNOE for Measuring Cardiometabolic Outcomes During Walking Exercise. *Journal of Functional Morphology and Kinesiology*. 10(2), 159.

This chapter has been presented in the below conference.

Kwok MMY, Ng SSM, Ngai CH, Lui KW, So BCL (2023 October). "Application of inexpensive 3D printed prototype for aquatic exercise aerobic fitness testing; a case study" In Australian Physiotherapy Association Conference (Poster Presentation)

5.1 Abstract

As AHIIT can be used to improve in cardio-metabolic health and physical functioning, and since DWR appears to be comparable to land-based training for improving CRF while providing advantages for persons who are weak, injured or in pain, DWR should be able to reduce the CMR for such persons and promote improvements in their cardio-metabolic capacity. Work was therefore undertaken to refine the methods for assessing the cardio-metabolic outcomes of DWR. That involved quantifying the validity and reliability of assessments using the portable PNOĒ metabolic analyser. That analyser is not water-resistant, but an inexpensive, 3D-printed, water-resistant adaptor was developed to make it so. The modified PNOĒ's measurements were then compared with those using a COSMED K5 system in treadmill walking experiments on land. The reliability and validity of the two system's readings were found to be comparable, so with its water-resistant case the PNOĒ should be a useful aid for sports scientists providing tailored exercise prescriptions.

5.2 Introduction

Cardio-metabolic data can be measured using either a metabolic cart or a portable device. Cardiopulmonary exercise testing (CPET) is a powerful technique for early detection of predisposing factors for major chronic cardiometabolic diseases (Macfarlane, 2017), but accurate measurement is essential if it is to be effective (Ross et al., 2016a). Technological advances have today produced portable systems which enable measurement of metabolic data in real time outside of the laboratory (Overstreet et al., 2016). This shift is important as laboratory testing can be cumbersome, labour intensive and expensive (Parvataneni et al., 2008). Overcoming these limitations is crucial to promoting widespread application of CPET assessments, but it requires a portable assessment system that is reliable, practical, cost-effective and gives valid results in evaluating various sports performances.

One such newly-developed portable metabolic measuring device is the PNOĒ system from ENDO Medical in Palo Alto, California. It is designed to measure cardiometabolic outcomes under both laboratory and field conditions. The PNOĒ device features an electrochemical fuel cell sensor for oxygen measurement, a non-dispersive infrared sensor for carbon dioxide measurement, and a hot film anemometer for gas flow measurements. The PNOĒ device operates in a breath-by-breath mode, allowing for continuous measurement of volume and simultaneous determination of expired gas concentrations. The advantage of this technology is its stable construction and the absence of moving parts. The PNOĒ system is not, however, water-resistant. It is designed for land-based testing. For this study's DWR experiments it was necessary to develop an adaptor that can be attached to the PNOĒ to make it water-resistant and suitable for aerobic fitness testing in aquatic environments. With that accomplished, it was also necessary to compare readings produced with the PNOĒ system to those produced using a conventional metabolism cart. Treadmill walking experiments were used, and the results showed that the accuracy and reliability of the PNOĒ system's readings are comparable to those of a portable metabolic cart.

Previous research has evaluated the reliability and validity of the PNOĒ device against the COSMED Quark CPET, a stationary metabolic cart (Tsekouras et al., 2019a).

The findings of that study indicated that the portable PNOĒ has accuracy comparable to that of a stationary metabolic cart. It is capable of precisely measuring healthy adults' respiratory variables across a wide range of exercise intensities under laboratory conditions (Tsekouras et al., 2019b).

Similarly, there is evidence supporting the accuracy of the COSMED K5 when compared to a metabolic cart during submaximal cycling exercise. The COSMED K5 demonstrated accuracy comparable to that of a metabolic cart in measuring VO_2, VCO_2, RER and EE, although it tended slightly to underestimate VO_2 and VCO_2 (by 6–7%) (Perez-Suarez et al., 2018). When comparing the COSMED K5 to the COSMED K4b2 portable metabolic analyser, a moderately strong relationship was observed in measuring VO_2, VCO_2, and EE across a range of walking speeds. Additionally, a strong correlation was found in measuring VO_2 and VCO_2 between the PNOĒ device and the COSMED-CPET metabolic cart, with the PNOĒ device demonstrating satisfactory repeatability (Tsekouras et al., 2019a). However, it is important to note that RER measurements may yield slightly different results due to variations in measurement and algorithms. Differences in measurement methodologies, such as variations in the accuracy and calibration of gas analysers and the time response of sensors at low to moderate exercise intensities can contribute to variations in RER measurement (Sato et al., 2011). Therefore, it is crucial to consider the specific characteristics and limitations in comparison.

5.3 Methods

5.3.1 Participants

The PNOĒ was tested including adults aged 20-35 years old who were clinically healthy, free of cardiorespiratory distress and not using any medication. Anyone with any cardiorespiratory or neurological pathology or who had suffered a fracture or had any surgical intervention done to the lower extremities in the previous six months was excluded. Due to the infection fear among elderly women participants during COVID-19 pandemic, we included students as our participants in this study. All participants were informed of the study's risks by an investigator, and signed an informed consent form prior data collection. The study was approved by the Hong Kong Polytechnic University's ethics committee for research involving human participants in accordance with the standards of the Helsinki Declaration (HSEARS20220318009).

5.3.2 Study design and procedures

The participants underwent an initial screening interview and familiarization process before providing informed consent for the study. The International Physical Activity Questionnaire was administered to assess each participant's physical activity levels for descriptive purposes (Helmerhorst et al., 2012).

The familiarization session was held one week prior to the experiments. In it the participants practiced using the PNOĒ face mask and flow sensor (Figure 5.1). They then attended two experiment sessions, with a gap of between 2 and 7 days between the sessions. The laboratory's temperature and humidity were recorded during each visit. During the first visit, resting heart rate, blood pressure, body mass (in kg), and height (in cm) were recorded.

During the second visit participants completed a 28-minute walk on a treadmill while wearing the portable cardio-metabolic device. Proper fitting of the metabolic test equipment was confirmed by checking for air leakage through maximal expiration before each test. The fit was continuously monitored during breath-by-breath measurements to ensure there was no lifting of the mask or whistling sounds indicating leakage.

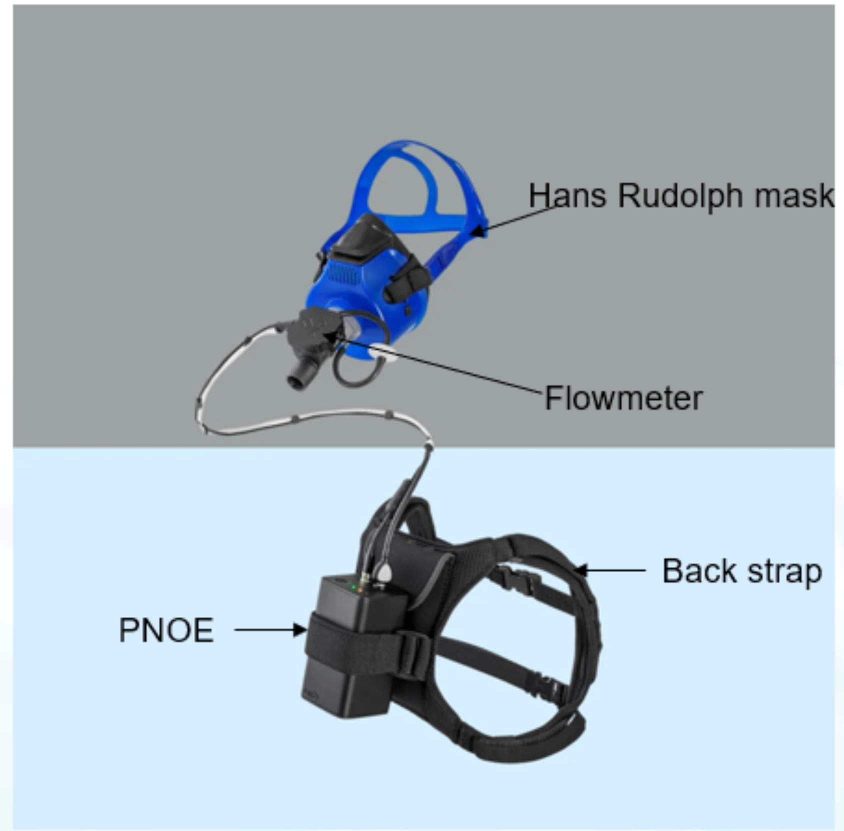


Figure 5.1 The PNOĒ system

The protocol began with 5 minutes of walking at 1.7 miles per hour with a 10% inclination, followed by a 2-minute rest period off the treadmill. That 5-minute walking and 2-minute rest cycle was repeated as the walking speed increased to 4.2 miles per hour with a 16% inclination (Bayles & Swank, 2018). Breath-by-breath data were collected continuously throughout the procedure. Participants performed the entire walking protocol twice, wearing the two proposed devices in a randomized and counterbalanced order (Figure 5.2).

Figure 5.2 Experiment set up

5.3.3 Instruments

The PNOĒ device operates on lithium batteries and weighs approximately 800g. The device is composed of a single housing (120mm tall × 110mm wide and 45mm long) carried in a shoulder harness (see Figure 5.2). The user wears a standard Hans Rudolph mask and breathes through a flow sensor (Hans Rudolph Inc., Kansas City). Figure 5.3 shows the comparable COSMED K5 device. (The unit tested had version 1.1 of the firmware.) It is also portable and worn on an anatomical harness. The device features a Galvanic fuel cell O_2 sensor and a digital infrared CO_2 sensor. The system transmits data via Bluetooth telemetry to the analysis software for visualization and real time measurement. The K5 also includes GPS sensing and an altimeter for use in the field. It measures of gaseous exchange on VO_2, volume of carbon dioxide (VCO_2), respiratory exchange ratio (RER), tidal volume (VT, metabolic equivalent (MET) and energy expenditure (EE).

Figure 5.3 The COSMED K5

5.3.4 *Validity assessment*

The validation protocol used an incremental version of the standard Bruce protocol (Pescatello et al., 2014). Each stage lasted for five minutes, starting from 1.7km/h with a 10% incline, then progressing to 2.5km/h with a 12% incline, followed by 3.4km/h with a 14% incline, and finally reaching 4.2 miles per hour with a 16% incline. The walking speed was estimated by visually inspecting the heart rate response and the BORG scale score. The intensity levels were classified to reflect relatively light to moderate cardio-metabolic demands, following the guidelines of the American College of Sports Medicine in consideration of wider applicability (Garber et al., 2011b). VO_2, VCO_2, RER, VT, MET and EE were the cardio-metabolic variables recorded, using values from the final minute of each five-minute stage. Measurements were obtained with both the COSMED K5 system and the PNOĒ, with the COSMED readings serving as the reference standard. Sequential gas sampling was performed to ensure accurate measurements.

5.3.5 *Reliability assessment*

The intra-rater reliability of the PNOĒ device was evaluated by measuring the cardio-metabolic variables of selected subjects with the same walking protocol on two separate days. The same experimental setup was followed in both visits. The test was performed at the same time of day by the same assessor, and subjects were instructed to wear similar clothing and the same walking shoes for the two trials.

5.3.6 *Adapting the PNOĒ for aquatic exercise fitness testing*

To address the limitations of the PNOĒ, which is not water-resistant and thus restricted to land-based testing, an inexpensive, 3D-printed water-resistant adaptor that can be attached to the PNOĒ was developed for aerobic fitness testing in aquatic environments. Collaborated with the University Research Facility in 3D Printing, a Polyjet resin was used in the 3D printing. The first fused deposition model (Figure 5.4) proved to be insufficiently water-resistant. The second iteration (Figure 5.4), however, remained watertight. The Poly-jet 3D printing allowed combining soft and hard material in a single piece and produced a smooth surface finish.

Figure 5.4 The first fused deposition iteration of the waterproof housing

Figure 5.5 The second-generation

The final prototype performed successfully in the aquatic exercise testing. It was easy to fabricate, and it provided a cost-effective, water-resistant instrument for performing aquatic exercise testing (Figure 5.6).

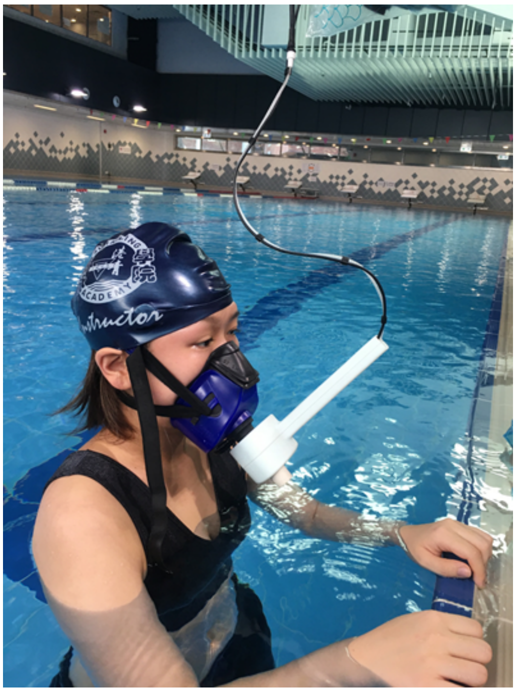


Figure 5.6 Application of the prototype during aquatic exercise tests

5.3.7 Statistical analysis

All results were normally distributed and are presented as mean values with standard deviations (mean ± SD). Statistical significance was accepted at the 5% level of confidence ($p \leq 0.05$). Reliability was assessed by calculating the coefficient of variation (CV). The test-retest reliability of the PNOĒ system (consistency) was assessed through two-way random-effects analysis of variance (ANOVA). Intraclass correlation coefficients quantified the level of agreement between the two instruments. A 95% confidence interval (CI) was used to describe the variation in the ICCs. An ICC of 0.50–0.75 was taken as indicating moderate reliability; good reliability was identified as an ICC= 0.75–0.90; an ICC >0.90 indicated excellent reliability (Koo & Li, 2016). The validity of the PNOĒ system's readings was assessed by comparing the data collected by the PNOĒ with that using the COSMED K5. Student's paired sample t-tests were used to assess the significance of the differences between the readings. To identify bias, an absolute mean difference was calculated. Pearson correlation coefficients (r) were used to evaluate the relationship between the devices. A moderate relationship was indicated by an r= 0.40–050; moderately high was r= 0.60–0.79; r≥ 0.80 was deemed high (Safrit & Wood, 1995). Bland Altman plots of the differences between the PNOĒ and COSMED K5 readings plotted against the average of the two measurements were prepared (Bland & Altman, 2010). All of the analyses were performed using version 23.0 of the Statistical Package for the Social Sciences.

5.4 Results

Twenty-one young healthy and active adults (15 men, 6 women) successfully completed the reliability and validity tests. The descriptive statistics for that sample are presented in Table 5.1.

Table 5.1 Descriptive characteristics of participants: (mean± SD)

	Total (n=21)	Women (n= 6)	Men (n=15)
Age (years)	22.76± 3.85	21± 3	23.47± 3.93
Height (cm)	169.38± 9.69	157.67± 3.77	174.07± 6.99
Body Mass (kg)	64.19± 11.92	50.17± 2.61	69.8± 9.28
BMI (kg/m^2)	22.20± 2.58	21.86± 1.44	22.33± 2.90
IPAQ level 1 (High)	28.6%	0%	40%
IPAQ level 2 (Moderate)	19.0%	33.3%	13.3%
IPAQ level 3 (Low)	52.4%	66.7%	46.7%

Note: IPAQ: international physical activity questionnaire; BMI: Body mass index

5.4.1 Reliability

Overall, the coefficient of variation for the HR, VT and RER readings was low at the faster walking speed and high at the slower speeds. This despite the fact that the RER data showed a high CV at the fastest walking speed. However, no specific pattern in the CVs was seen with the VO_2, VCO_2 and EE data at the different speeds. The CV ranged from 3.4% to 20.8% for all of the variables across all of the speeds (**Table 5.2**). The ICC was found to be moderate (0.50–0.75) across HR levels 1 and 2, for level 4 of the RER and level 1 of VT. For the rest of the variables at all speeds the ICCs generally remained at the good level (0.75–0.9). In particular, the ICCs at VT levels 3 and 4 exceeded 0.9, indicating excellent reliability. Most of the ICCs for VCO_2 were in the good range. Those for RER from level 1 to level 3 and for VT at levels 2–4 indicate good to excellent reliability.

Table 5.2 Test-retest reliability of metabolic variables with the PNOĒ (n=21)

Bruce protocol	Level 1 1.7mph x 10%	Level 2 2.5 x 12%	Level 3 3.4 x 14%	Level 4 4.2 x 16%
HR (bpm)				
visit 1	111.47 ±14.70	123.28 ±17.46	141.44 ±19.48	163.97 ±21.20
Visit 2	113.06 ±12.16	123.43 ±12.50	142.35 ±15.38	165.21 ±16.61
ICC (95% CI)	0.51 (0.12-0.77) *	0.65 (0.32-0.84) *	0.84 (0.64-0.93) *	0.90 (0.77-0.96) *
CV (%)	12.4	10.5	7.4	5.5
VO_2 (mL/min/kg)				
Visit 1	18.03±5.59	24.41 ±6.96	31.52±8.81	39.27±10.91
Visit 2	17.65±5.91	22.66±6.97	30.84±9.33	37.79±11.68
ICC	0.80 (0.57-0.91) *	0.82 (0.61-0.92) *	0.86 (0.68-0.94) *	0.81 (0.58-0.92) *
CV (%)	18.9	15.9	16.2	19.0
VCO_2 (ml/min/kg)				
Visit 1	13.66±4.43	19.32±5.71	26.77±7.72	36.81±10.39
Visit 2	13.79±4.90	18.28±5.84	26.77±8.26	36.15±11.12
ICC	0.81 (0.59-0.91) *	0.87 (0.70-0.94) *	0.86 (0.69-0.94) *	0.82 (0.61-0.92) *
CV (%)	18.4	15.1	15.1	17.9

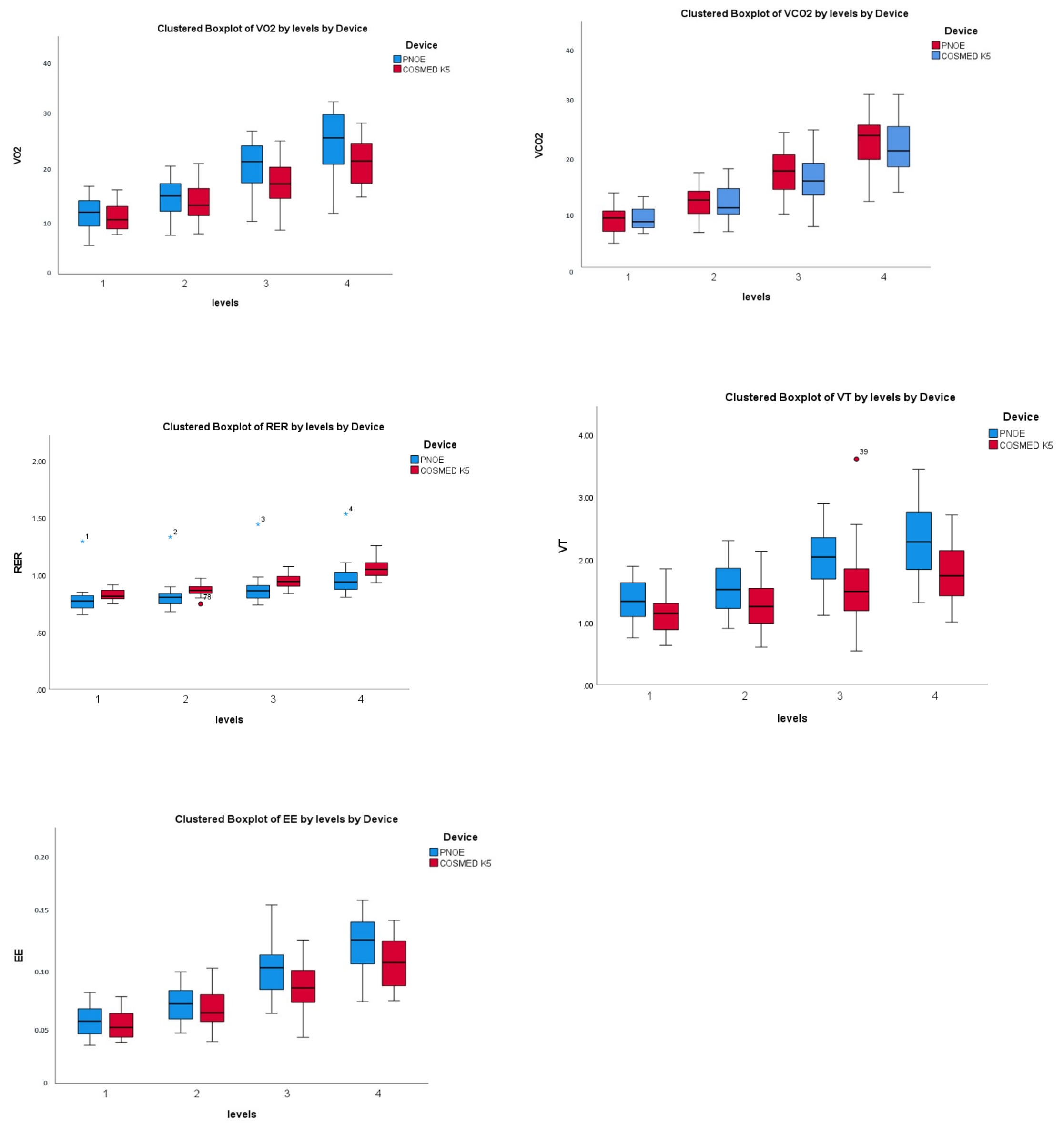
RER				
Visit 1	0.76 ±1.07	0.80 ±1.03	0.85 ±1.09	0.97 ±0.18
Visit 2	0.79 ±0.13	0.82 ±0.13	0.88 ±0.14	0.97 ±0.15
ICC	0.79 (0.55-0.91) *	0.85 (0.67-0.94) *	0.85 (0.66-0.94) *	0.51 (0.11-0.77) *
CV (%)	10.3	8.0	7.8	17.5
Tidal Volume (L)				
Visit 1	1.24 ±0.38	1.56 ±0.34	1.98 ±0.52	2.35 ±0.60
Visit 2	1.35 ±0.34	1.56 ±0.41	2.01 ±0.53	2.35 ±0.60
ICC	0.72 (0.43-0.88) *	0.88 (0.73-0.95) *	0.93 (0.84-0.97) *	0.99 (0.98-1.0) *
CV (%)	20.8	11.4	8.1	3.4
EE (kcal/min/kg)				
Visit 1	0.08±0.03	0.12±0.03	0.15±0.04	0.19±0.05
Visit 2	0.08±0.03	0.11±0.03	0.14±0.04	0.18±0.05
ICC	0.81 (0.59-0.92) *	0.83 (0.62-0.93) *	0.88 (0.72-0.95) *	0.81(0.58-0.92) *
CV (%)	15.8	13.9	12.5	16.0

Notes: ICC, Intraclass correlation coefficient; 95%CI, 95% confidence interval. *indicates a value significant at the $p \leq 0.05$

5.4.2 Validity

Paired t-tests showed that there were no significant differences between the mean HR, VO_2, VCO_2, RER values measured with the two devices. That was also true of the VT readings at speed level 3 and EE at levels 1-3. There were, however, significant mean differences in VT at the 3 walking speeds and in the EE data at one speed (Figure 5.7). The mean VT was significantly higher ($\boldsymbol{p} \leq 0.05$) at speeds 1, 2 and 4 when measured by the PNOĒ compared with the K5. Similarly, the mean EE was significantly higher ($\boldsymbol{p} \leq 0.05$) at the highest speed with the PNOĒ compared with the K5.

Figure 5.7 Comparison of cardio-metabolic variables across levels of exercise between the PNOĒ and COSMED K5 instruments



Note: The box and whisker plots present the medians and 25th and 75th percentiles for the metabolic variable readings.

The Pearson correlation coefficients between the PNOĒ and K5 readings were moderately high (r=0.60–0.79). RER was one exception with values out of the moderate range. On the other hand, the HR data at levels 3 and 4 and the VT data at level 4 showed correlation coefficients exceeding 0.8 (Table 5.3).

The absolute mean percentage differences reveal that the PNOĒ tends to be biased towards higher values for all metabolic variables compared with the K5 system. The absolute mean percentage differences ranged between 0.01 and 25.53%. The greatest absolute mean percentage difference was in VT at all speeds, but particularly at the fastest speed of 4.2mph. RER exhibited only small differences (0.01 to 0.05).

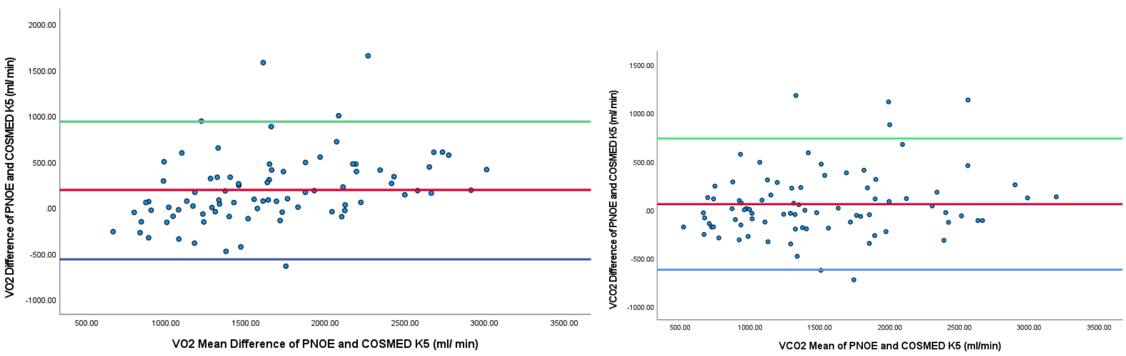
Table 5.3 Validity of the PNOĒ readings for select metabolic variables (compared with the K5) (n=21)

Bruce protocol	Level 1 1.7mph x 10%	Level 2 2.5 mph x 12%	Level 3 3.4 mph x 14%	Level 4 4.2 mph x 16%
HR (bpm)				
Pearson's r	0.57*	0.74*	0.89*	0.95*
Absolute Mean % Difference	0.55	1.50	0.37	0.18
VO_2 (mL/min/kg)				
Pearson's r	0.67*	0.65*	0.53*	0.58*
Absolute Mean % Difference	6.54	6.07	12.90	14.00
VCO_2 (mL/min/kg)				
Pearson's r	0.66*	0.67*	0.55*	0.62*
Absolute Mean % Difference	1.40	0.66	5.50	5.49
RER				
Pearson's r	0.12	-0.07	-0.30	-0.27
Absolute Mean % Difference	0.01	0.03	0.04	0.05

VT (L)				
Pearson’s r	0.62*	0.74*	0.62*	0.86*
Absolute Mean % Difference	17.16	16.67	21.39	25.53
EE (kcal/ min/kg)				
Pearson’s r	0.69*	0.69*	0.58*	0.63*
Absolute Mean % Difference	6.97	5.97	13.07	14.40

Notes: ***indicates a value significant at the *p*≤0.05**

Bland- Altman plots for the relationships between the two instruments are shown in Figure 5.8. Bland-Altman plots look for evidence of proportional bias. They are a simple way to evaluate any bias between mean differences, and to estimate an agreement interval within which 95% of the differences between the PNOĒ and COSMED K5 data will fall. Cases outside the upper and lower confidence intervals should also be noted. Almost all of the data (except for a few VT readings) fell within the plots’ upper and lower 95% confidence intervals.



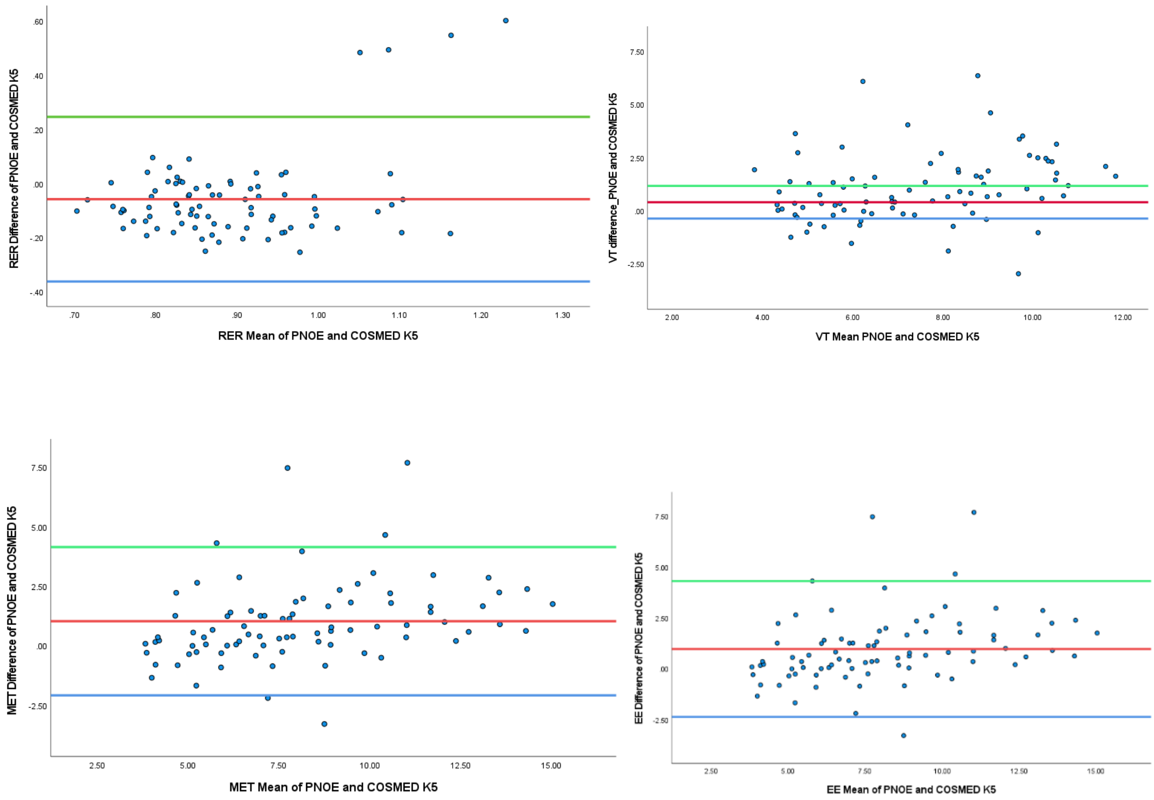


Figure 5.8 Agreement between the PNOĒ and COSMED K5 readings

5.5 Discussion

Ensuring reliable and valid measurements is crucial when using portable metabolic devices to assess cardio-metabolic outcomes. This has been the first study to rigorously compare reliability of the PNOĒ and COSMED K5 devices and the validity of the cardio-metabolic data they generate in the context of treadmill walking.

In this study, the reliability of the metabolic variables was assessed using the CV. Lower CV values, particularly for RER and VT at higher speeds, were considered more favourable as they indicated less variability around the mean (Hodges et al., 2005). While the VT showed moderate reliability at slower speeds, the PNOĒ device demonstrated good to excellent reliability in measuring VO2, VCO2, RER, METs, and

EE at higher speeds. These findings suggest that the PNOĒ device may be less reliable at slower speeds compared to other speeds, possibly due to greater variations in step length and increased metabolic costs associated with slower speeds (Rock et al., 2018). Consequently, this could potentially result in lower reliability for the PNOĒ device at slower speeds.

In terms of validity, we utilized a Bland-Altman plot analysis Figure 5.8 to evaluate agreement between the PNOĒ and COSMED K5 portable metabolic analysers. Our findings revealed good agreement with an acceptable bias between the two devices for VO_2, VCO_2, RER, METs, and EE across the four levels of the incremental protocol. The Bland-Altman plots demonstrated that the differences between the two devices remained consistent across the entire range of exercise intensities, indicating similarity. However, for VT, there was a lack of similarity as the values fell outside the upper and lower 95% confidence interval between the two devices. It is important to note that VT is an absolute measure that tends to minimize individual variability, resulting in a restricted spread of values for this variable. Previous research by Leprete et al. (2012) also reported a strong relationship between respiratory variables obtained simultaneously using two commercially available portable metabolic systems (Leprêtre et al., 2012). They found no significant differences in measures of VO_2 and VT; however, there was a notable difference in RER and VCO_2 at maximal exercise intensity. These differences could be attributed to participants' characteristics or mask properties, such as dead space and resistance to air flow. Another potential explanation for these discrepancies could be methodological differences between the protocols, such as treadmill versus cycling.

The validity findings strongly indicate that the PNOĒ metabolic system is acceptable for measuring VO_2, VCO_2, RER, MET, and EE across the four levels of incremental exercise intensities although there were significant differences in VT measurements between the two devices. This discrepancy may be attributed to the PNOĒ device's sensitivity to ambient CO_2 levels, highlighting the impact of environmental factors on the PNOĒ 's ability to reliably and accurately assess metabolic data (DeBlois et al., 2021). The observed r values for VO_2, VCO_2, VT, and EE indicated moderately high associations (r=0.60-0.79). These findings are somewhat consistent with previous studies comparing VO_2 measurements between the PNOĒ and a metabolic cart (Tsekouras et al., 2019a). The significant correlations observed between VO_2, VCO_2, VT, and EE provide further support for the validity of measuring cardiometabolic responses using both the PNOĒ and COSMED K5 devices.

These findings align with previous research that highlights the significance of aerobic contributions to the energy demands of an incremental walking protocol (DeBlois et al., 2021). Assessing the aerobic contribution in a graded walking protocol is valuable as it helps avoid potential issues related to mask displacement and thermal strain. This insight can provide exercise specialists and coaches with important considerations regarding the role of aerobic measurements using different portable metabolic devices. The moderate to high associations observed between the PNOĒ and COSMED devices is consistent with the experimental design of PNOĒ and COSMED-Quark CPET, which are designed to examine cardiometabolic responses to exercise. It is worth noting that in the current study, a strong relationship (r = 0.86) was observed between metabolic variables, particularly VT, at higher speeds when measured by both the PNOĒ and K5 systems.

This study is novel in several respects. The evaluation of aerobic power and metabolic outcomes is important for exercise specialists as it provides a fundamental baseline measurement and serves as a monitoring and motivational tool to evaluate exercise progression. Understanding that the reliability and validity results of both the PNOĒ and COSMED K5 systems were comparable to each other can inform objective measurement considerations for these portable metabolic devices. This practical evaluation process will greatly assist sports scientists in providing individualized exercise prescriptions (Sartor et al., 2013). Therefore, the development of a reliable and accurate test for assessing metabolic outcomes using the PNOĒ and COSMED K5 portable metabolic devices is an essential step in evaluating cardiometabolic fitness of individuals seeking to increase their aerobic power for sports performance or overall health improvement.

This study has several limitations. First, food intake was not strictly controlled or measured prior to the assessments, which may have affected participant compliance and potentially influenced the measured metabolic variables. Additionally, not all participants performed the assessments at the same time of day, which could introduce variability in the outcomes between participants. Furthermore, it is important to acknowledge that the participants in this study were healthy and physically active young adults. Therefore, generalizing the true validity of the PNOĒ and COSMED K5 systems using these data should be done cautiously, as temporal changes related to physical training or overall health status may have influenced the results. Another limitation is that the study only investigated treadmill walking at low to moderate intensity, and it is important to explore other modes and intensities of exercise. Future research should involve testing the accuracy of the systems using different modes of exercise at various intensities under different environmental conditions.

5.6 Conclusions

The PNOĒ, a portable metabolic device, demonstrated an overall acceptable test-retest reliability. It exhibited performance comparable performance to that of the COSMED K5 in measuring some of the cardio-metabolic variables. In the course of the study an inexpensive water-resistant adaptor was developed for the PNOĒ device that allows it to test aquatic exercise.

Chapter 6 Establishing the AHIIT protocol: Incremental exercise on land and in the water with and without added resistance

This chapter has been published as below:

Kwok MMY, Poon ETC, Ng SSM, Lai MCY, So BCL. Effects of Aquatic versus Land High-Intensity Interval Training on Acute Cardio-metabolic and Perceptive Responses in Healthy Young Women. *International journal of environmental research and public health. 2022;19(24):16761.*

Kwok, M. M. Y., Ng, S. S. M., Ng, Y. M., Tan, G. C. C., Huang, P. P., Zhang, Y., & So, B. C. L. (2024). Acute effect of resistive aquatic high-intensity interval training on metabolic costs in adults [Original Research]. *Frontiers in sports and active living*, *6*. https://doi.org/10.3389/fspor.2024.1421281

This chapter has been presented in the below conference.

Kwok MMY, Ng SSM, Lai MCY, So BCL (2021, December). "Effects of Aquatic versus Land High-Intensity Interval Training on Acute Cardio-metabolic and Perceptive Responses in Healthy Young Women" In Hong Kong Physiotherapy Association Conference (Poster Presentation)

Kwok MMY, Ng YM, Ng SSM, Tan GCC, So BCL (2023, June). "The effect of single bout aquatic high-intensity interval training (AHIIT) and resistive aquatic high-intensity interval training (R-AHIIT) on resting energy expenditure and respiratory ratio in healthy adults" *In World Physiotherapy Congress* (Poster Presentation)

Kwok MMY, Ng YM, Ng SSM, Tan GCC, So BCL (2024, June). "The effect of single bout aquatic high-intensity interval training (AHIIT) and resistive aquatic high-intensity interval training (R-AHIIT) on resting energy expenditure and respiratory ratio in healthy adults" International Conference on Evidence Based Aquatic Therapy, *ICEBAT-UK 2024* (Poster Presentation)

6.1 Abstract

An AHIIT protocol was developed by matching intensity with L-HIIT and comparing the cardio-metabolic and perceptual responses. The matching involved adding resistance in the AHIIT. Twenty healthy women performed 10 one-minute treadmill runs at 90% of their maximum heart rates with active recovery in between. Another twenty other healthy adults (9 female, 11 male) performed followed the same regimen in water. Heart rate and oxygen pulse were found to be significantly increased in the AHIIT compared to on land. Those running in the water with additional resistance to achieve the matching (termed the R-AHIIT group) also reported a significantly higher subjectively perceived rate of exertion (RPE). Their RPE was moderately correlated (r= 0.53 with their respiratory exchange ratios (RERs). Matching intensity between AHIIT and L-HIIT thus allows effective prescribing of aquatic exercise for improving cardio-metabolic health and to monitor exercise intensity.

6.2 Introduction

At a given exercise intensity, AHIIT is associated with lower perceived exertion during and after training as measured by the Borg Rating of Perceived Exertion scale (Andrade et al., 2022). Additionally, AHIIT has demonstrated faster recovery, with a more than 10% reduction in post-exercise heart rate reserve (Faíl et al., 2022), and favourable effects on oxygen consumption and energy expenditure (Depiazzi et al., 2018). However, it remains unclear to what extent training in an aquatic environment offers more favourable cardio-metabolic and perceptual outcomes compared with training at the same intensity on land. Identifying the appropriate exercise intensity each individual is essential (Iannetta et al., 2020), and people's heart rate and VO_2 responses can differ significantly in water compared to on land (Antunes et al., 2015).

How following an R-AHIIT program might affect basal metabolism today still remains unclear. Basal metabolism reflects energy expenditure at rest and is indicative of physical fitness and general health status (Schaun et al., 2018). Previous research has shown that HIIT can increase post-exercise resting energy expenditure (REE) and the respiratory ratio, suggesting improvements in basal metabolism and fat oxidation (Paoli et al., 2012). Water's viscosity generates greater muscle strength through exercise, as water resistance is approximately 900 times that of air (McGinnis, 2020). The effects of resistance training on resting metabolic rate (RMR) remain less than clear, so AHIIT's potential to increase RMR and daily energy expenditure merits further investigation (Kirk et al., 2009).

In addition to the physiological benefits of AHIIT, exercise can positively influence perceptual responses, which in turn influence exercise compliance (Ekkekakis et al., 2012). Enjoyment naturally encourages participation (Wankel, 1993), and studies have shown that HIIT with rest intervals can foster positive perceptions, leading to better exercise compliance (Thum et al., 2017). Identifying perceptual responses to exercise is therefore important.

In specifying the AHIIT protocol to be used in this research, it was considered important to identify a matched level of exercise intensity relating AHIIT with L-HIIT. Any effects of AHIIT could then be objectively compared with those of L-HIIT in terms of both cardio-metabolic and perceptive responses. And the training effects on basal metabolism of AHIIT with or without an added resistance could then be objectively documented.

6.3 Designing the HIIT protocol

6.3.1 Participants

Twenty young, healthy and active women were recruited from the university community through poster advertising. They were required to be between 20 and 35 years of age, clinically healthy and not pregnant. Anyone with a chronic medical condition, a fear of water or a skin disease was excluded. All of the participants were informed of the study's risks by an investigator, and signed an informed consent form prior to the data collection. They then completed the International Physical Activity Questionnaire to assess their physical activity levels for descriptive purposes (Helmerhorst et al., 2012). The participants received no rewards for the trial.

Version 3.0.10 of the G*power software was used to estimate the required sample size. Targeting an effect size of 0.64 and assuming power of 0.8 at an alpha level of 0.05 suggested a sample size of at least 16 per group (Haynes et al., 2020a). Allowing for a 20% attrition rate, 20 subjects per condition was considered adequate to detect any differences between the groups. The study was approved by the Research Ethics Committee of the Hong Kong Polytechnic University (HSEARS20210522001). The study's protocols conformed to the Declaration of Helsinki for studies involving humans.

Each subject's resting heart rate, blood pressure, body mass and height were recorded as additional indicators of their habitual levels of physical activity (Puthoff et al., 2006). All of the subjects completed a familiarization session before the experiments, during which the details of the exercises were explained, including the range of movement involved and the use of the mouthpiece and the Hans Rudolph valve. A registered aquatic physiotherapist provided feedback and instruction to the subjects during the trials and the experiments.

6.3.2 Matching the intensity of the aquatic and land incremental tests

Incremental tests on land and in the water were performed prior to the exercise interventions to confirm an individualized cadence required to yield matched exercise intensity. Each participant's body mass and height in cm were first recorded using a BC-730b electronic scale (TANITA, Issaqua) and a stadiometer. In the test's stationary running the participants were asked to flex the hip and knee to as close to 90° as comfortable while maintaining control, and then to push to straighten the hip and knee) (Figure 6.1). Prior to the testing, the exercise was demonstrated first, then practiced once. Participants were monitored continuously and recorded at a frequency of 1Hz using a Polar OH1 heart rate sensor (Polar, Kempele). That HR sensor has been shown to provide valid and reliable HR data (Bergamin et al., 2015a). Gas exchange data were obtained during the tests using the PNOĒ. The incremental protocol increased the exercise load from 85 beats per minute in 15bpm increments every 2 minutes (Ana Carolina Kanitz et al., 2015). An IMT 300 metronome (Intelli, Korea was used to prompt the speed of movement throughout the trial. The HR, VO_2 and rate of perceived exertion were recorded each minute. Maximum oxygen capacity (VO_{2max}) was determined in both the aquatic and land incremental tests with stationary running to volitional exhaustion. VO_{2max} was considered to have been attained when one of the following was met: (1) a respiratory exchange ratio ≥1.10; (2) failure of the heart rate to increase with increases in the workload; (3) post-exercise blood lactate ≥8.0mmol·L^{-1} (Liguori et al., 2022); (4) clear signs of exhaustion (facial flushing, unsteady gait); or (5) refusal to carry on despite strong verbal encouragement. HR, percentage of VO_{2max} (%VO_{2max}), percentage of HR max (% HR_{max}), percentage of VO_2 reserve (%VO_2R), and percentage of HR reserve (%HRR), were compared between the two environments. Blood lactate was measured via capillary blood sampling (approximately 25µL) from the fingertips with a Lactate Plus portable analyser (Nova Biomedical, Waltham) (American College of Sports & Medicine, 2018). The Lactate Plus analyser provides accurate and reproducible measurements of blood lactate concentration that can be used to estimate workloads corresponding to absolute lactate concentrations (Hart et al., 2013). The Data collected in the incremental test were used to determine the intensity required in the exercise experiments for each participant.

Figure 6.1 Aquatic and land-based stationary running incremental tests

6.3.3 Study design and procedures

A randomized crossover design was used. Please refer to Figure 6.2. The order in which each participant completed the AHIIT and L-HIIT trials was randomly assigned. The women were asked not to indulge in any aerobic training for 48 hours prior to any testing session to limit the influence of prior exercise on the study outcomes. Each participant attended two sessions at the pool and two sessions on land in the laboratory. Incremental testing was completed first, either at the pool or on land, followed by the AHIIT and L-HIIT. For the AHIIT, immersion was chest deep (xiphoid-sternal depth or up to five centimetres deeper). The water temperature in the 0.95–1.40m deep hydrotherapy pool was 29°C (Becker et al., 2009). For the L-HIIT the room temperature was maintained at 23°C.

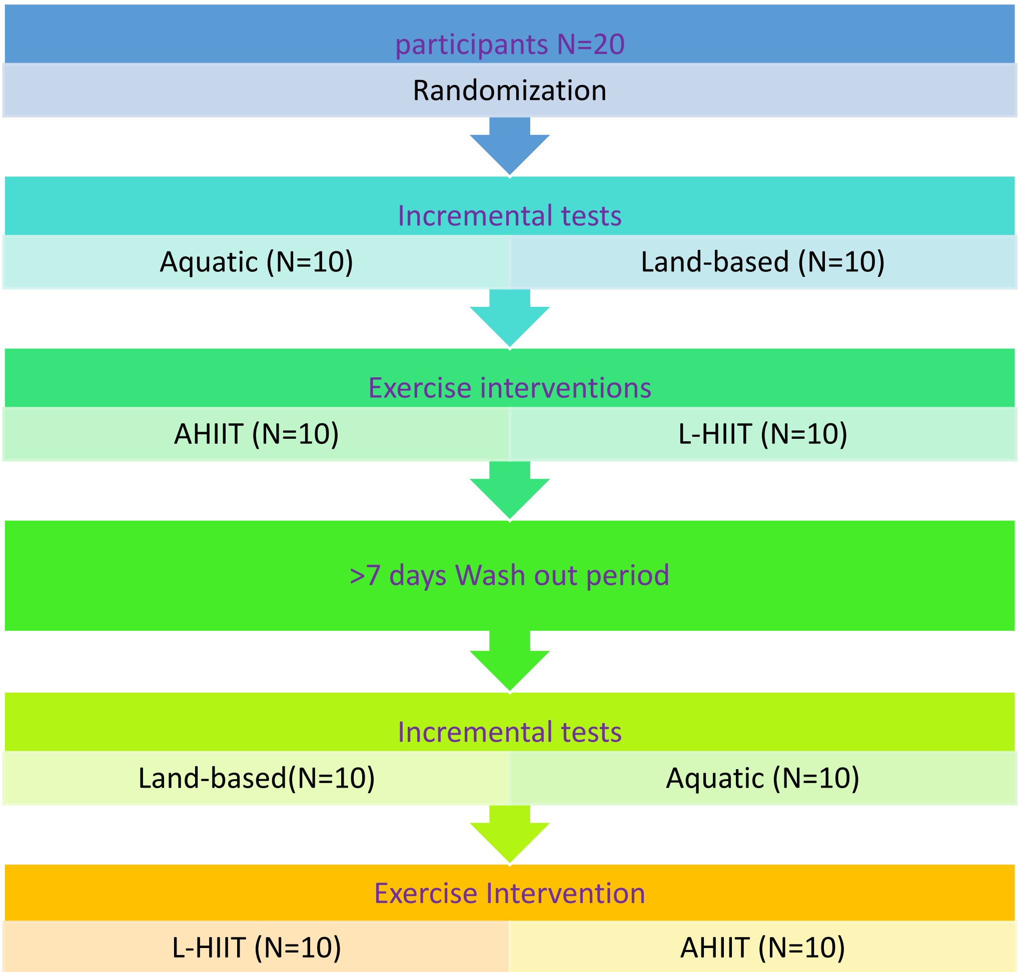


Figure 6.2 Activity flow for the AHIIT and L-HIIT trials

The experiments all involved stationary running at 90% of each individual's maximum heart rate. That rate was determined in an incremental test. Both the AHIIT and R-AHIIT exercises were performed at 130–150 beats per minute, guided by an MA-30 digital metronome (Korg, Tokyo). Each exercise session lasted for 10 minutes and was preceded by a 5-minute warm-up and followed by a 5-minute cool-down. During the 10-minute exercise period, there were 5 sets of stationary running, with each set consisting of 1 minute at 90% of HR_{max} followed by 1 minute of dynamic rest at 70% of HR_{max}. The AHIIT and R-AHIIT protocols were identical in setup, except for the use of resistance boots in the R-AHIIT protocol and the cadence for the R-AHIIT exercise (Figure 6.3).

Brand name :THERABAND Aquafins	The resistance boots are wrapped around the distal shin of subjects with the 2 yellow fins in sagittal plane. The drag force is determined by the surface area and orientation of the fins in contact with water as well as cadence.
Manufacturer: Aquafins Company	
Country: Columbia, Maryland	

Figure 6.3 Details of the resistance boots and their adjustment

The incremental exercise test was a stationary run immersed in the pool (Figure 6.4). Heart rate and breath-by-breath gas analysis were continuously monitored using the a Polar OH 1 sensor and the PNOĒ, respectively. The incremental test was terminated when the subject reached her maximum heart rate. That was determined based on the HR plateauing, VO_2 plateauing, or the subject's inability to maintain the required cadence.

The AHIIT and R-AHIIT experiments themselves involved 5 work-rest cycles lasting a total of 10 minutes. REE, RER, and VO_{2max} were continuously recorded using the PNOĒ breath-by-breath analyser. Maximum and average HR were recorded using the Polar OH sensor connected to the PNOĒ, and RPE was recorded 15 seconds before transitioning to the next work/rest stage. The procedure is summarised in (Figure 6.5).

Figure 6.4 Aquatic incremental test

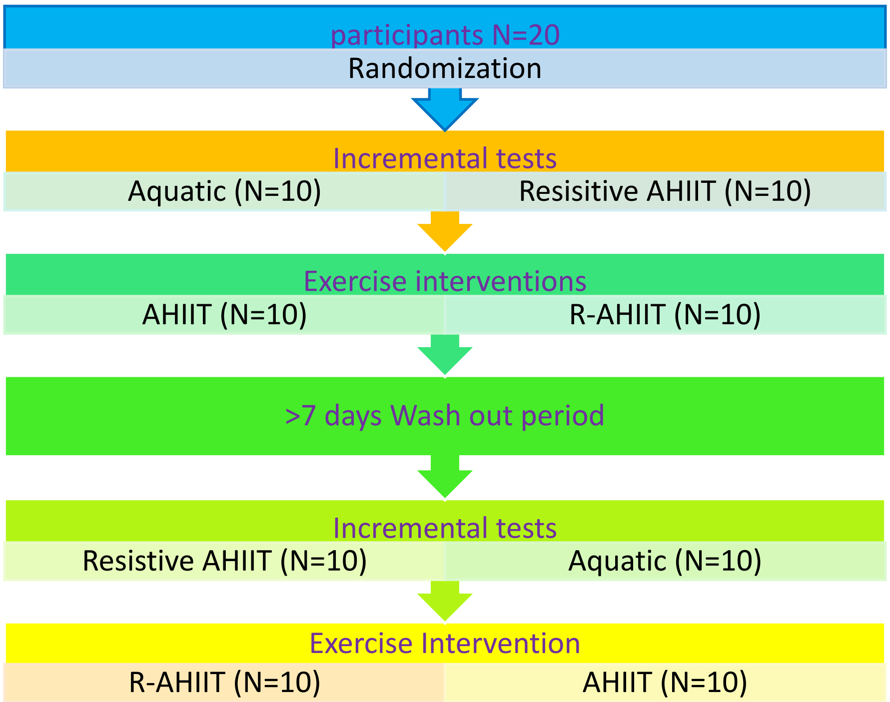


Figure 6.5 Procedure for the AHIIT and R-AHIIT trials

6.3.4 *The AHIIT and L-HIIT exercises*

In the experiments themselves the participants first performed a standardized 3-minute warm-up stretching at 50% of their individual HR_{max}. The instructor then asked the participant to run in place at the cadence determined from the prior incremental tests, guided by the audible metronome. The AHIIT and L-HIIT protocols both consisted of 10 single 1min bouts of stationary running at 90% of HR_{max}, separated by 1 min active recovery intervals at 70% of HR_{max}. The total exercise time for each condition was thus 20 minutes. All of the sessions were held at the same time of the day to avoid variations related to circadian rhythms. No external stimuli such as music or verbal encouragement were provided during either intervention.

6.4 Outcomes

During the AHIIT and L-HIIT at matched intensity, VO_2, oxygen pulse, respiratory exchange ratio, minute ventilation (VE) and HR were measured by the PNOĒ device. When resistance was added during the AHIIT, the primary outcomes were resting energy expenditure (REE) and respiratory exchange ratio (RER). REE accounts for 60–75% of daily energy expenditure, and increasing REE in overweight or obese individuals could help improve their energy imbalance (Paoli et al., 2012). RER is the ratio of carbon dioxide production to oxygen uptake, directly measured at the mouth. Values of 0.7, 0.8, and 1.0 represent respiratory quotient values for fat, protein, and carbohydrates, respectively (Fonseca et al., 2018; Patel et al., 2018). Also recorded were VO_{2max}), HR_{max}, mean heart rate, total energy expenditure (TEE), and perceived exertion. For a man, 42–

46ml/kg/min is considered a good level of VO_{2max}, but among women it is 33–37ml/kg/min (Scribbans et al., 2016). VO_{2max}, HR_{max}, mean HR and TEE were recorded to reflect the subjects' cardio-metabolic status, while RPE was monitored to quantify their perceived exertion. It was measured immediately after each one-minute interval with reference to the Borg 6-20 scale (Borg & Kaijser, 2006). A board with the scale was shown to the participants.

In addition, measures of the participants' energy expenditures (EEs), cumulative EE and metabolic equivalents (METs) were used when matching the AHIIT and L-HIIT. They were quantified by the PNOĒ via indirect calorimetry.

Exercise enjoyment was assessed by administering the 18-item Physical Activity Enjoyment Scale (PACES) scale immediately after each intervention. The respondents indicated their level of enjoyment by responding to the 18 different items using 7-point Likert scales ranging from 1 ("I enjoy it") to 7 ("I hate it'). The possible total response range was 0 to 126, and higher scores indicate a greater enjoyment during the exercise session. Evidence supports the reliability and validity of the PACES for assessing enjoyment of physical activity (Teques et al., 2020).

Efficacy self-perceptions were another outcome recorded. Perceived efficacy is a belief in one's ability to successfully execute some course of action (Hu et al., 2007). In this study the participants' perceptions of their efficacy were assessed using a 5-item questionnaire designed to determine their confidence about being able to repeat either the AHIIT or the L-HIIT. That questionnaire has demonstrated good internal consistency ($\alpha = 0.9$) (Poon et al., 2020). All questions included the same stem: 'How confident are you that you can…' The five items were: 'perform (one to five) session(s) of exercise per week for the next four weeks that is similar to the one you completed during the intervention. The responses were scored at a percentage in 10% increments, with 0%

representing (Not at all) and 100% (Extremely confident). The percentages were averaged over the five items. The participants were asked to complete the scale immediately after each session.

6.5 Statistical analysis

Descriptive statistics were first computed for the demographic data and the study variables. A series of Shapiro-Wilk tests were then performed to evaluate the normality of the data distributions. The continuous data were summarized with means and SDs. Repeated measures analyses of variance were evaluated to assess the main effect of exercising in the water or on land, the main effect of the stages in the incremental tests and any interaction of water or land and the stages in the incremental tests. The predictors tested were HR, $\%HR_{max}$, VO_2, $\%VO_{2max}$, $\%VO_2R$, %HRR and RPE. Paired t-tests assessed the significance of any within-group differences in the cardio-metabolic and perceptual variables in the AHIIT and L-HIIT.

6.6 Results

6.6.1 Participants

The descriptive statistics for the participants in the incremental testing are presented in Table 6.1.

Table 6.2 reports the number of participants who reached each stage in the incremental testing.

The descriptive statistics for the 11 men and 9 women who participated in the AHIIT and R-AHIIT trials are presented in Table 6.3. Again, all of the participants completed 4 stages of the incremental tests and the training protocol.

Table 6.1 Descriptive characteristics of participants in the AHIIT and L-HIIT trials (mean± SD)

Participants

N	20
Sex	F
Age (in years)	21.95±2.35
Height (cm)	160.95±5.76
Body Mass (kg)	53.95±8.08
Body mass index (kgm^{-2})	20.10±3.2
International physical activity levels (%)	
Level 1 (inactive)	0%
Level 2 (minimally active)	85%
Level 3 (active)	15%

Table 6.2 Number of participants who reached each stage of the aquatic and on-land incremental tests

Increment	Cadence (bpm)	Aquatic increments N (%)	Land increments N (%)
1	85	20 (100%)	20 (100%)
2	100	20 (100%)	20 (100%)
3	115	20 (100%)	20 (100%)
4	130	20 (100%)	20 (100%)
5	145	19 (95%)	16 (80%)
6	160	11 (55%)	12 (60%)
7	175	9 (45%)	8 (40%)
8	190	8 (40%)	7 (35%)
9	205	6 (30%)	3 (15%)
10	220	4 (20%)	0 (0%)

Table 6.3 Characteristics of the subjects (mean ± SD)

Number of subjects	20
Gender	Male: 11; Female: 9
Age (years)	Male: 24.27±6.59; Female: 25.44±4.22
Height (cm)	Male: 174.27±6.40; Female: 161.44±4.50
Body Mass (kg)	Male: 66.64±9.11; Female: 57.28±9.02

6.6.2 The effects of exercise intensity in the incremental tests

As the cadence increased in the incremental tests, the anticipated increases in HR, $\%HR_{max}$, %HRR, VO_2, $\%VO_2max$ and $\%VO_2R$ as well as the subjective RPE were observed (Figure 6.6 Aquatic and land incremental test results All were statistically significant at the $p \leq 0.001$ level of confidence. Comparing the two sets of incremental tests, there was a significant main effect of the medium on HR, %HRR and RPE ($p \leq 0.05$) (Figure 6.6), but the immersion showed no significant interaction with any of the outcome variables. The observations are detailed in Table 6.4.

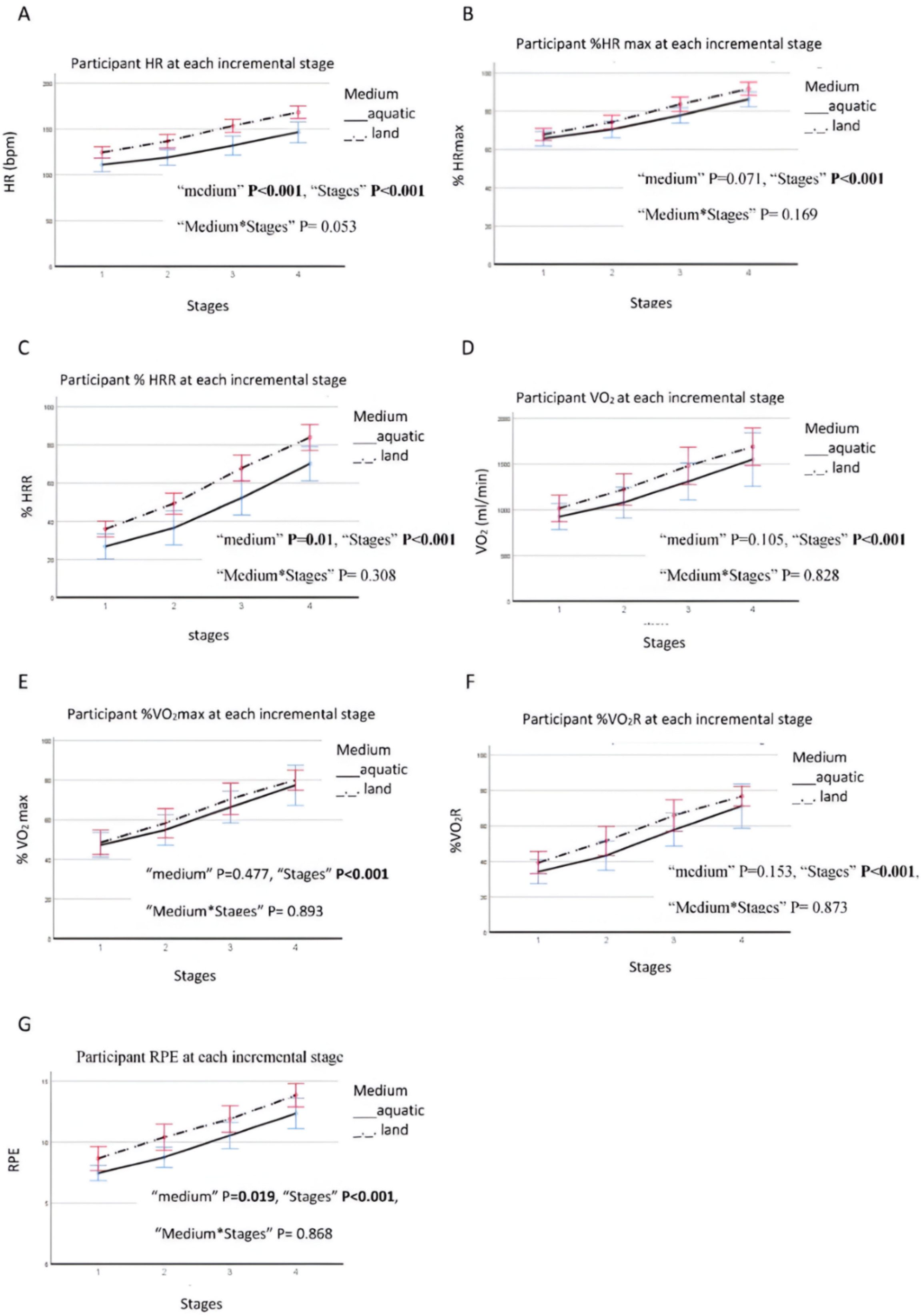

A
Participant HR at each incremental stage
HR (bpm)
Medium
___aquatic
_._. land
"medium" P<0.001, "Stages" P<0.001
"Medium*Stages" P= 0.053
Stages
B
Participant %HR max at each incremental stage
% HRmax
Medium
___aquatic
_._. land
"medium" P=0.071, "Stages" P<0.001
"Medium*Stages" P= 0.169
Stages
C
Participant % HRR at each incremental stage
% HRR
Medium
___aquatic
_._. land
"medium" P=0.01, "Stages" P<0.001
"Medium*Stages" P= 0.308
stages
D
Participant VO2 at each incremental stage
VO2 (ml/min)
Medium
___aquatic
_._. land
"medium" P=0.105, "Stages" P<0.001
"Medium*Stages" P= 0.828
Stages
E
Participant %VO2max at each incremental stage
% VO2 max
Medium
___aquatic
_._. land
"medium" P=0.477, "Stages" P<0.001
"Medium*Stages" P= 0.893
Stages
F
Participant %VO2R at each incremental stage
%VO2R
Medium
___aquatic
_._. land
"medium" P=0.153, "Stages" P<0.001,
"Medium*Stages" P= 0.873
Stages
G
Participant RPE at each incremental stage
RPE
Medium
___aquatic
_._. land
"medium" P=0.019, "Stages" P<0.001,
"Medium*Stages" P= 0.868
Stages

Figure 6.6 Aquatic and land incremental test results compared

Table 6.4 Observations in the four stages of the incremental tests in the pool and on land (mean ±SD)

Stage	Medium	HR	%HR max	%HRR	VO_2	$\%VO_2max$	$\%VO_2R$	RPE
1	Aquatic	110.9±16.2	65.7±8.0	26.8±14.1	926.0±303.8	47.4±13.4	34.1±14.7	7.5±1.3
	Land	136.7±15.8	67.8±6.7	36.1±8.9	1016.7±305.1	49.6±13.2	39.3±13.3	8.7±2.1
2	Aquatic	119.0±18.2	70.5±9.4	36.6±19.3	1078.3±357.3	54.9±16.4	43.2±17.6	8.8±1.8
	Land	153.6±15.3	74.3±7.5	49.2±11.7	1221.4±366.7	58.3±15.8	51.5±17.5	10.4±2.3
3	Aquatic	132.0±21.9	77.8±8.6	52.2±19.0	1309.2±431.7	65.5±17.1	57.8±20.0	10.6±2.3
	Land	168.5±14.5	83.6±8.1	67..9±14.5	1479.8±436.3	70.6±17.0	65.9±18.9	11.9±2.3
4	Aquatic	146.6±24.5	86.3±8.4	70.2±19.2	1548.6±625.3	77.4±21.6	71.1±26.5	12.4±2.7
	Land	168.5±14.5	91.7±7.2	83.9±14.5	1689.7±438.7	80.0±11.0	76.7±11.9	13.9±2.0

Note: HR = heart rate; VO_{2max} = maximum oxygen uptake; HR_{max} = maximum heart rate; RPE = perceived rate of exertion; HRR = heart rate reserve; VO_2R = VO_2 reserve

The AHIIT group showed a significantly lower HR and $\%HR_{max}$ during both the work and the recovery intervals ($p \leq 0.01$). Their average oxygen pulse was significantly higher ($p \leq 0.05$). There was no significant difference between the AHIIT and L-HIIT groups in terms of the other cardiorespiratory parameters Table 6.5.

The two groups' average EE, MET and cumulative EE readings also did not show any significant difference. There was also no significant difference in their average blood lactate concentration changes (AHIIT 6.08 ±2.86mmol/L and L-HIIT 5.84±2.42 mmol/L (Table 6.5).

Table 6.5 Cardio-metabolic outcomes in the AHIIT and L-HIIT groups (mean ±SD)

Measure	AHIIT	L-HIIT	*p*-value
HR max (bpm)	162±19.1	179.1±14.3	<0.01*
HR (bpm)	W149.62±18.88	W166.75±16.41	<0.01*
	R 139.26±17.90	R158.07±15.78	<0.01*

$\%HR_{max}$ (%)	W92.71±4.25	W93.26±3.98	0.693
	R 86.40±6.60	R 88.75±3.47	0.177
VO_{2max} (mL·kg^{-1}·min^{-1})	35.78±6.58	36.14±7.24	0.819
VO_2 (mL·min^{-1})	W1758.57±348.76	W1829.18±287.67	0.293
	R1383.19±280.37	R1462.29±318.55	0.273
$\%VO_{2max}$ (%)	W91.4±5.98	W95.38±11.29	0.091
	R72.18±10.15	R76.66±11.02	0.173
Oxygen pulse (ml/beat)	W11.81±2.05	W11.05±1.78	0.038*
	R9.92±1.55	R9.27±1.78	0.078
RER	W0.93±0.09	W0.94±0.06	0.460
	R1.05±0.09	R1.01±0.08	0.178
VE (L/min)	W61.31±15.31	W62.93±11.22	0.621
	R52.40±13.63	R51.35±8.81	0.704
EE (kcal/min)	W8.64±1.70	W9.02±1.44	0.257
	R6.99±1.44	R7.33±1.57	0.340
MET	W9.22±1.50	W9.79±1.52	0.122
	R7.28±1.48	R7.77±1.35	0.189
Cumulative EE (kcal)	W609.81±235.53	W618.70±246.65	0.897
	R663.98±255.72	R667.07±265.39	0.967
Lactate change (mmol/L)	6.08±2.86	5.84±2.42	0.572

Notes: W = work period, R = active recovery period, VO_2 = oxygen uptake, VO_{2max} = maximum oxygen uptake, RER = respiratory exchange ratio, VE = minute ventilation, EE = energy expenditure, MET = metabolic equivalents, RPE= perceived rate of exertion. * highlights a significant inter-group difference

No significant differences in average RPE were observed in either the work or the recovery intervals. In both the AHIIT and L-HIIT exercises the participants responded similarly in terms of enjoyment, self-perceptions of efficacy and muscle soreness (Table 6.6).

Table 6.6 Perception outcomes of the AHIIT and L-HIIT exercising (mean ±SD)

Measure	AHIIT	L-HIIT	*p* value
RPE (range 6–20)	W13.18±2.0 R11.66±2.18	W12.86±1.84 R11.71±1.89	0.60 0.948
Enjoyment (score of 126)	68.55±7.53	68.55±9.24	1.00
Self -efficacy (score of 100)	37.8±27.48	45.75±20.91	0.072
Muscle soreness index (range 0–6)	5.3±2.07	5.4±1.7	0.873

Note: RPE=rate of perceived exertion, W = work period, R = active recovery period

Within the AHIIT and R-AHIIT groups, however, average REE was found to be significantly different pre- and post-exercise ($p \leq 0.001$ in each case) Figure 6.7. The change in average RER was not, though, significantly different between the groups (F (1,38) = 0.615,Figure 6.7 Pre- and post-exercise resting energy expenditure in the AHIIT and resisted AHIIT groups (mean ± SD)

). Group-by-time interactions revealed no significant differences in REE and RER between the AHIIT and R-AHIIT groups, on average (Table 6.7).

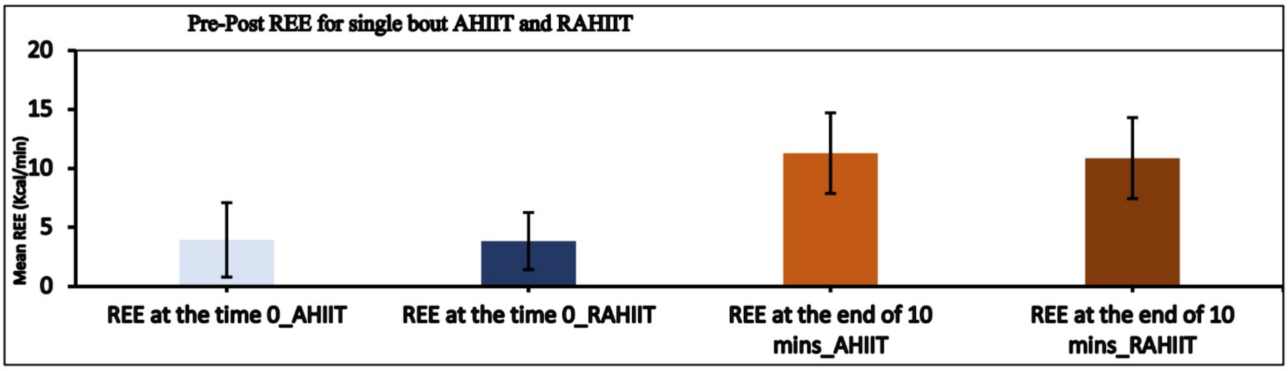


Figure 6.7 Pre- and post-exercise resting energy expenditure in the AHIIT and resisted AHIIT groups (mean ± SD)

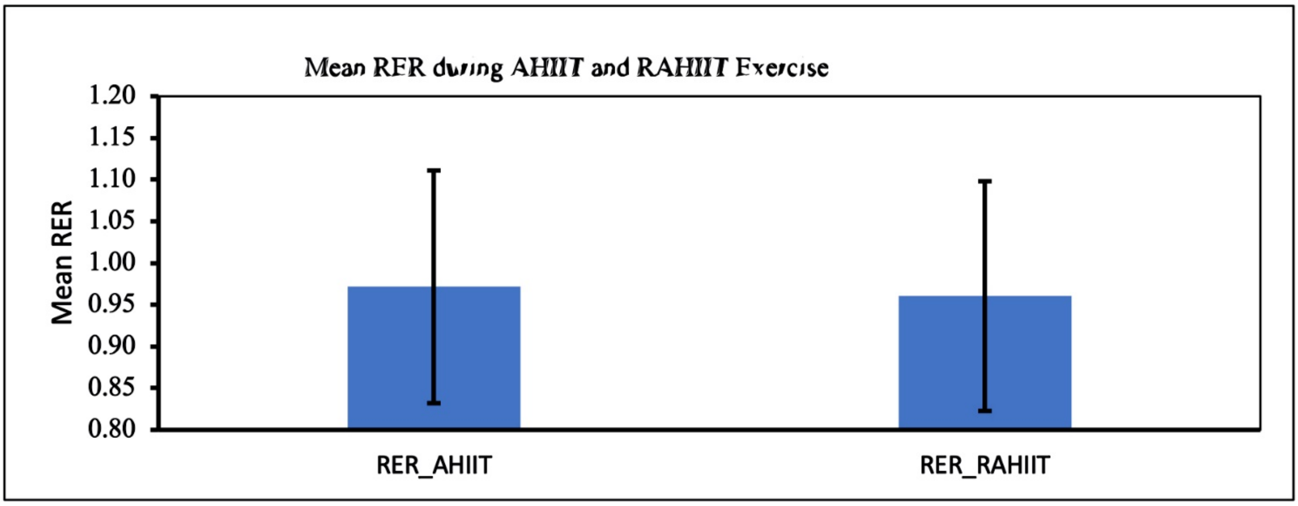


Figure 6.8: Mean respiratory exchange ratio during AHIIT and resisted AHIIT exercise (mean ± SD)

Table 6.7: Primary outcomes of the AHIIT and resisted AHIIT exercise (mean ± SD)

	AHIIT (n=20)		Resisted AHIIT (n= 20)		Time effect			Group*time effect		
	Pre-intervention	Post-intervention	Pre-intervention	Post-intervention	F	*p* value	ES	F	*p* value	ES
REE (kcal/min)	3.95±3.15	11.30±3.42*	3.85±2.42	10.88±3.43*	14.99	<0.01	0.29	0.17	0.76	0.01
RER	1.1±0.15	0.97±0.14*	1.1±0.16	0.97± 0.13*	7.04	<0.05	0.17	0.02	0.88	0.01

***Highlights a significant time effect difference upon pairwise comparison ($p \leq 0.05$)**
Note: REE, resting energy expenditure; RER, Respiratory exchange ratio; ES, effect size

The average total energy expended in 10 minutes of AHIIT or resisted AHIIT was not found to be significantly different (Figure 6.9). HR_{max} and HR_{mean} also did not differ significantly during the 10 minutes(Figure 6.10). And none of the secondary outcomes exhibited a significant difference in the group-by-time interactions or between groups. There was, however, a significant interaction shown in the TEE data when gender was added as a covariate (effect size (ES): 0.38, $p \leq 0.01$).

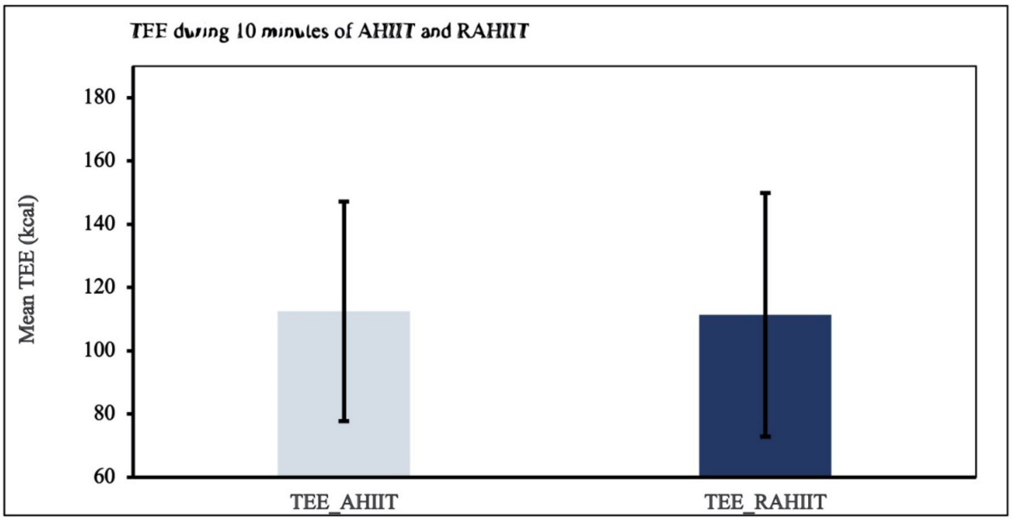


Figure 6.9: Total energy expended during ten minutes of AHIIT or R-AHIIT

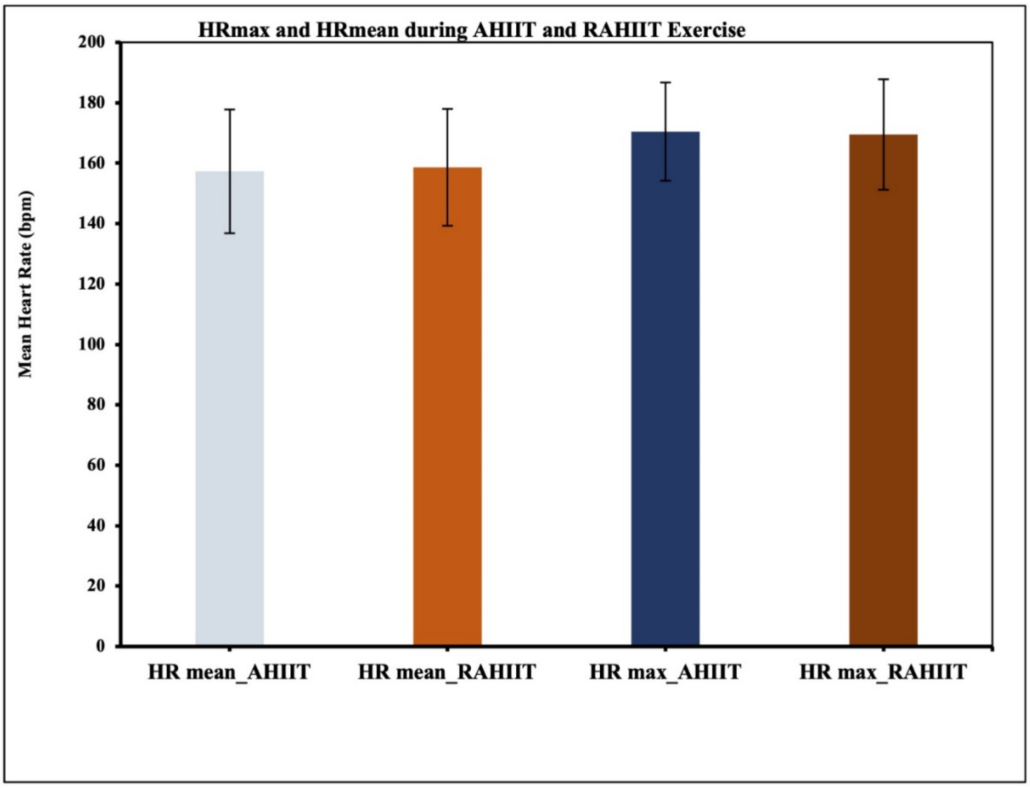


Figure 6.10: Maximum and mean heart rate during AHIIT or resisted R-AHIIT

Peak VO_2 utilisation during 10 minutes of AHIIT or resisted AHIIT exercise was not found to differ significantly on average (Figure 6.11). Reported perceptions of the exertion involved before and after exercise were, though. significantly different when the AHIIT was resisted ($p \leq 0.01$). AHIIT and resisted AHIIT exercise were not found to have significant group-by-time interaction difference (Table 6.8), but there was a significant interaction shown in the peak VO_2 data when gender was added as a covariate (ES: 0.37, $p \leq 0.01$)

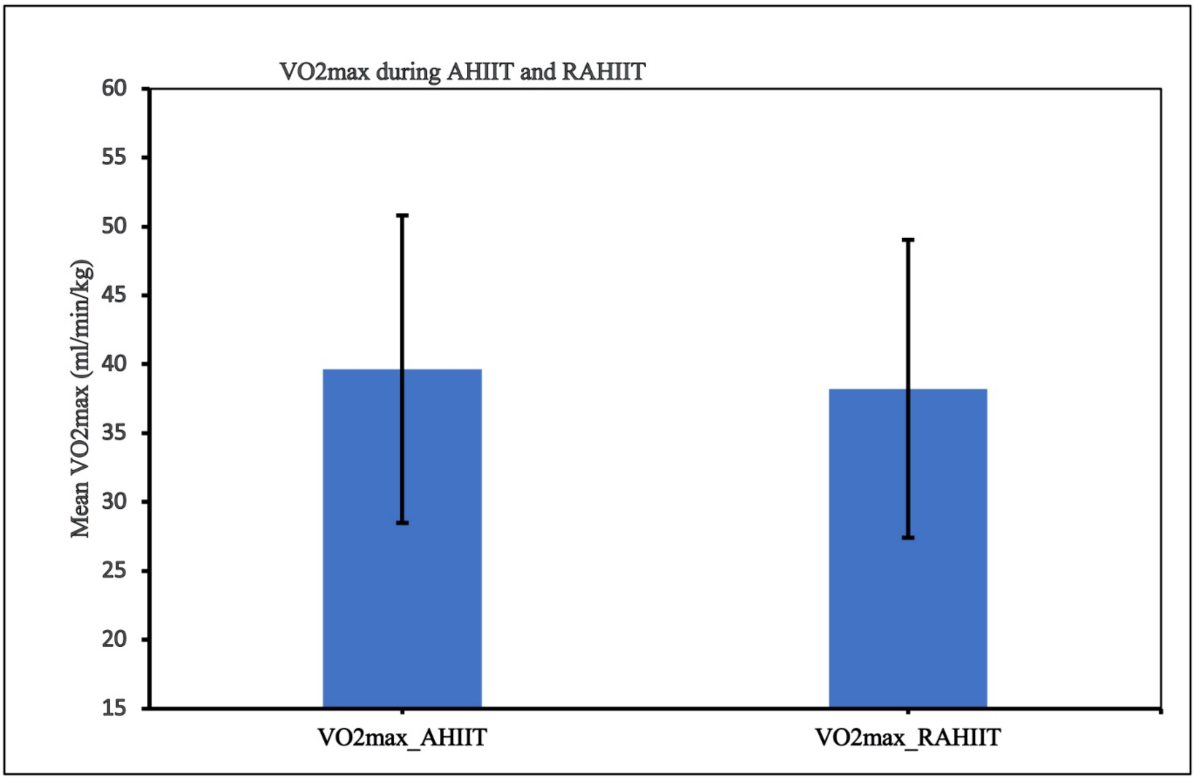


Figure 6.11: Peak oxygen utilisation during AHIIT and R-AHIIT

Table 6.8: Secondary outcomes in AHIIT and R-AHIIT (mean ± SD)

Parameters	AHIIT (n=20)		Resisted AHIIT (n= 20)		Time effect			Group*time effect		
	Pre-intervention	Post-intervention	Pre-intervention	Post-intervention	F	*p* value	ES	F	*p* value	ES
TEE (kcal)	113.26±57.84	101.20±37.66	123.84±60.32	103.72±35.74	0.78	0.23	0.04	0.81	0.59	0.001
HR max (bpm)	178.20±14.94	170.45±16.25	173.20±15.52	169.75±18.04	2.73	0.15	0.07	0.01	0.50	0.012
HR mean (bpm)	146.3±16.43	160.25±18.19	143.70±17.39	161.70±16.53	0.52	0.20	0.05	0.08	0.48	0.52
VO_2 peak (ml/min/kg)	42.61±9.98	39.65±11.16	43.21±11.08	39.61±9.57	0.27	0.63	0.24	0.00	0.81	0.06
RPE	6.00±0.00	13.70±1.72*	6.00±0.00	14.75±2.45*	35.76	<0.01	0.49	2.63	0.11	0.07

*highlights a time effect difference upon pairwise comparison significant at the $p \leq 0.05$ level of confidence.

Note: TEE, total energy expenditure; HR_{max}, maximum heart rate; HR_{mean}, mean heart rate; VO_{2peak}, peak oxygen consumption; RPE, perceived rate of exertion; ES, effect size

Simple linear regression was used to predict RER from RPE. The linear regression model showed the best adjustment in all of the analyses, with a significant relationship ($p \leq 0.05$) between the subjective and metabolic variables RPE and RER (Figure 6.12). Using the criteria suggested by Safrit and Wood (1995), the R (0.543) was considered statistically significant.

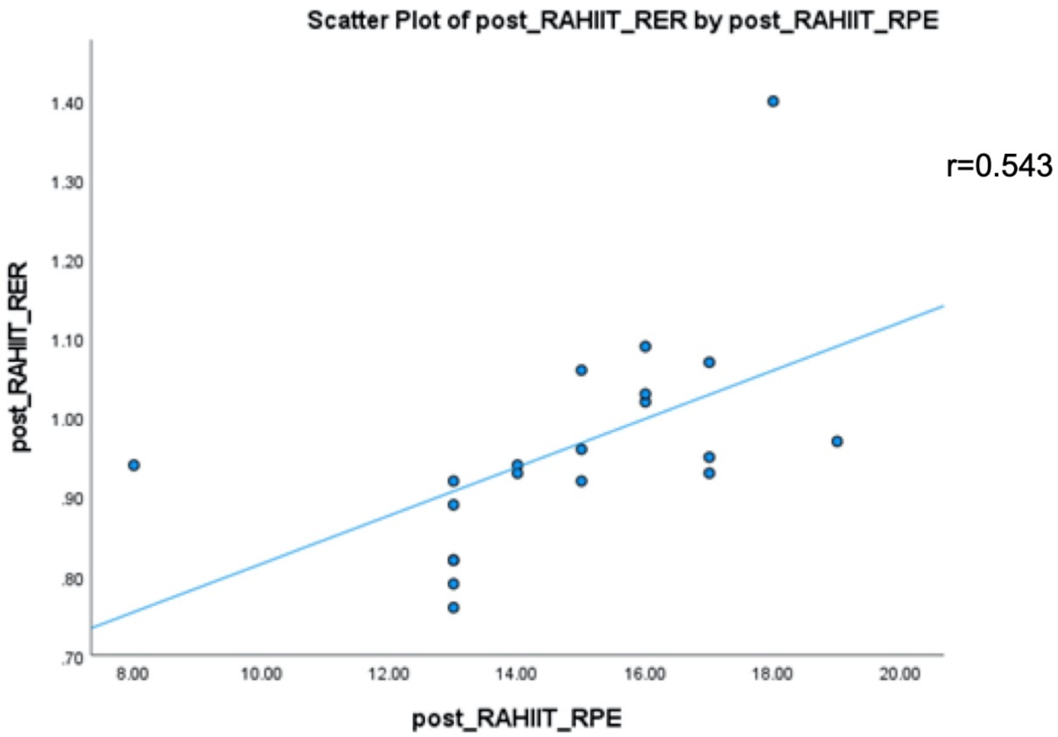


Figure 6.12: Scatter Plot

6.7 Discussion

The major objective of these experiments was to establish the AHIIT protocol by matching intensity and comparing acute cardio-metabolic and perceptual responses between AHIIT and L-HIIT, including the effects of adding resistance in AHIIT. That was successfully achieved. AHIIT is, though, associated with a lower HR, HR_{max} and higher oxygen pulse in healthy young women, with a similar perceptual response.

The incremental tests performed in both environments showed that as the exercise cadence increases, HR, $\%HR_{max}$, VO_2, $\%VO_{2max}$, %HRR, $\%VO_2R$ and RPE all increase as would be expected (Hall et al., 2004a). Instructors and coaches can use the matched intensity revealed between aquatic and land incremental tests to efficiently and precisely prescribe HIIT training sessions.

The experiments confirmed the hypothesised reduction in HR_{max} and in work and recovery HR during aquatic AHIIT compared to L-HIIT. The results also provide more evidence about the accuracy of exercise prescription when intensity was monitored solely by HR. These findings were similar to those of previous aquatic and land-based studies, where the heart rate decreased with water immersion (Alberton et al., 2013).

A significant increase in oxygen pulse was demonstrated in AHIIT compared with L-HIIT. This could be explained by a more effective ventilation at peak exercise in an aquatic environment as a result of decreases in physiological dead space. That, in turn, promotes greater cardiorespiratory efficiency during progressive increases in the exercise work rate while immersed (Kelly S. Chu & Edward C. Rhodes, 2001). Another possible reason for the higher oxygen pulse observed could be increased breathing frequency that occurs while submerged to the chest in water (Silvers et al., 2007). Greater ventilatory drive may have been required to overcome the hydrostatic pressure on the thoracic cavity, causing an increased residual volume, and decreased tidal volume and vital capacity. As a result, the higher oxygen pulse in AHIIT compared to L-HIIT observed in this study could potentially have challenged the participants' cardiorespiratory fitness further.

The other cardiorespiratory outcomes (VO_2, VO_{2max}, $\%VO_{2max}$, RER and VE) showed no significant differences between the AHIIT and L-HIIT trials. The VO_{2max} averages were little different, as Masumoto (Masumoto et al., 2007) and Greene (Greene et al., 2011) have previously reported. Silver's group report similar findings from incremental treadmill tests in both environments (Silvers et al., 2007). Alberton has suggested that stationary running does not consistently produce a significant change in VO_{2max} because it depends heavily on the muscle mass involved (Alberton et al., 2009). The water environment might have reduced VO_{2max} by reducing vital capacity, total lung capacity and pulmonary elasticity, causing oxygen to be consumed by the respiratory muscles and reducing its availability to other muscles. That would tend to reduce VO_{2max}. This aligns with the findings of a previous review of stationary water running and cycling on land and in the water (Ana Carolina Kanitz et al., 2015). Therefore, a determining factor for the VO_{2max} pattern is the mode of exercise performed rather than the inherent physical properties of water.

In these experiments, the total EE for a 20-minute AHIIT or L-HIIT session was between 610 and 667kcals (7–9kcals/min; 7–9 METs). That EE was similar to those reported in previous studies involving aquatic interval training (Nagle et al., 2013). The comparatively high EE achieved could be explained by the water's resistance causing a higher EE at a given speed and intensity while maintaining the same ranges of movement (Colado et al., 2009). The EE observations here are in line with those of previous aquatic and land-based studies, where HIIT elicited a comparatively greater EE when compared with constant intensity or continuous exercise regimes (Kruel et al., 2013b).

The mean values of blood lactate in AHIIT were similar immersed and in the L-HIIT. The blood lactate level was the net lactate difference between lactate production and elimination, which is positively associated with post-exercise fatigue (Fiorenza et al., 2019). A possible reason for the observed lack of any significant difference between two media could have been the 60sec active recoveries. The active recovery period could compensate for the energy consumed and facilitate the removal of metabolites. That would help to decrease the post-exercise lactate concentration and offset the influences of the environment on the production of lactate. This is also supported by the study in which Chien's group compared AHIIT and L-HIIT and found no difference in the lactate level between the two environments immediately post-exercise (Chien et al., 2020).

As for the perceptual variables, no significant group differences in RPE, enjoyment, efficacy self-perceptions or muscle soreness were found. HIIT combines time efficiency, diversity and fun. Ample research has demonstrated the physiological benefits brought by HIIT, but there is still on scholarly consensus about the perceptive responses it elicits (MacInnis & Gibala, 2017). The result here agree with those reported by Ma and his colleagues which suggest that aquatic and land interval training elicited similar changes in RPE among women (Ma et al., 2017). Obese men have been reported to find AHIIT the more affective and enjoyable (Sriton et al., 2022b). The diverse results may arise from differences in the water depth, subject characteristics, gender, exercise intensity and/or the intervals used in the AHIIT.

Turning to the R-AHIIT results, both AHIIT and R-AHIIT were associated with higher resting energy expenditure following a single session, with no significant differences between the two groups. There were, however, significant differences in terms of perceived exertion, with R-AHIIT eliciting higher ratings, as intended. There were significant differences within both the AHIIT and R-AHIIT groups ($p \leq 0.01$), as well as between them ($p \leq 0.01$). And there was a moderate correlation between RPE and RER in the R-AHIIT group (r=0.543). This result aligns with other reports that highlight RPE as an indicator of exercise intensity (Andrade et al., 2022). Both the within-group and between-group analyses revealed significant pre-post differences in the various outcomes, but there were no significant differences between the two groups in terms of maximum heart rate, mean heart rate, maximum oxygen consumption or total energy expended.

Since AHIIT and R-AHIIT demonstrated comparable effects on resting energy expenditure and cardio-metabolic responses, but participants perceived a lower exertion level in AHIIT compared to R-AHIIT at the same exercise intensity, AHIIT can be preferred as a training program to enhance exercise compliance and overall health. This finding also suggests that instead of progressing subjects with a resistive component in a water-based environment, the focus could be on monitoring a subject's RPE and maintaining appropriate heart rates during AHIIT (M. M. Kwok et al., 2022).

R-HIIT might have important results in terms of other outcomes such as muscle fibre capillarization, muscle morphology and succinate dehydrogenase activity. A group led by (Leuchtmann et al., 2020) organised a randomized and controlled trial with twenty older but recreationally active men who were assigned to either 12 weeks of observation followed by 12 weeks of resistance training (RT), or 12 weeks of high-intensity interval training (HIIT) followed by 12 weeks of RT (Leuchtmann et al., 2020). The results showed that both programmes were equally effective in improving capillarization and oxidative enzyme activity, as assessed through biopsies of the vastus lateralis muscle.

The incremental testing allowed for accurate intensity matching between the exercise protocols tested in the rest of this research. The aquatic incremental testing is particularly noteworthy, as it demonstrates a more appropriate methodology for exercise prescription in water compared to land-based tests.

The incremental testing also allowed for the standardization of conditions in the aquatic environment, allowing for effective comparisons between non-resisted and resisted exercise. The matched intensity based on heart rate allowed for the individualisation of training intensity, which is crucial for tailoring interventions to participants' fitness levels. As such, the precise intensity matching served as a foundation for determining individuals' baseline fitness levels, enabling specification of tailored AHIIT protocol in the balance of the research.

The sample was small, however, and it consisted entirely young people due to the pandemic. Additionally, both studies employed a cross-sectional design, focusing solely on the immediate effects of the AHIIT, L-HIIT and R-AHIIT exercise without addressing any long-term impacts on cardio-metabolic outcomes. Consequently, while the results provide valuable practical guidelines for matching exercise intensity in the water and on land, further research with larger, more diverse samples and longitudinal designs is needed to fully understand the efficacy of these interventions across different demographics and over time.

6.8 Challenges

These data were collected between August 2021 and August 2022, a period when the covid-19 pandemic posed significant challenges.

- All public and private swimming pools in Hong Kong were closed from the onset of the pandemic in 2020 until May 2023. Pools were among the first facilities to close and the last to reopen during the five waves of the epidemic.

- The campus of the Hong Kong Polytechnic University and the surrounding community implemented social distancing measures, making subject recruitment difficult.
- Due to surges in covid-19 cases, strict infection control measures were enforced in Hong Kong. Many aged women were anxious about the risk of infection, further complicating the recruitment process.
- The logistics of conducting DWR sessions were complex, requiring a specific water depth of 2 metres. Limited access to swimming pools made it challenging to develop the AHIIT protocol using DWR.

Despite these adverse social constraints, the Hong Kong government's social distancing guidelines were adhered to throughout. But that required three key changes.

1. With all pools closed, access to a swimming pool was only available for one hour per day.
2. Concerns about wearing face masks and cross-infection made it impossible to recruit any aged women as subjects for this part of the research. Students were recruited instead.
3. Stepping exercises can be performed with minimal setup, making them more practical for intensity matching when designing the AHIIT protocol. Given the controlled environment and the reduced skill requirements compared to DWR, stepping was used in the incremental testing, allowing for better intensity matching.

6.9 Conclusions

In summary, the results of the incremental tests suggest that AHIIT demonstrates distinct advantages over L-HIIT in terms of modulating heart rate and oxygen pulse, but it shows no significant differences in several other metabolic and perceptual variables immediately following a single exercise session. With both AHIIT and R-AHIIT yield comparable results in terms of cardio-metabolic benefits, both AHIIT and R-AHIIT reveal significant differences in perceptions of exertion, indicating that RPE can effectively guide exercise intensity prescription. The addition of resistance in R-AHIIT yields results similar to those achieved through the natural drag force of water, reaffirming the efficacy of both training modalities. Despite all the challenges we faced during data collection, we have overcome obstacles through solutions, ultimately achieving the project's goals.

Chapter 7 The Effects of Aquatic High-Intensity Interval Training on the Cardio-metabolic Health and Cognitive Responses of Physically Inactive Aged Women

This chapter has been published as below:

KWOK, M. M. Y., NG, S. S. M., MYERS, J., & SO, B. C. L. (2024). Aquatic High-Intensity Interval Deep Water Running Influence on Cardio-metabolic Health and Cognitive Psychological Responses in Women. *Medicine & Science in Sports & Exercise*, *56*(11), 2203-2210. https://doi.org/10.1249/mss.0000000000003500

This chapter has been presented in the below conference.

Manny, M. Y. Kwok, Shamay, S. M. Ng, Jonathan Myers, Billy C. L. So. (2024 May) Effects Of Aquatic High Intensity Interval Training (AHIIT) DWR On Cardio-metabolic Health In Elderly Women **ACSM 2024** (Poster Presentation)

7.1 Abstract

This study was designed to determine whether or not DWR can improve cardio-metabolic health and psychological responses among physically inactive aged women. The effects of 8 weeks of DWR training were compared with those of matched HIIT performed on land.

The DWR sessions involved ten 2-minute exercise bouts at 80-90% of each woman's maximum heart rate, with 1-minute active recovery at 70% of HR_{max} between bouts. There were two sessions per week, for 8 weeks. The L-HIIT group performed treadmill running at the same intensity.

Both groups showed similar cardiovascular fitness, maximum aerobic capacity, maximum HR and respiratory exchange ratio improvement over the course of the training, but the DWR group showed a significantly greater improvement in average minute ventilation, metabolic equivalents and O_2 pulse over the 8- week intervention. Those training in the water and on land both significantly decreased their blood triglycerides, total cholesterol and high- and low-density lipoprotein, on average. No significant inter-group differences in cognitive functioning were revealed by the Mini-mental State Examination or the Montreal Cognitive Assessment. Both groups showed reported similar enjoyment and similar self-perceptions of efficacy with good adherence (>90%).

7.2 Introduction

Exercising in the water minimizes the body mass's impact on joint stress, bones, and muscles, so it is commonly integrated into rehabilitation programs (Faíl et al., 2022).

Interval training in the water has previously been reported as producing significant aerobic and cardiorespiratory benefits (Nagle, Sanders, & Franklin, 2017). Deep water running is one exercise that has gained prominence. In some of the rehabilitation settings it is performed wearing a floatation vest which keeps the body upright and prevents the feet from touching the bottom of the pool (Bojan Jorgic et al., 2012; Reilly et al., 2003). The vest makes DWR easier, resulting in better exercise compliance (Planna et al., 2019; Reichert et al., 2016). DWR can thus be an effective AHIIT protocol for older adults, helping them to train at higher intensities than on land, and especially for physically inactive women who have difficulty performing L-HIIT.

Exercise is well understood to benefit middle-aged and elderly persons in terms of to some extent preventing the cardio-metabolic diseases associated with ageing (Liguori et al., 2022). Older women are particularly at risk. Aerobic capacity declines 10% per decade of life beyond 20 years of age (Bartlett et al., 2011). This is relevant to cardio-metabolic functioning and related to functional capacity, which is an important predictor of mortality (Myers et al., 2017). Stimulating cardio-metabolic functioning thus helps to decrease mortality in such populations. That is why this study focused on physically inactive aged women.

Perceptions of exercise will influence the compliance with any exercise program (Sylvester et al., 2016). Anticipated health benefits and the perceived competence of those administering the exercises are also known to be important for adherence among older adults (Whaley & Schrider, 2005). It has been suggested that self-perceptions of efficacy and enjoyment of exercise also predict exercise adherence, and that lack of time is the most frequently reported barrier to physical activity (Trost et al., 2002). DWR may potentially improve the enjoyment of exercise (Barbosa et al., 2009).

High-level mental processing such as perceiving, conceiving, remembering, reasoning, judging, imagining and problem solving are known to have critical implications for synthesising and integrating thoughts and experiences (Williams et al., 2009). While cognitive control abilities generally decline with age, physical exercise can enhance performance at any stage of life (Bull et al., 2020). Work by a group led by Farinha has shown improvements in cognitive functioning (as reflected in MMSE scores) after aquatic exercise compared with a control group (Farinha et al., 2021). Aquatic exercise programs are potentially beneficial for older individuals' cognition.

Despite the increasing popularity of AHIIT, research investigating its cardio-metabolic health benefits and comparing the psychological responses to DWR with those seen in L-HIIT has not yet been reported. There is well-established evidence about the health benefits of L-HIIT, but similar evidence regarding DWR is lacking (Batacan et al., 2017). This study was therefore designed to investigate the effects of 8 weeks of AHIIT based on DWR among physically inactive aged women and to compare them with the results of L-HIIT in terms of cardio-metabolic health and psychological outcomes. The null hypothesis was that 8 weeks of DWR training is as effective as a matched L-HIIT exercise routine in improving cardio-metabolic health and cognition.

7.3 METHODS

7.3.1 Participants

Seventy inactive but healthy aged women were recruited from the community through poster advertising. All claimed that they were free of any cardiorespiratory or neurological pathology and that they had not suffered a fracture or had any surgical intervention in the lower extremities in the previous six months. None of the participants were taking any medications. All of those included provided written informed consent before the start of the experiments.

The effect sizes were calculated in terms of Cohen's d. The sample size required was calculated based on the primary outcome of the incremental testing (Karen Davidson & Lars McNaughton, 2000). Using G*power software and based on the effect size of 0.28 obtained, the sample size computed was 30 or more subjects per group assuming 5% type I error and 80% power. Anticipating a 20% attrition rate, the total enrolled sample size for each group required to ensure adequate statistical power was 36.

7.3.2 Study design

This was a parallel, two-group, randomized and controlled set of experiments which conformed to the Consolidated Standards for Reporting of Trials (CONSORT) protocols (Schulz et al., 2010). The participants were randomly assigned to perform either DWR or L-HIIT using Research Randomizer software. Figure 7.1 presents a flow chart of the study's design.

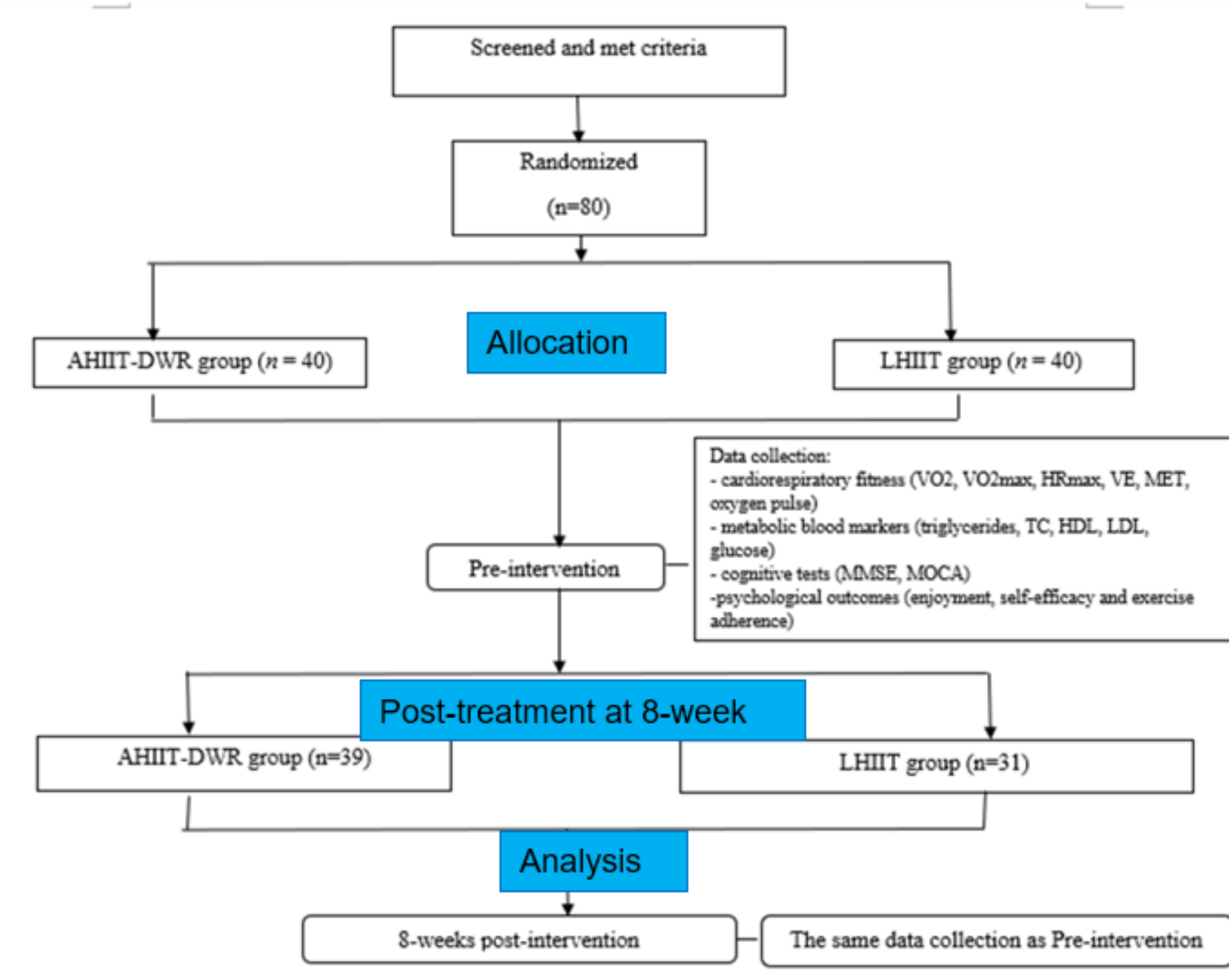


Figure 7.1 Eight weeks Study design

7.3.3 Experimental Procedures

The participants gave their informed consent after a screening and familiarization interview in which they completed the International Physical Activity Questionnaire (Craig et al., 2003). A woman was classified as physically inactive if she reported performing less than 150min of moderate or 75min of vigorous physical activity per week. Resting heart rate, blood pressure, body mass and height were also measured as part of the screening. All of the women then completed a DWR familiarization session in the pool a week before the experiments began.

Each woman completed a program of incremental tests in the water and on land (on a treadmill) to confirm an individualized exercise intensity match (Ana Carolina Kanitz et al., 2015). The exercises were demonstrated first, and then practiced once. The women's HRs were monitored continuously at a frequency of 1Hz using the Polar OH1 heart rate sensor. Gas exchange data were obtained simultaneously using the PNOĒ. The PNOĒ acquired data breath-by-breath and continuously measured ventilation volume and determined expired gas concentrations simultaneously.

The DWR incremental protocol increased the exercise load from 85 beats per minute by 15bpm every 2 minutes (Ana Carolina Kanitz et al., 2015). The treadmill increments on land followed the Bruce protocol (American College of Sports & Medicine, 2018) paced by the Intelli metronome. VO_2, and the perceived rate of exertion were also recorded each minute.

The incremental testing and experimental sessions always began with a standardized 3min warm-up and were followed by a 3min cool down that included pool walking or jogging at 50% of each woman's heart rate reserve, and stretching against a wall.

7.3.4 The exercises

The testing involved ten 2min bouts of DWR at 80% of each woman's individual HRR with 1min active recovery intervals at 60% HRR between bouts. Each woman participated in two 30-minute sessions a week for 8 weeks (a total of 16 sessions). The AHIIT-DWR was supervised by an experienced aquatic fitness instructor. The participants wore a flotation vest to prevent their feet from touching the bottom of the pool floor, and to keep their trunk straight with the chest out. The body angle was adjusted so it was slightly leaning forward in the sagittal plane. The arms were swung in a relaxed manner with the elbows flexed at 90 degrees while keeping the thumbs below the surface. The shoulder was flexed and extended to bring the elbow back and forth to complete the running cycle. The running stride began by flexing the hip to 70–80 degrees while maintaining the knee at about 90 degrees. Both legs made cyclic movements by alternating hip flexion and hip extension to complete the running cycle Figure 7.2. The quality of the movement was closely monitored by the aquatic fitness instructor.

Figure 7.2 AHIIT- DWR form

The treadmill running was also performed in 2-minute bouts at 80% HRR with 1min of active recovery at 50%HRR between bouts. The women trained in 30-minute sessions twice a week for 8 weeks just like those training in the water Figure 7.3. The quality of the movement was closely monitored by an experienced exercise instructor.

Figure 7.3 L-HIIT- Treadmill running

During the first 2 weeks the training both in the water and on land was 80–85% HRR. During weeks 3-5 the training was at 85–90% HRR of the AHIIT-DWR, and during weeks 6-8 it was at 90–95% of the individual's initial HRR. The recovery intervals were 1 minute at 50% of HRR throughout. Please refer to Table 7.1.

Table 7.1 Training periodization

Weeks	Volume and Intensity	Total time
1 and 2	10 x (2min 80–85% HRR + 1min 50% HRR)	30 min
3–5	10 x (2min 85–90% HRR + 1min 50% HRR)	30 min
6–8	10 x (2min 90–95% HRR + 1min 50% HRR)	30 min

7.3.5 Outcomes

Cardio-metabolic markers were measured at baseline (pre -ex) and at least 48 hours following but within 5 days after the final session of the 8-week intervention. For each pre and post exercise assessment, the session lasted for 30 minutes.

7.3.6 Cardiorespiratory fitness

Gas exchange data were obtained using the waterproofed PNOĒ device after the incremental tests Figure 7.4.

Figure 7.4 Land and aquatic incremental tests

7.3.7 Blood metabolic markers

A qualified nurse performed took 20mL venous blood samples after a 12-hour fasting period. Glucose levels were measured using the enzymatic-amperometric method, which has a coefficient of variation of 0.5% (Solnica et al., 2011). The instrument used was a Biosen-C from EKF Diagnostics (Cardiff). The lipid profile was assessed using commercially available RX Monza kits from Randox Biosciences (Crumlin). Total cholesterol levels were determined using the cholesterol oxidase, esterase, and peroxidase colorimetric method, with an intra-assay CV of 1.3%. High-density lipoprotein cholesterol levels were measured using the polyethylene glycol direct method, while low-density lipoprotein cholesterol levels were assessed using the direct method.

Those techniques have intra-assay CVs of 0.7% and 1.3% respectively. Triglyceride levels were measured using the enzymatic method without glycerol blanking, a method with an intra-assay CV of 1.3%. Insulin levels were determined using an enzyme-linked immunosorbent assay with an instrument from Mercodia AB (Uppsala). Duplicate measures were taken for each marker, and the average value is reported here. Insulin resistance was estimated using the homeostasis assessment model for insulin resistance (Matthews et al., 1985).

7.3.8 Psychological responses

Cognition was assessed before and after the 8 weeks of exercise intervention using the Mini-mental State Examination (MMSE) and the Montreal Cognitive Assessment (MOCA). MOCA is a 10-minute 30-point cognitive screening tool designed to evaluate cognitive function (Nasreddine et al., 2005). It is developed based on eight domains assessing the short-term memory recall, visuospatial abilities, executive functions, attention, concentration, working memory, language and also orientation. MMSE is a preliminary screening task for cognitive impairment and it takes about 5–10min to administer which shows good reliability and validity in clinical applications (Helen et al., 1994). Both instruments are validated instruments suitable for use as a mediator variable for devising interventions for promoting cognition. Perceptions of exercise will influence whether one continues with an exercise program, tries a different program instead, or stops exercising completely. It has been observed that the perception of exercise variety is an important factor for adherence (Sylvester et al., 2016).

7.3.9 Statistical analysis

The statistical analyses were performed using version 22.0 of the Statistical Package for the Social Sciences (SPSS, Inc., Chicago). A confidence limit ≤5% was taken as indicating adequate statistical significance. All of the continuous variables are presented as means and standard deviations. Mean differences between the AHIIT-DWR and L-HIIT groups for each cardio-metabolic and psychological variable were tested by evaluating mixed repeated measures ANOVA models. Group (AHIIT-DWR vs L-HIIT) and time (0 vs 8 weeks) and their interaction were tested for their ability to predict cardio-metabolic and cognitive outcomes. Tukey's post-hoc test was applied to analyse the within-group and between-group comparisons.

The effect sizes were calculated in terms of Cohen's d. The sample size was calculated based on the primary outcome of a previous study comparing the effects of interval DWR and land trainings on aerobic fitness (Karen Davidson & Lars McNaughton, 2000).

7.4 Results

The women's demographics are presented in Table 7.2.

Table 7.2 Anthropometric observations (mean ± SD)

Variable	AHIIT-DWR (n= 39)	L-HIIT (n= 31)
Age (yr)	66.33±4.99	65.68±6.19
Height (m)	156.31±6.12	154.65±5.67
Body Mass (kg)	62.97±10.05	57.069±9.05
BMI (kg/m^2)	25.73±3.55	23.84±3.37

7.4.1 Fitness

The maximum exercise performances of both training groups in the cardiopulmonary exercise test pre- and post-intervention are presented in Table 7.3. Significant improvements in absolute VO_{2max} ($p \leq 0.01$), relative VO_2 ($p \leq 0.01$), HR_{max} ($p \leq 0.01$), peak O_2 pulse ($p \leq 0.01$), RER ($p \leq 0.01$), VE ($p \leq 0.01$) and METs ($p \leq 0.01$) were detected after both AHIIT-DWR and L-HIIT. Group-by-time interactions revealed a significant difference in relative VO_2 (ES:0.06, $p \leq 0.05$); VE (ES: 0.11, $p \leq 0.01$); METs (ES: 0.06, $p \leq 0.01$) and O_2 pulse (ES: 0.07, $p \leq 0.05$) between AHIIT-DWR and L-HIIT. There was, however, no significant difference in VO_{2max}, HR_{max} or RER.

Table 7.3 Cardiovascular fitness after 8 weeks of AHIIT-DWR or L-HIIT (mean ± SD)

	AHIIT-DWR (n= 39)		L-HIIT (n= 31)		Time effect		Group *time effect	
	Pre-training	Post-training	Pre-training	Post-training	*p* value	ES	*p* value	ES
HR max	136.75±20.18	145.36±17.97*	138.88±21.06	142.88±19.56*	<0.01	0.15	0.21	0.02
VO_2	1299.19±303.98	1738.22±468.10*#	1253.19±357.70	1529.29±448.14*	<0.01	0.55	0.04	0.06
RER	0.97±0.12	1.13±0.12*	1.01±0.11	1.11±0.15*	<0.01	0.44	0.07	0.05
VE	45.58±11.59	66.05±17.70*#	47.29±14.11	59.32±20.60*	<0.01	0.64	<0.01	0.11
MET	5.83±1.43	7.60±1.92*#	6.36±1.66	7.62±1.80*	<0.01	0.46	<0.05	0.06
O_2 pulse	8.79±2.17	11.85±3.37*#	9.16±2.35	10.67±2.51*	<0.01	0.23	<0.05	0.07
VO_{2max}	20.76±4.14	26.62±6.73*	21.92±5.75	26.57±6.37*	<0.01	0.5	0.35	0.01

#indicates group*time effect difference upon pairwise comparison significant at the $p \leq 0.05$ level of confidence

7.4.2 Metabolic blood markers

None of the metabolic blood markers exhibited any significant difference in the group-by-time interactions or between groups except the fasting glucose levels ($p \leq 0.05$, ES=0.07) (Table 7.4). However, simple effects testing revealed both AHIIT- DWR and L-HIIT significantly decreased triglycerides ($p \leq 0.05$, ES= 0.07).

Table 7.4 Cardio-metabolic blood markers after 8 weeks of AHIIT-DWR or L-HIIT (mean ± SD)

	AHIIT-DWR (n= 39)		L-HIIT (n= 31)		Time effect		Group effect		Group* time effect	
	Pre-training	Post-training	Pre-training	Post-training	*p*-value	ES	*p*-value	ES	*p*-value	ES
TC (mmol/L)	5.53±0.95	5.50±1.09	5.36±1.06	5.55±0.91	0.06	0.06	0.40	0.01	0.83	0.00
HDL (mmol/L)	1.60±0.34	1.76±0.52	1.68±0.44	1.78±0.39	0.09	0.05	0.25	0.02	0.27	0.02
LDL (mmol/L)	3.26±0.86	3.09±1.0	3.48±0.92	3.30±0.81	0.05	0.06	0.36	0.02	0.93	0.00
Triglycerides (mmol/L)	1.47±0.71	1.32±0.56*	1.26±0.64	1.11±0.37*	0.04	0.07	0.16	0.03	0.10	0.00
Glucose (mmol/L)	5.38±0.44	5.39±.0.51	5.25±0.71	5.08±0.64	0.52	0.01	0.21	0.04	0.05	0.07

7.4.3 Cognitive effects

There was a significantly greater improvement in the average MOCA score after the AHIIT-DWR than after the L-HIIT ($p \leq 0.01$, ES=0.22) (Table 7.5). The MMSE scores, however, remained similar after the aquatic and land-based training. No significant differences or interactions were shown in either cognitive test.

Table 7.5 Cognitive testing results after 8 weeks of AHIIT-DWR or L-HIIT

Test	AHIIT-DWR (n= 39)		L-HIIT (n= 31)		Time effect		Group effect		Group* time effect	
	Pre-training	Post-training	Pre-training	Post-training	*p*-value	ES	*p*-value	ES	*p*-value	ES
MMSE (Score of 30)	29.08±1.29	29.10±1.11	29.18±1.23	28.97±1.08	0.95	0.00	0.95	0.07	0.55	0.01
MOCA (Score of 30)	27.36±2.25	28.49±1.85	27.16±2.45	28.52±2.31	<0.01	0.22	0.73	0.35	0.69	0.00

There were also no significant differences in self-reported enjoyment or efficacy perceptions between the two exercise regimens, on average. And the exercise adherence was similar in both groups (>90% of the sessions completed) (Table 7.6). There were no adverse events associated with the exercises.

Table 7.6 Psychological outcomes after 8 weeks of AHIIT-DWR or L-HIIT (mean ± SD)

	AHIIT- DWR (n=39)	L-HIIT (n=31)	*p*-value
Enjoyment (score out of 126)	110.5±8.5	104.6±13.5	0.88
Self-efficacy (score out of 100)	68.7±13.7	65.7±15.2	0.57
Exercise adherence (%)	93.26±6.69	92.29±11.04	0.81

7.5 Discussion

The major finding is that 8 weeks of training improved the average cardiorespiratory fitness of those in both groups. AHIIT-DWR did, however, tend to improve VO_2, HR, VE and oxygen pulse better than L-HIIT. A significant inter-group difference was observed only in the reduction of blood triglycerides, with AHIIT-DWR more effective, on average. There were also no significant inter-group differences in the cognitive responses measured.

The data do confirm that DWR can improve the cardiovascular fitness of inactive older women. That extends the findings of previous research because of the incremental tests performed to match each individual's exercise intensity on land and in the water. The HRR used takes into account differences between individual's resting heart rates, and it has been recommended over percentage of maximum HR by the A(American College of Sports & Medicine, 2018). There were significant improvements in all of the cardiorespiratory parameters after both interventions, but DWR produced significantly greater improvements in VO_2, VE, METs and O_2 pulse, on average. This finding agrees with the majority of previous studies comparing the efficacy of aquatic exercise and land-based exercise for cardiorespiratory improvement (Bunæs-Næss et al., 2023). Mechanistically, it has been suggested that the responses in water are caused by increased venous return to the heart with enhanced peripheral venous blood pressure due to the compression of the lower body by water pressure.

Low cardiovascular fitness, indicated by low maximum oxygen uptake (VO_{2max}), is one typical consequence of a physically inactive lifestyle, and is a powerful predictor of premature cardiovascular mortality (Myers et al., 2015). Despite the exercises using different media, they induced similar improvements in absolute VO_{2max} and HR_{max} in both groups. The aerobic fitness in both groups was significantly elevated. This suggests that the aerobic fitness of physically inactive elderly individuals can be effectively improved by either type of exercise.

Previous studies have shown that HIIT is beneficial for improving aerobic fitness, either on land or in the water (Ramos et al., 2015; Tanaka, 2009). The AHA has specified in a scientific statement that an increase in VO_{2max} or just one MET is valuable for increasing health outcomes and survival (Ross et al., 2016b). Two other trials have reported an increase in VO_{2max} of 3.5mL/kg/min or more resulting from aquatic exercise (Delevatti et al., 2020; Samadi et al., 2019). Since DWR is as beneficial as L-HIIT, that provides physically inactive aged women another option for effective HIIT. Some may find the pool a more attractive environment, which would encourage them to start and continue with high-intensity training. If so, the physiological benefits of hydrostatic pressure and the enabling effect of buoyancy may facilitate such training's effectiveness. Traditional L-HIIT exercises may not be suitable for all physically inactive aged women given that it requires a greater skill, imposes greater impact, and demands a certain level of physical functioning to perform.

Both forms of exercise markedly reduced triglycerides, but there were no significant inter-group differences in any of the blood markers or cognitive outcomes, on average. The lack of differences between the groups could be due to the timeframe of the study, as some previous evidence suggests that a minimum of 8–12 weeks may be required for high intensity interval training to demonstrate a positive impact on physiological adaptions that improve metabolic health (Batacan et al., 2017; Kessler et al., 2012). Another potential reason for that finding could be that all of the participants' blood markers were already within the normal range at baseline. That would have reduced the likelihood of observing notable differences. Further research with different clinical populations over a longer timeframe may be required to determine if the effects of DWR and L-HIIT differ in some way.

The data show that both forms of exercise may be useful for improving the cognitive ability of older women. The lack of difference between the groups may again be due to the training's short duration. A recent review (Ludyga et al., 2020) reported the largest effects following interventions with longer sessions and a longer duration (Ludyga et al., 2020; Northey et al., 2018). The improvements in both groups may be explained by increased cerebral blood flow and the improvements in aerobic capacity, cardiac output, oxygen transport and metabolism. All would tend to improve neurotransmitter functioning and brain health (Dustman et al., 1990). That higher cardiac output is associated with higher cerebral blood flow suggests that HIIT in any form will have a positive effect on cognitive ability (Mekari et al., 2020). Another possible mechanism is related to the increased level of brain-derived neurotrophic factor (BDNF) in the brain. Acute aerobic exercise increases BDNF levels, which are an important component of the brain’s neuroplasticity (Ferris et al., 2007). Physical exercise can improve BDNF circulation, which has beneficial neurotrophic, neuroprotective and cognitive effects (Walsh et al., 2020).

An important consideration is how the general population, particularly inactive and less fit aged women, perceive HIIT and whether they can adhere to it over the long term (Biddle & Batterham, 2015). The results here agree with those of a previous study suggesting that single bouts of AHIIT or L-HIIT (matched in exercise intensity) elicit similar enjoyment, similar self-perceptions of efficacy and similar exercise adherence (M. M. Y. Kwok, E. T. C. Poon, et al., 2022). However, there were also conflicting findings, with one study suggesting that obese men found AHIIT more effective and enjoyable than L-HIIT (Sriton et al., 2022a). Such mixed results are likely to have arisen from differences in water depth, subject characteristics, gender, exercise intensity and the intervals used as much as on the exercise modality. Certainly, in this study both AHIIT and L-HIIT showed excellent exercise compliance (>90%), suggesting that either can be a practical exercise option for physically inactive aged women in terms of enjoyment and feelings of efficacy.

These findings provide valuable evidence that both DWR and HIIT on land can be a time-efficient and efficacious approach for health professionals when designing individualized programs targeted at cardio-metabolic health benefits. DWR may be more appropriate for individuals who need a lower weight bearing alternative to L-HIIT.

These were randomized and controlled experiments that compared the impact of DWR and L-HIIT performed at matched intensities. Nevertheless, it is important to bear in mind that only aged women were recruited. Caution is called for in generalising the findings to men, or even to younger women. In addition, the relatively short 8-week intervention may to some extend limit the applicability of the findings. Longer HIIT may better distinguish the effects of DWR and L-HIIT. But the findings nevertheless provide valuable insights regarding the potential applications of DWR as an alternative to conventional HIIT. From a practical point of view, the results suggest that DWR can be a useful alternative to L-HIIT for some healthcare professionals designing programs that target cardio-metabolic health benefits, metabolic blood markers, cognitive functioning and perceptions among physically inactive aged women. Future studies on a larger scale for longer duration seem to be warranted.

7.6 Conclusions

The results of the eight-week DWR and L-HIIT programs showed no significant inter-group differences in the various outcomes measured. DWR did, though, improve the women's oxygen capacity, metabolic equivalents, oxygen pulse and minute ventilation. This suggests that a practical program of DWR can offer cardio-metabolic health benefits, especially cardiovascular fitness benefits, for inactive aged women comparable to those attainable using L-HIIT. DWR also generated similar enjoyment and feelings of efficacy. Both exercise programs had high adherence. Further research in different populations with longer duration is required to determine how DWR compares to L-HIIT in terms of overall cardio-metabolic health and psychological benefits.

Chapter 8 Summary and conclusions for the research as a whole

8.1 Summary

Globally, 27.5% of adults fail to achieve the recommended level of physical activity. Women are particularly inactive, with 30.7% inactive compared with 23.7% of men (Guthold et al., 2018). Previous studies have suggested that while light activity is beneficial, it does not produce the same benefits as activity of moderate to vigorous intensity (Chastin et al., 2019). HIIT is well known to improve aerobic capacity and endurance by stimulating cardio-metabolic changes (Gibala & Jones, 2013) resulting in better cardiorespiratory fitness and VO_{2max} gains, even among middle-aged and older adults (Poon et al., 2021). Research by (Nagle, Sanders, & Franklin, 2017) has shown that some individuals are more likely incorporate AHIIT into their lifestyles than HIIT performed on land For example, persons whose movements are limited by pain or other symptoms may prefer the aquatic environment.

Despite this, no previous study has rigorously compared the benefits of AHIIT with those of L-HIIT or other types of aquatic exercise. And its benefits for aged women have never previously been explicitly documented. In particular, the benefits of DWR for cardiorespiratory fitness, physical functioning, and quality of life have never previously been systematically investigated. And its psychological impact has received no scholarly attention at all.

Previous studies comparing land- and water-based exercise have generated little evidence specifically in terms of cardio-metabolic and perceptual responses. And the AHIIT experiments reported have not incorporated resistance, leaving unclear which variations produce the most beneficial metabolic effects.

A meta-analysis of the previous research in this area revealed that AHIIT can indeed improve certain markers of cardio-metabolic health. The results of 13 randomized and controlled trials revealed a moderate but significant beneficial effect (in terms of peak oxygen utilisation, resting heart rate and sit-to-stand test times) compared to control groups who did not exercise. AHIIT can be a valuable alternative with added value for HIIT for physically inactive aged women, especially those dealing with barriers to exercise performance such as pain or weakness. The aquatic environment may also offer people with limited mobility the ability to train at higher intensities than on land (Waller et al., 2014). There may be a potential for reducing some of the barriers to land-based exercise in the water, which would encourage better longer-term adherence to training programs. But these advantages have not yet be demonstrated in DWR, a potential mode of exercise for AHIIT application in our main study which warrants examining in the next systematic review.

DWR was used in cardiovascular training programs for various clinical populations (Dale, 2007; Bojan Jorgic et al., 2012) and although several clinical trials have evaluated the effects of DWR on numerous quality-of-life (QoL) domains (B. Jorgic et al., 2012; Planna et al., 2019) , there has not yet been a systematic examination of the effects of DWR on cardiorespiratory fitness, physical functioning and QoL. Hence in our systematic review, we reviewed eleven studies of DWR generated results similar to those from exercise on land. Only quality of life showed a significant improvement compared with no exercise. That review suggested a need for correlational studies applying biopsychosocial approaches to investigate relationships between measured outcomes of DWR with a larger and more homogenous group of participants.

The general methodology is presented in Chapter 6. To ensure accurate measurement of cardio-metabolic outcomes, previous studies have employed laboratory-based (or sometimes portable) metabolic analysers. However, findings from these studies have often been inconclusive, making it difficult to reliably establish valid and generalizable cardio-metabolic outcomes. There is therefore a need to assess whether other portable metabolic analysers can be used interchangeably with the COSMED K5, currently regarded as the “golden standard” portable (Perez-Suarez et al., 2018). Completing it first required validating the cardio-metabolic outcomes measured using a PNOĒ by comparing them with the output of a COSMED K5 instrument in cardiorespiratory testing. The results showed that the devices produced very similar results. That finding should greatly assist sports scientists in providing individualized exercise prescriptions. To use the PNOĒ instrument in aquatic exercise testing, however, it was first necessary to design and fit a water-resistant case. As a result, a portable unit suitable for use with water resistant property is badly needed.

With a valid tool to measure cardiometabolic outcomes, it is crucial to develop the AHIIT protocol. Matching intensity prescriptions on land and in the water also remains a work in progress (Bunæs-Næss et al., 2023). The lack of clarity in ensuring matched intensity between these modalities constitutes a significant research gap, with limited evidence quantifying the intensity responses.

Two cross-sectional studies establish the AHIIT protocol used in the exercise testing. A first set of incremental tests matched the intensity of running in the water and on land. Those tests showed that AHIIT exhibits distinct differences from L-HIIT in terms of heart rate and oxygen pulse, despite showing no significant differences in several cardio-metabolic and perceptual variables immediately following an acute bout of exercise. This suggests that AHIIT can provide cardio-metabolic benefits and perceptual responses comparable to L-HIIT, making it a valuable adjunct or alternative for women, particularly those unable to exercise on land or those who wish to incorporate water training for assessment or rehabilitation purposes.

Additionally, designing a specific AHIIT program that optimizes cardio-metabolic outcomes requires the application of resistance, which can be achieved by increasing cadence and drag on the limbs (Pöyhönen et al., 2002). No study has yet systematically investigated the cardio-metabolic benefits of adding resistance in an AHIIT program. Ensuring matched exercise intensity prescriptions definitely needs further research.

In the second set of experiments, resistance was added to the aquatic exercise regimen. That enhanced its beneficial effects on metabolic health while also reducing perceived effort with the healthy adults tested. The findings indicate that incorporating a resistive component into aquatic HIIT can yield results comparable to using water's drag force as the sole medium for resistance, confirming that AHIIT continues to offer cardio-metabolic benefits when compared to R-AHIIT while RPE can effectively guide exercise intensity prescription.

And finally, a review of the prior research shows that the cognitive responses to AHIIT and L-HIIT have never been properly compared. There is well-established evidence about the effects of L-HIIT on cardio-metabolism, physical health or perception, but similar evidence regarding AHIIT, and specifically DWR, is lacking (Batacan et al., 2017). Chapter 7 described randomized and controlled experiments which demonstrated that DWR can elicit improvements similar to those of L-HIIT in terms of cardiorespiratory health, metabolic blood markers, cognition when pursued by previously inactive aged women. After exercising for 8 weeks, cardiorespiratory fitness had improved in both the DWR and L-HIIT groups, but the DWR produced significantly better increases in oxygen utilisation, HR, VE and oxygen pulse than intensity-matched running on land. AHIIT can therefore be recommended as an alternative to L-HIIT, and considered by healthcare professionals when designing programs that target cardio-metabolic health benefits, metabolic blood markers, cognitive functioning and the perceptions of physically inactive aged women.

8.2 Limitations and directions for future research

The findings of this research are limited by several factors. First, the closure of all public and private swimming pools in Hong Kong from the onset of the covid19 pandemic until May 2023 hindered data collection. That was compounded by social distancing measures implemented by the Hong Kong Polytechnic University and the surrounding community which made subject recruitment difficult and hence students were recruited when developed the AHIIT protocol. Additionally, strict infection control measures created anxiety among aged women regarding cross-infection, further complicating recruitment efforts.

The 8-week duration of the training may have been insufficient to discriminate the effects of L-HIIT from those of DWR. The study's focus on aged women also raises concerns about the generalisability of the findings.

The research did not identify a potential mechanism to explain the observed outcomes. Nevertheless, the findings provide valuable insight into potential applications of DWR instead of L-HIIT. They certainly show that the effects on cardio-metabolic variables, cognition and perceptions are comparable. From a practical point of view, the results suggest that AHIIT-DWR could be a useful alternative to L-HIIT for healthcare professionals designing programs that target cardio-metabolic health benefits, metabolic blood markers, cognitive function and perception in physically inactive aged women.

The data suggest three priorities for future research. Studies on a larger scale and lasting longer are clearly warranted. An 8-week training course might not be sufficient to generate all the possible beneficial effects. Then, the mechanism accounting for the observed benefits still remains to be explained from a neurophysiological perspective. Future studies in that direction are definitely required. For example, a future study might use functional near-infrared spectroscopy to examine blood flow in the prefrontal cortex to better define how AHIIT affects cognition.

Despite the differences in physiology and biomechanics in water, AHIIT appears to provide an adequate stimulus for cardiovascular training. In future research, it would be useful to examine the link between AHIIT and L-HIIT exercise capacity and key patient-related outcomes, barriers to AHIIT, L-HIIT and the ongoing independent commitment to exercise. Follow-up periods after intervention are an area of interest that could be tested in the future.

8.3 Significance and Implications for clinical practice

The meta-analysis underscores the potential of Aquatic High-Intensity Interval Training (AHIIT) as a viable exercise alternative for improving cardio-metabolic health in physically inactive older women, particularly those limited by pain, weakness, or mobility constraints. By demonstrating moderate yet significant enhancements in markers like peak oxygen utilization and resting heart rate, the findings highlight the unique capacity of aquatic environments to mitigate barriers to high-intensity exercise (e.g., joint stress) and enable training at intensities often unattainable on land. This suggests AHIIT could be prioritized in clinical or community programs to promote adherence and accessibility for aging populations. However, the underexplored utility of Deep Water Running (DWR) as a specific AHIIT modality underscores the need for future systematic reviews to validate its efficacy and long-term benefits. Addressing this gap could inform standardized protocols, enhance exercise programming for at-risk groups, and guide policy decisions to expand aquatic infrastructure, ultimately bridging the gap between theoretical advantages of aquatic exercise and practical, sustainable health interventions.

Significantly, our findings revealed that DWR produces outcomes comparable to land-based exercise, with QoL emerging as the sole measure showing marked improvement over non-exercise controls, underscoring its value for enhancing well-being in populations with mobility limitations. Clinically, this positions DWR as a viable low-impact alternative for individuals unable to tolerate traditional exercise. However, the lack of conclusive evidence for cardiorespiratory or functional gains highlights a critical research gap, necessitating future large-scale, homogenous studies using biopsychosocial frameworks to clarify relationships between DWR outcomes. These implications urge methodological refinements to validate DWR's therapeutic role and inform its integration into tailored rehabilitation programs for at-risk groups.

By validating the PNOĒ against the gold-standard COSMED K5—demonstrating their interchangeable accuracy—the findings provide sports scientists with a practical tool for individualized exercise prescriptions, enhancing precision in training and rehabilitation programs. However, the lack of aquatic compatibility for the PNOĒ underscores the urgency to develop water-resistant cases, which would expand its utility to aquatic exercise testing, a growing area of interest for populations requiring low-impact interventions. This advancement could bridge the gap between laboratory-grade accuracy and real-world applicability, fostering innovation in portable metabolic technology and improving accessibility to tailored exercise regimens across diverse settings.

AHIIT as a viable alternative or adjunct to traditional land-based training, particularly for women facing barriers to terrestrial exercise—such as joint pain, injury rehabilitation, or mobility limitations—while still achieving similar acute physiological benefits. The significance lies in expanding accessible, low-impact exercise options for underserved populations, potentially improving adherence and reducing injury risk. Clinically, this supports integrating AHIIT into rehabilitation or fitness programs The observed cardiovascular differences underscore the need for further research to optimize AHIIT protocols and clarify long-term adaptations, ensuring its efficacy aligns with land-based standards. Additionally, by demonstrating that adding resistance enhances metabolic health outcomes while reducing RPE in healthy adults, the findings challenge the assumption that water's natural drag force alone is sufficient for optimizing AHIIT protocols. The significance lies in validating resistance (via cadence or external tools) as a scalable method to intensify workouts without compromising the low-impact advantages of aquatic training, thereby broadening its applicability to populations requiring joint-friendly interventions (e.g., older adults, rehabilitation patients). Importantly, the efficacy of RPE in guiding intensity prescriptions simplifies practical implementation, fostering adherence. These implications advocate for refining AHIIT protocols, while highlighting the need for future research to establish standardized intensity-matching criteria for diverse populations.

The main study findings reveal that DWR as a form of AHIIT can be an alternative to L-HIIT which healthcare professionals should consider when designing programs that target cardio-metabolic health benefits, metabolic blood markers, cognitive functioning and perception in treating physically inactive aged women.

Additionally, DWR generates similar enjoyment and feelings of efficacy to L-HIIT. And it stimulates similarly good adherence. These results can provide valuable insights regarding the potential applications of AHIIT and L-HIIT. L-HIIT could be a time-efficient and effective approach for health professionals to use. The unique physical properties of water make DWR appropriate for individuals who need a lower weight bearing alternative to L-HIIT. The findings are significant as this positions DWR as a compelling alternative for aging populations who face barriers to land-based exercise (e.g., joint pain, mobility limitations), while expanding evidence for its cognitive benefits, a previously understudied area.

Clinically, these implications urge healthcare professionals to integrate AHIIT-DWR into rehabilitation and wellness programs targeting cardio-metabolic health, cognitive function, and exercise adherence. Furthermore, the results advocate for broader recognition of aquatic exercise in public health guidelines, particularly for older women, and highlight the need for long-term studies to validate its sustainability and psychosocial impacts.

8.4 Conclusions

In conclusion, this research establishes AHIIT, particularly Deep Water Running (DWR), as an unique exercise modality for inactive older women, offering cardio-metabolic, cognitive, and perceptual benefits comparable to—and in some cases superior to L-HIIT. By validating the efficacy of low-impact aquatic training in improving oxygen utilization, metabolic markers, and cognitive function, these findings challenge traditional exercise paradigms and provide a scientifically grounded alternative for populations restricted by pain, mobility limitations, or joint stress. The development of a 3D printed adaptor in situ with the PNOĒ metabolic analyzer and a matched intensity and resistance-enhanced AHIIT protocols further underscores the potential to democratize access to personalized, joint-friendly exercise regimens. These advancements not only empower healthcare professionals to design inclusive, evidence-based programs but also highlight urgent priorities for future research: standardizing aquatic exercise protocols, exploring long-term adherence, and integrating biopsychosocial frameworks to optimize holistic health outcomes for physically inactive aged women.

Appendices

9.1 Accepted Manuscript by Journal of Exercise Science and Fitness (Chapter 3)

The effect of aquatic High Intensity Interval Training on cardio-metabolic and physical health markers in women: A systematic review and meta-analysis.

Introduction

Cardio-metabolic health is an important issue to address. Identifying cardio-metabolic risk (CMR) factors, mortality, morbidity, and disability can reduce 73% of all deaths and 60% of the global health burden(Organization, 2014; World Health, 1995). Globally, more than a quarter of the world's adult population (1.4 billion adults) is insufficiently active(Pedro C. Hallal et al., 2012b). The association between physical inactivity and increased mortality appears with a reduction in cardio-metabolic health(Ralph S. Paffenbarger et al., 1986). Thus, identifying and reducing modifiable CMR factors—namely, physical inactivity—will have an essential effect on diseases that would otherwise raise enormous public or health concerns.

Women are relatively less physically active than men. Among adults aged 18 years or older, 31.7% of women and 23.4% of men fail to achieve physical activities up to or exceeding the WHO recommended level of at least 600 metabolic equivalent (MET) minutes weekly(Guthold et al., 2018). In addition, women have a higher prevalence of lower limb osteoarthritis and obesity; women over 45 years old were found to be much more obese than the rest of the population, with a body mass index (BMI) surpassing 25 kg/m^2 in 47% of them and surpassing 30 kg/m^2 in 9% of them(L. Andrade et al., 2020; Ng et al., 2014). Recent public health promotions have highlighted interval training as an alternative exercise strategy that has gained popularity among the general population(Thompson, 2019). High-intensity interval training (HIIT), for example, has numerous significant health benefits for cardio-metabolic and physical health(Karlsen et al., 2017). Performing HIIT in an aquatic environment (AHIIT) can be an alternative to land-based HIIT. AHIIT has emerged for individuals who may be more likely to incorporate HIIT into their lifestyles while immersed in an aquatic environment(Nagle, Sanders, & Franklin, 2017). It offers a great option for cardio-metabolic and physical training. Those who have participated in AHIIT studies were predominantly women. Our review focused on female populations who are believed to have a higher CMR(Campbell et al., 2003; Depiazzi et al., 2018).

Lack of time is a commonly cited reason for people failing to participate continuously in a traditional exercise program; it thus restricts physical activities. HIIT is focused on the inverse relationship between intensity and duration of training(Gibala, 2007). Therefore, interspersing intervals of high levels of exercise with periods of rest or low to moderate levels of exercise can be a more time-efficient and effective option for physical activity than continuous training(Batacan et al., 2017; Gibala, 2007; Nagle, Sanders, & Franklin, 2017; Weston et al., 2014). AHIIT is considered to be effective because many physiological benefits are related to the physical properties of water(Becker et al., 2009). Water buoyancy reduces discomfort and stress placed on the joints. In addition, the hydrodynamic nature of water acts as a form of resistance to movements, optimising the development of muscle strength(Harrison et al., 1992; Rana et al., 2007). With this water cushioning effect, AHIIT can be a safe option for many, as it minimises some of the hindrances to exercise associated with land-based training, such as pain, fear of movement, and balance(Barker et al., 2014b).

There is well-established evidence about the effects of HIIT on cardio-metabolic or physical health, while similar evidence regarding AHIIT is lacking(Batacan et al., 2017). To the best of our knowledge, one systematic review has been conducted on the effects of AHIIT. However, the outcome measures concerned aerobic capacity, muscle strength, and body composition(Depiazzi et al., 2018). Evidence regarding other cardio-metabolic health markers, including lipid profile, blood pressure, and bone mineral density (BMD), is lacking. Given this knowledge gap and lack of evidence, alongside the growing application of AHIIT, the objective of this study is to investigate the effects of AHIIT on the improvement of cardio-metabolic and physical health markers in women.

Methods

A complete search of randomised controlled trials was performed up to January 2021. The reporting in this study adheres to the preferred reporting items for systematic reviews and meta-analysis guidelines (PRISMA)(Moher, Liberati, Tetzlaff, & Altman, 2009). This review protocol was registered in the PROSPERO database of systematic reviews (CRD42021229631).

Study selection

Two reviewers (MK and BS) screened the titles and abstracts of the articles. The reviewers independently reviewed the full texts of the articles and agreed on their eligibility for inclusion in the review. If disagreement occurred, a third reviewer (SN) resolved any discrepancies between the two reviewers or the article was removed from the review.

Search strategies

An exhaustive search of the literature was conducted to identify publications related to the effectiveness of AHIIT. Articles were retrieved from seven electronic bibliographic databases: MEDLINE, CINAHL, PubMed, SPORTDiscus, PsycINFO, Embase, and Cochrane Library. The search strategy comprised the following medical subject headings (MeSH) or keywords: (1) aquatic exercise, aquatic or water exercise, hydrotherapy, water immersion, or head-out aquatics; (2) high-intensity interval training, intermittent exercise, or interval training; (3) cardio-metabolic health, cardiorespiratory or metabolic markers, physical, or physical health; and (4) women or female. An example of the search strategy can be found in Appendix A.

Inclusion criteria

The articles included were based on the following criteria: 1) All participants must be women who were ≥ 18 years old; 2) the studies were published in full and were written in English; 3) the articles included must be randomised control trials; 4) the outcome measures must include cardio-metabolic or physical health markers; and 5) head-out aquatic exercise was assessed. For the intervention to be considered high intensity, the work rate prescribed needed to elicit: i) maximal oxygen capacity (VO_2 max) > 75%]; ii) HRmax > 80–95%; and iii) rate of perceived exertion (RPE) of 15 or more on the BORG exertion scale. The definitions used in the present review are based on a general classification scheme for interval training suggested by Weston and colleagues(Weston et al., 2014). Control groups included were defined as not receiving exercise training.

Exclusion criteria

The studies were excluded if: 1) the participants were men; 2) the intervention was conducted for less than two weeks (the minimum time requirement to observe meaningful physiological changes in interval training); or 3) the intervention was not interval training.

Outcome measures

The outcome measures for this study were cardio-metabolic and physical health markers. Cardio-metabolic health markers include peak oxygen uptake (VO_2 peak), resting heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), body fat percentage, high-density lipoprotein (HDL), and low-density lipoprotein (LDL). In addition, bone mineral density (BMD), knee flexion and extension strength, and the chair to stand (CS) test were the physical health markers measured.

Quality assessment

Two authors (MK and BS) independently graded the quality of the included studies using the Physiotherapy Evidence Database (PEDro) scale.[18] Items related to internal validity and quality of reporting were scored across 10 criteria; "yes" was given a score of one and "no" a score of zero. When data were unavailable or unclear, the item was assigned a score of zero. Reliability and validity for the PEDro scale have been established.[19]

Data extraction

Two authors (MK and BS) independently extracted data regarding the methodology and outcome measures using a standardised data extraction sheet.

Meta-analysis

A further meta-analysis was carried out using comprehensive meta-analysis software (Version 3.0; Biostat, Englewood, NJ). The mean value and standard deviation of each study were inputted into the software. If the values were not available, the authors were contacted by email to obtain the raw data. If no raw data were available, the study was excluded from the meta-analysis. A random-effects model was used to estimate the distribution of observed effect sizes.

Effect size

The effect size in terms of Hedges' g and its 95% confidence interval (CI) was computed in all meta-analyses as various methods between trials assessed cardio-metabolic and physical health markers. Hedges' g is a variation of Cohen's d, which corrects for a possible bias in small sample sizes(Higgins, 2019). Effect size was quantified as large (> 0.8), medium (0.5–0.8), small (0.2–0.5), or non-significant (< 0.2) as a general rule of thumb, as suggested by Cohen (1988).

Publication bias

Egger's regression asymmetry test was performed to assess the existence of publication bias. A P-value of < 0.1 (two-tailed test) indicated the presence of publication bias(Egger et al., 1997).

Results

Study selection

The review search identified 1,520 articles published between 1994 and January 2021, of which 1,278 articles were excluded because they were not relevant to the scope of this review or were duplicates. Out of the remaining 242 articles, 151 were excluded when the abstracts were reviewed, and 51 articles were excluded after reading the full texts. A total of 18 articles were ultimately identified as being relevant to the study;(Aboarrage Junior et al., 2018b; L. Andrade et al., 2020; L. S. Andrade, S. S. Pinto, et al., 2020b; Bento & Rodacki, 2015a; Gi Broman et al., 2006; Connolly et al., 2016; Costa et al., 2018; Denise Fernandes Moreira et al., 2013; Junior et al., 2018; Mohr et al., 2015; Mohr et al., 2014; Moreira et al., 2014; Munukka et al., 2020; Munukka et al., 2016; Nordsborg et al., 2015; Rýzková et al., 2018; Samadi et al., 2019; Waller et al., 2017) 13 of these articles were included in the final analysis *(*Figure 1).

[Insert Figure 1]

Study characteristics

Descriptions of the participants and outcomes of the studies are summarised in Appendix B. All of the included studies employed AHIIT as an intervention. The characteristics of the interventions are summarised in Table 1.

[Insert Table 1]

Quality assessment

Quality assessment of all the studies achieved a moderate quality median PEDro score of 5.5 out of 10 for all papers; the highest score was eight(Munukka et al., 2020; Munukka et al., 2016) and the lowest was four(Bento & Rodacki, 2015a; Gi Broman et al., 2006) (Table 2). There was no blinding of subjects in any of the studies reviewed due to the nature of intervention studies. PEDro scores of seven and above are considered high-quality studies; scores of five and six are considered to be of moderate quality; scores lower than four are considered poor quality(Moher, Liberati, Tetzlaff, & Altman, 2009). Four studies were considered high-quality, with PEDro scores of seven and above, mainly due to the intention to analyse the data(Costa et al., 2018; Moreira et al., 2014; Munukka et al., 2020; Munukka et al., 2016). Twelve studies were of moderate quality, in which allocations were not concealed and the assessors were not blinded(Aboarrage Junior et al., 2018b; L. Andrade et al., 2020; L. S. Andrade, S. S. Pinto, et al., 2020a; Connolly et al., 2016; Junior et al., 2018; Mohr et al., 2015; Mohr et al., 2014; Moreira et al., 2014; Nordsborg et al., 2015; Rýzková et al., 2018; Samadi et al., 2019; Waller et al., 2017). Two studies were of low quality, as a result of a lack of information about the follow-up procedures and blinding of assessors during the intervention(Bento & Rodacki, 2015a; Gi Broman et al., 2006).

[Insert Table 2]

Effect of AHIIT on cardio-metabolic health markers

Our analysis of five studies(L. S. Andrade, S. S. Pinto, et al., 2020b; Gi Broman et al., 2006; Costa et al., 2018; Munukka et al., 2016; Samadi et al., 2019) revealed a significant ($P < 0.001$) moderate effect size point estimate of 0.610 in favour of AHIIT compared with the control for peak oxygen uptake (VO_2 peak) (95% CI 0.277 to 0.943), with no significant heterogeneity found ($I^2 = 26.69\%$, $P = 0.244$) (Figure 2a). Four studies(L. S. Andrade, S. S. Pinto, et al., 2020b; Gi Broman et al., 2006; Mohr et al., 2014; Rýzková et al., 2018) indicated a significant (*P* = 0.009) moderate effect size point estimate of -0.495 (95% CI -0.866 to -0.124) in favour of reducing resting heart rate (HR), without significant heterogeneity across studies (I^2 = 0%, *P* = 0.599) (Figure 2b). However, our analysis of three studies(Gi Broman et al., 2006; Junior et al., 2018; Mohr et al., 2014) on SBP and DBP indicated insignificant moderate effect size point estimates of -0.044 (95% CI -0.964 to 0.076) (*P* = 0.095) (I^2 = 33.898%, *P* = 0.22) (Figure 2c) and -0.328 (95% CI -0.938 to 0.281) (*P* = 0.291) (I^2 = 51.539%, *P* = 0.127) (Figure 2d), respectively, in favour of AHIIT, compared with the control.

For body fat percentage, three studies(Junior et al., 2018; Mohr et al., 2014; Rýzková et al., 2018) indicated an insignificant (*P* = 0.082) moderate effect size point estimate of -0.735 (95% CI -1.562 to 0.093) in favour of AHIIT compared with the control. There was significant moderate heterogeneity across studies (I^2 = 69.197%, *P* = 0.039) (Figure 2e). Although our analysis of two studies(Costa et al., 2018; Mohr et al., 2014) indicated that both blood lipid measurement regarding HDL and LDL had insignificant (P = 0.273) and small (P = 0.327) effect size point estimates of 0.240 (95% CI -0.189 to 0.669) and -0.285 (95% CI -0.853 to 0.284), respectively, there was no significant heterogeneity across studies: (I^2 = 0%, *P* = 0.688) (Figure 2f) and (I^2 = 42.376%, *P* = 0.188) (Figure 2g), respectively.

Effect of AHIIT and physical outcomes

For total body BMD and total femur BMD, our analysis of three studies(Junior et al., 2018; Mohr et al., 2014; Moreira et al., 2014) indicated an insignificant (*P* = 0.224) moderate effect size point estimate of 0.627 (95% CI -0.384 to 1.638) and a small effect size point estimate of -0.110 (95% CI -0.409 to 0.188, *P* = 0.468) in favour of AHIIT compared with the control. There was significant large heterogeneity (I^2 = 87.722%, *P* =

0.000) (Figure 2h) and no significant heterogeneity across studies (I^2 = 0.000%, *P* = 0.402) (Figure 2i), respectively.

Our analysis of three studies(L. S. Andrade, S. S. Pinto, et al., 2020b; Bento & Rodacki, 2015a; Munukka et al., 2016) on knee extension strength indicated an insignificant (*P* = 0.098) small effect size point estimate of 0.261 (95% CI -0.048 to 0.571) without significant heterogeneity across studies (I^2 = 0.000%, *P* = 0.945) (Figure 2j). Our analysis of two studies(Bento & Rodacki, 2015a; Munukka et al., 2016) on knee flexion strength indicated an insignificant (*P* = 0.232) small effect size point estimate of 0.262 (95% CI -0.168 to 0.691) in favour of AHIIT compared with the control group. There was no significant heterogeneity across studies (I^2 = 31.181%, *P* = 0.228) (Figure 2k). Furthermore, our analysis of two studies(L. Andrade et al., 2020; Junior et al., 2018) on the chair to stand test indicated a significant (*P* = 0.042) moderate effect size point estimate of 0.548 (95% CI 0.019 to 1.077) in favour of AHIIT compared to the control group; no significant heterogeneity was found (I^2 = 0%, *P* = 0.552) (Figure 2l).

[Insert Figure 2]

Publication bias

According to the results of Egger's test, no significant publication bias was observed in the meta-analysis of cardio-metabolic health markers (*P* = 0.249) and physical outcomes (*P* = 0.855).

Discussion

To the best of our knowledge, this systematic review with meta-analysis is the first to examine the effects of AHIIT on cardio-metabolic and physical health markers in women. Our main findings revealed AHIIT resulted in a moderate improvement in cardio-metabolic markers in regard to VO_2 peak, resting HR, and physically in the CS test.

VO_2 peak is considered to be directly reflective of maximal oxygen capacity (VO_2 max), the highest value of VO_2 attained upon a high-intensity exercise test(Whipp et al., 1990). The test of VO_2 peak is designed to bring the subject to the limit of tolerance, a gold standard measure of cardiorespiratory fitness and a solid indicator that can predict mortality and improve prognosis(Keteyian et al., 2008). Our findings demonstrated that the VO_2 peak significantly increased with a moderate effect when AHIIT was compared to control groups(L. S. Andrade, S. S. Pinto, et al., 2020b; Gi Broman et al., 2006; Costa et al., 2018; Munukka et al., 2016; Samadi et al., 2019). With training, centrally, the left ventricle of the heart can stretch to allow for more forceful contractions, which increase stroke volume and result in increased VO_2 peak(McDaniel et al., 2020). HIIT is also able to increase the VO_2 peak rapidly via increasing mitochondrial density, resulting in the generation of more adenosine triphosphate (ATP) for working muscles(Gibala & Jones, 2013). Another possible explanation is that VO_2 is influenced by a restriction in chest expansion when inspiratory muscle contractions are unable to equal or overcome the force of hydrostatic pressure(McDaniel et al., 2020). It is suggested that central hypervolaemia influences lung volumes by reducing lung compliance and promoting gas trapping, which narrows the airways(McNamara et al., 2013). With such unique physiological adaptations during aquatic training, minute ventilation and breathing frequency were significantly increased when compared with land-based training with an equivalent exercise intensity(Silvers et al., 2007).

Our findings also revealed an overall moderate effect in reducing resting HR in women following AHIIT, consistent with another study(Plotnick et al., 1986). With increased water immersion due to hydrostatic gradient, AHIIT increases stroke volume, which induces increased cardiac contractability and reduced heart rate(Helgerud, Hoydal, et al., 2007). It has been proposed that the reduction of heart rate is due to an increase in parasympathetic activity and a decrease in the sympathetic activity of the heart(Carter et al., 2003). With an increase in resting HR response indicating a risk factor for all-cause mortality, a reduction in resting HR through AHIIT minimises risk factor mortality(Jensen et al., 2013). Such improvements in VO_2 peak and resting HR provided by AHIIT are crucial, since both are independent predictors of cardio-metabolic disease, particularly cardiovascular disease mortality(Lau et al., 2019). It has been shown that every 1-MET increase in VO_2 peak is associated with 10–25% improvements in survival rate and an 18% reduction in events related to cardiac-related diseases(Myers et al., 2002). Hence, improving VO_2 peak and resting HR to improve cardio-metabolic health can be easily achieved with the help of AHIIT.

Blood pressure is another common cardio-metabolic measure. A lack of significant reduction in both SBP and DBP was observed. This lack of significance could be because the included participants in the studies were exclusively hypertensive aged women, and it is known that SBP increases progressively with age(Strandberg & Pitkala, 2003). Similarly, changes in lipid profile were not significant in this review. A possible reason for this result is that lipid metabolism reflects HDL and LDL levels, and depends on the concentration of other enzymes(Costa et al., 2018). Due to the limited number of review papers on lipid profile, the mechanism regarding the effect of AHIIT is still unknown.

HIIT appears to promote a significant reduction in body fat percentage in overweight men(Poon et al., 2020). Our results did not show an overall significant effect of AHIIT on body fat percentage in female populations. Likewise, there was a review that showed no overall effects on body composition utilising AHIIT among non-athletic populations(Depiazzi et al., 2018). Despite these findings, when trained at the same relative intensity, a higher VO_2 training value could be achieved in water than on land.

This could potentially result in a higher caloric cost and, hence, a higher reduction in body fat percentage(Hall et al., 2004b).

When considering physical health markers, the hydrostatic pressure in the water created by resistance in movement at high speed promotes muscle action(Pöyhönen et al., 2002). High speeds promote the recruitment of type II fast-twitch muscle fibres(Wing-Hoi et al., 2010). However, our results showed no significant difference in overall knee extension and flexion strength following AHIIT. Our results are contrary to the results of the meta-analysis conducted by Depiazzi and colleagues, but are similar to the findings of Heywood and colleagues, which showed no significant improvement in muscle strength(Depiazzi et al., 2018; S. M. Heywood et al., 2016). This discrepancy in muscle strength could result from a difference in movement speed and resistance loading exerted on the muscles. The improvement in muscle strength remains controversial at this stage and warrants further investigation.

Our results revealed no overall significant changes in total body BMD or total femur BMD. Impact or resistive exercise in an aquatic environment results in different loadings exerted on bone cells; according to Wolff's law, mechanical loading is considered to be an active osteogenic stimulus(Wolff et al., 1999). Nonetheless, no significant results were found in total body BMD and total femur BMD. This finding could be explained by the level of immersion during AHIIT exercises (e.g., exercises performed at chest level associated with reduced mechanical loading on the bones)(Martyn-St James & Carroll, 2009).

Our results showed a significant effect on functional improvement in the CS test despite only two studies on the CS test being included in the analysis(Aboarrage Junior et al., 2018b; L. Andrade et al., 2020). These studies indicate that the CS test has good test-retest reliability ($R \geq 0.8$) and reasonably good criterion-related validity relative to knee extensor strength(Bohannon, 2009). Our review, however, showed a significant improvement in the CS test without a substantial improvement in knee extensor strength. This result may be because the CS test is significantly associated with the eccentric contraction of the knees, instead of the maximal dynamic strength measured(Vaidya et al., 2017). Given the improvement in the ability of women to perform the CS test after AHIIT, it seems to be a reliable tool with which to assess lower-body strength and performance(Zanini et al., 2015). It might therefore be considered an alternative tool to determine maximal dynamic strength and potentially simulate daily living tasks, resulting in a functional gain.

Finally, data presented in this analysis indicate that AHIIT is safe for female populations in regard to safety issues. This result is consistent with other reviews, which also show a trivial number of adverse events associated with HIIT and aquatic training(Barker et al., 2014b; Weston et al., 2014).

Study limitations

Although the synthesised evidence in this review is encouraging, this study has several limitations. Discrepancies exist in defining high intensity across different kinds of training, using maximal oxygen uptake, and HR or RPE scales. The relatively small number of studies included in each outcome resulted in a small sample size, and limiting the sample to female populations may affect the generalisability of the results. Our literature search was also limited to databases and publications in English peer-reviewed journals. The quality of the majority of studies included is between low and moderate in terms of reporting. Although no other intervention groups were used for comparison in this study, the data of the involved studies in the present analysis were carefully and systematically assessed. Overcoming the shortcomings of this research should be considered in the design of future studies.

Conclusion

This is the first systematic review and meta-analysis of the overall effectiveness of AHIIT interventions in improving cardio-metabolic health markers in regard to VO_2 peak, resting HR, and physical outcomes in the CS test among female populations. Although AHIIT appears to be effective, future research is needed to compare AHIIT with other exercise interventions and to develop optimal AHIIT parameters to benefit cardio-metabolic and physical health.

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Declaration of interests

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Figure and Table Legends

Figures

Figure 1 PRISMA Flow Diagram

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Figure 1 PRISMA Flow Diagram

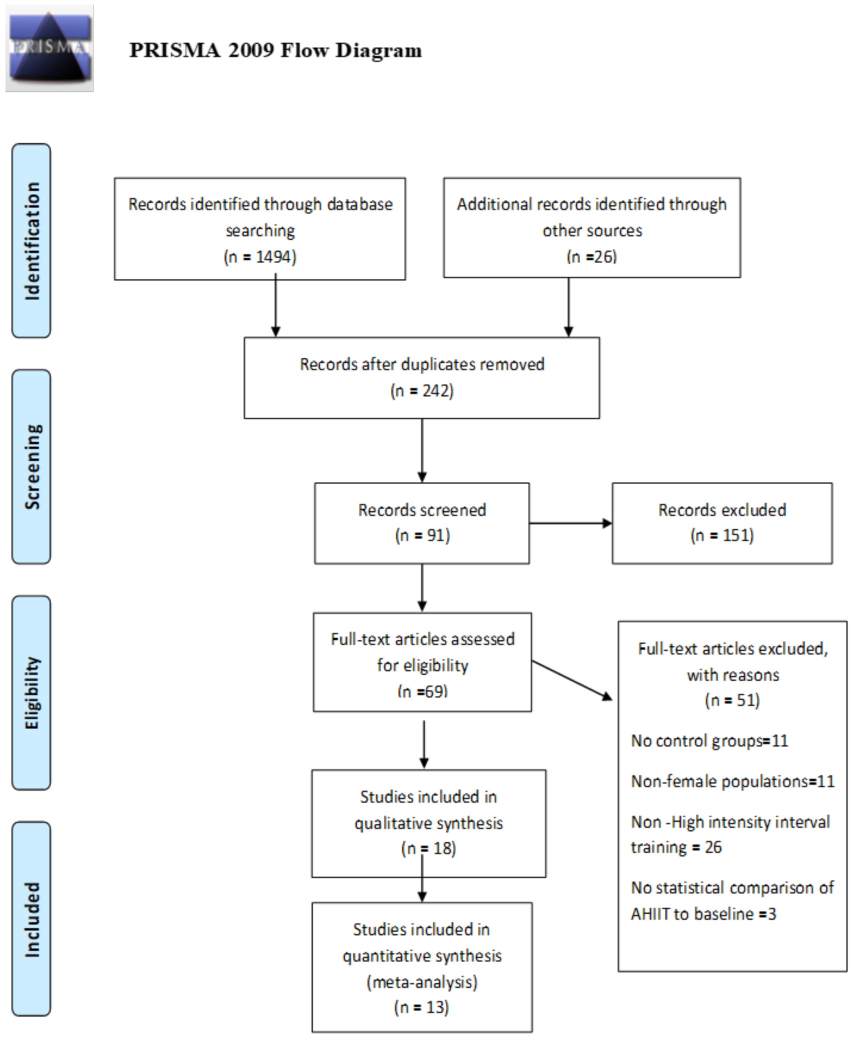
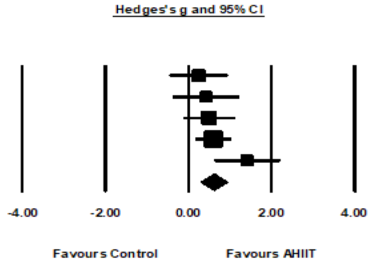


Figure 2 Meta-analysis and forest plots of AHIIT on VO2 peak

AHIIT overall effect om VO2 peak

Study name	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Andrade 2020	Vo2 peak	0.246	0.357	0.128	-0.455	0.946	0.687	0.492
Broman 2006	Vo2 peak	0.424	0.412	0.169	-0.383	1.231	1.030	0.303
Costa 2018	Vo2 peak	0.492	0.315	0.099	-0.125	1.109	1.562	0.118
Manukka 2016	Vo2 peak	0.599	0.221	0.049	0.166	1.033	2.711	0.007
Samadi 2019	Vo2 peak	1.415	0.399	0.160	0.632	2.198	3.542	0.000
		0.610	0.170	0.029	0.277	0.943	3.587	0.000

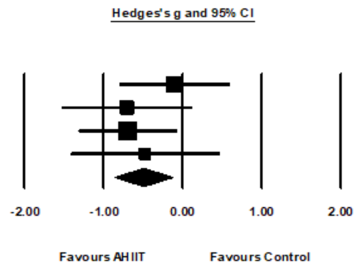


Heterogeneity I^2= 26.56%, p= 0.244
Test for overall effect Z= 3.587 p= 0.000

Figure 3 Meta-analysis and forest plots of AHIIT on resting HR

AHIIT overall effect on resting HR

Study name	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Andrade 2020	resting HR	-0.101	0.356	0.127	-0.799	0.597	-0.284	0.777
Broman 2006	resting HR	-0.706	0.420	0.176	-1.529	0.116	-1.682	0.092
Mohr 2014	resting HR	-0.694	0.316	0.100	-1.313	-0.075	-2.197	0.028
Ryzkova 2017	resting HR	-0.473	0.480	0.230	-1.414	0.468	-0.985	0.325
		-0.495	0.189	0.036	-0.866	-0.124	-2.614	0.009

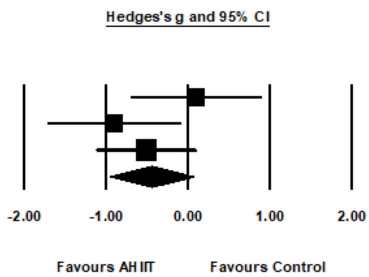


Heterogeneity I^2= 0.000%, p= 0.599
Test for overall effect Z= -2.614 p= 0.009

Figure 4 Meta-analysis and forest plots of AHIIT on SBP

AHIIT overall effect on systolic blood pressure

	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Broman 2006	SBP	0.096	0.407	0.166	-0.702	0.895	0.237	0.813
Junior 2018 b	SBP	-0.902	0.415	0.172	-1.716	-0.089	-2.174	0.030
Mohr 2014	SBP	-0.511	0.312	0.097	-1.121	0.100	-1.639	0.101
		-0.444	0.266	0.070	-0.964	0.076	-1.672	0.095

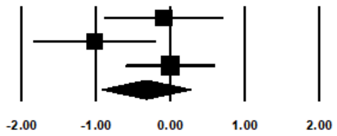


Heterogeneity I^2= 33.90%, p= 0.220
Test for overall effect Z= -1.671 p= 0.095

Figure 5 Meta-analysis and forest plots of AHIIT on DBP

AHIIT overall effect on diastolic blood pressure

Study name	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Broman 2006	DBP	-0.088	0.407	0.166	-0.887	0.710	-0.217	0.828
Junior 2018 b	DBP	-1.017	0.421	0.177	-1.841	-0.193	-2.418	0.016
Mohr 2014	DBP	0.000	0.306	0.094	-0.601	0.601	0.000	1.000
		-0.328	0.311	0.097	-0.938	0.281	-1.056	0.291

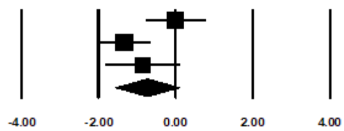


Heterogeneity I^2= 51.54%, p= 0.127
Test for overall effect Z= -1.056 p= 0.291

Figure 6 Meta-analysis and forest plots of AHIIT on body fat percentage

AHIIT overall effect on body fat percentage

Study name	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Junior 2018	Body fat percentage	0.000	0.395	0.156	-0.774	0.774	0.000	1.000
Mohr 2014	Body fat percentage	-1.324	0.340	0.115	-1.990	-0.659	-3.901	0.000
Ryzkova 2017	Body fat percentage	-0.847	0.496	0.246	-1.819	0.124	-1.709	0.087
		-0.735	0.422	0.178	-1.562	0.093	-1.741	0.082

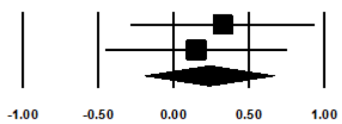


Heterogeneity I^2= 69.20%, p= 0.039
Test for overall effect Z= -1.741. p= 0.082

Figure 7 Meta-analysis and forest plots of AHIIT on HDL

AHIIT overall effect on High Density Lipoprotein

Study name	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Costa 2018	HDL	0.329	0.312	0.097	-0.282	0.941	1.055	0.291
Mohr 2014	HDL	0.154	0.307	0.094	-0.448	0.755	0.501	0.617
		0.240	0.219	0.048	-0.189	0.669	1.097	0.273

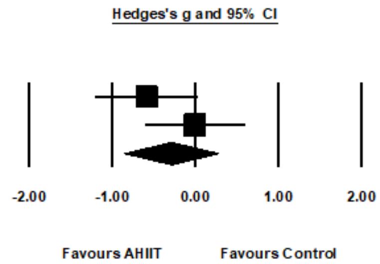


Heterogeneity I^2= 0.000% , p= 0.688
Test for overall effect Z= 1.097 p= 0.273

Figure 8 Meta-analysis and forest plots of AHIIT on LDL

AHIIT overall effect on Low Density Lipoprotein

Study name	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Costa 2018	LDL	-0.580	0.317	0.100	-1.201	0.040	-1.833	0.067
Mohr 2014	LDL	0.000	0.306	0.094	-0.601	0.601	0.000	1.000
		-0.285	0.290	0.084	-0.853	0.284	-0.981	0.327

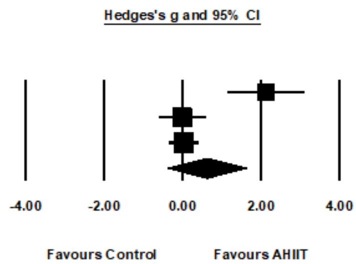


Heterogeneity I^2= 42.376% , p= 0.188
Test for overall effect Z= -0.981 p= 0.327

Figure 9 Meta-analysis and forest plots of AHIIT on total body BMD

AHIIT overall effect on Total Body Bone Marrow Density

	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Junior 2018	total BMD	2.141	0.498	0.248	1.166	3.116	4.303	0.000
Mohr 2015	total BMD	0.000	0.306	0.094	-0.601	0.601	0.000	1.000
Moreira 2014	total BMD	0.038	0.194	0.038	-0.343	0.419	0.195	0.845
		0.627	0.516	0.266	-0.384	1.638	1.216	0.224

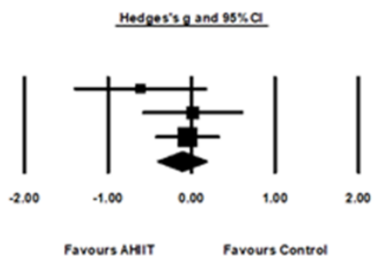


Heterogeneity I^2= 87.72% , p= 0.000
Test for overall effect Z= 1.216 p= 0.224

Figure 10 Meta-analysis and forest plots of AHIIT on total femur BMD

AHIIT overall effect on total femur bone marrow density

	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Junior 2018	BMD Total femur	-0.612	0.404	0.163	-1.404	0.180	-1.513	0.130
Mohr 2015	BMD Total femur	0.017	0.306	0.094	-0.583	0.618	0.057	0.955
Moreira 2014	BMD Total femur	-0.046	0.194	0.038	-0.427	0.335	-0.236	0.813
		-0.110	0.152	0.023	-0.409	0.188	-0.726	0.468

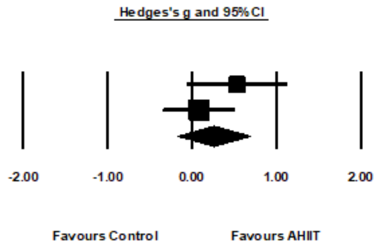


Heterogeneity I^2= 0%, p= 0.402
Test for overall effect: Z= -0.726 p= 0.468

Figure 11 Meta-analysis and forest plots of AHIIT on knee flexion strength

AHIIT overall effect on Knee flexion strength

Study name	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Bento 2015	knee flexion strength	0.538	0.304	0.093	-0.059	1.134	1.767	0.077
Manukka 2016	knee flexion strength	0.088	0.216	0.047	-0.336	0.512	0.405	0.685
		0.262	0.219	0.048	-0.168	0.691	1.194	0.232

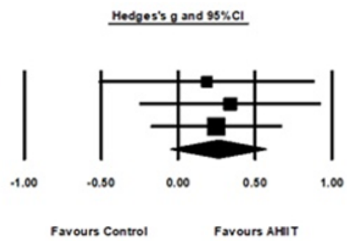


Heterogeneity I^2= 31.18% , p= 0.228
Test for overall effect Z= 1.194 p= 0.232

Figure 12 Meta-analysis and forest plots of AHIIT on knee extension strength

AHIIT overall effect on knee extension strength

Study name	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Andrade 2020 a	knee extension strength	0.187	0.357	0.127	-0.512	0.886	0.524	0.600
Bento 2015	knee extension strength	0.339	0.301	0.091	-0.251	0.929	1.125	0.261
Manukka 2016	knee extension strength	0.248	0.217	0.047	-0.177	0.674	1.144	0.253
		0.261	0.158	0.025	-0.048	0.571	1.654	0.098

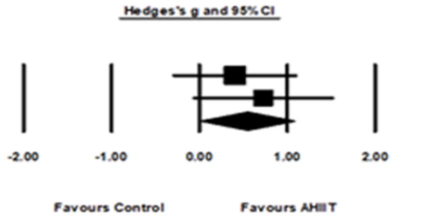


Heterogeneity I^2= 0%, p= 0.945
Test for overall effect: Z= 1.654 p= 0.098

Figure 13 Meta-analysis and forest plots of AHIIT on chair to stand test

AHIIT overall effect on chair to stand test

Study name	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Andrade 2020 b	CS	0.407	0.360	0.129	-0.298	1.111	1.131	0.258
Junior 2018	CS	0.730	0.408	0.167	-0.070	1.530	1.789	0.074
		0.548	0.270	0.073	0.019	1.077	2.032	0.042



Heterogeneity I^2= 0%, p= 0.552
Test for overall effect: Z= 2.032 p= 0.042

Table 1 Characteristics of Aquatic High Intensity Interval training

Author	Type of water training	Characteristics of AHIIT intervention			Pool (Type & temperature)	Depth (immersion level)	Description of AHIIT (work-rest ratio)	High intensity measurement	Adverse events recorded
		Times per week	Number of weeks	Time for each session (min)					
L. S. Andrade et al., 2020a	Water based exercise program (stationary running, frontal kick and cross-country skiing)	2	12	44	Pool of a sports club linked to university of water temp 30º C -32º C,	Between xiphoid process and shoulder	2min-2min	RPE 17.3-18.9 corresponds to 80-89% VO2 peak	Nil
L. S. Andrade et al., 2020b	Water based exercise program (stationary running, frontal kick and cross-country skiing)	2	12	44	Pool of a sports club linked to university of water temp 30º C -32º C,	Between xiphoid process and shoulder	2min-2min	RPE 17.3-18.9 corresponds to 80-89% VO2 peak	Nil
Bento et al 2014	1. Upper and lower limbs water aerobic exercise 2. Strengthening lower limb muscles using aquatic resistance devices	3	12	60	Indoor swimming pool of water temp 28º C -30º C	Xyphoid process	1.40s-20s 2.2-3 sets of exercise with 8-12 RM and 2 mins rest	Exercises executed without the feet contacting the bottom of the pool to increase the exercise intensity	NR
Broman et al 2006	Deep water running with vest	2	8	48	27º C	Shoulder level	10min-2min	>75% maximal HR	Nil
Connolly et al, 2016	All out free style swimming (front crawl)	3	15	15-25	NR	Head out swim	30s-2min	Mean HR and peak HR are 86 ±3 and 96±1 % HR max in high intensity interval group	Nil

Costa et al 2018	Water aerobic exercises (9 upper limbs and lower limbs exercises)	2	12	45	Swimming pool of the university	Xiphoid process	NR	BORG 15	NR
Aboarrage Junior et al 2018	Jump based aquatic exercise program	3	24	30	Aquatic centre of pool temp 29 º C	Xiphoid process	30s-30s	All-out intensity, self-selected maximal intensity	Nil
Junior et al 2018	Aquatic exercises (a) jumping jacks (b) horizontal adduction and abduction of the shoulder; (c) stationary running with knee	NR	NR	45	Pool temp 29 º C	Xiphoid process	30s-1min	60-89% HRR (Vigorous intensity)	Nil
Mohr et al 2015	All out free style swimming (front crawl)	2.9 (0.1)	15	15-25	NR	Head out swim	30s-2min	Mean HR and peak HR are 86 ±3 and 96±1 % HR max in high intensity interval training group	Nil
Mohr et al 2014	High Intensity Sprint swimming	2.9 (0.5)	15	15-25	NR	Head out swim	30s-120s	Mean HR and peak HR are 86 ±3 and 96±1 % HR max in high intensity interval training group	Nil
Moreira et al 2014	Strength and power exercise and aquatic cardiorespiratory training	3	24	50-60	Covered swimming pool, with water temp 30-31º C	Water depths 1.1-1.3m	1. Two sets of 30s each 2. Three sets of 20 s each, 3. Four sets of 15 s each, 4. 5 sets of 10s each	1. 60% HR max in 16 mins of session 5-9, 2. 70% HR max in 13 mins of session in weeks 10-14, 3. 80% HR max in 9 mins session in weeks 15-19, 4. 90% HR max in 7 mins session in weeks 20-24,	Nil

Moreira et al 2013	Strength and power exercise and aquatic cardiorespiratory training	3	24	50-60	Covered swimming pool, with water temp 30-31º C	Water depths 1.1-1.3m	1. Two sets of 30s each 2. Three sets of 20 s each, 3. Four sets of 15 s each, 4. 5 sets of 10s each	1. 60% HR max in 16 mins of session 5-9, 2. 70% HR max in 13 mins of session in weeks 10-14, 3. 80% HR max in 9 mins session in weeks 15-19, 4. 90% HR max in 7 mins session in weeks 20-24,	Nil
Munukka et al 2019	Lower limbs aquatic resistance training	2.6(0.5)	16	60	Heated pool, 30-32º C	NR	2 sets x 30 reps to 3 sets x 30-45 reps, with rest period 30-45s	-Average Intensity of each session RPE 15 (12-17), HRmax 144 (12) bpm -Intensity of training set at "as hard and fast as possible	-Two medical consultations (bilateral knee pain and dyspnoea) after training
Munukka et al 2016	Lower limbs aquatic resistance training	2.6(0.5)	16	60	Heated pool, 30-32º C	NR	2 sets x 30 reps to 3 sets x 30-45 reps, with rest period 30-45s	-Average Intensity of each session RPE 15 (12-17), Max HR144 (12) bpm -Intensity of training set at "as hard and fast as possible	-Two medical consultations (bilateral knee pain and dyspnoea) after training
Nordsborg et al 2015	All out free style swimming (front crawl)	3	15	15-25	NR	Head out swim	30s-2min	Mean HR and peak HR are 86 ±3 and 96±1 % HR max in high intensity interval training group	Nil

Ryzkova et al 2018	Aqua-fitness program: HIIT program	2	10	50	Pool at the university of water-temperature of 28 º C	Central chest area up to shoulder	20s-10s	High intensity training zone >80% HR reserve	NR
Samadi et al 2019	AHIIT training (20 min, quick movements of body)	3	12	30	NR	NR	20s-10s	80-95% HR max	NR
Waller et al 2017	Lower limbs aquatic resistance training	2.6 (0.5)	16	60	Heated pool, 30-32º C	NR	2 sets x 30 reps to 3 sets x 30-45 reps, with rest period 30-45 s in between sets	-Average Intensity of each session RPE 15 (12-17), HR max144 (12) bpm -Intensity of training set at "as hard and fast as possible"	-Two medical consultations (bilateral knee pain and dyspnoea) after training

BORG, Borg scale of perceived exertion; bpm, beats per minute; HIIT, High Intensity Interval Training; HR, heart rate; HRmax, maximal heart rate; Min, minute; NR, not recorded; temp, temperature; RPE, rate of perceived exertion; reps, repetitions; s, second

Table 2 The PEDro Score

	PEDro Criterion											
Study	A	B	C	D	E	F	G	H	I	J	K	Total Score
L. S. Andrade et al., 2020	1	1	0	1	0	0	1	0	1	1	1	6
L. Andrade et al., 2020	1	1	0	1	0	0	1	0	1	1	1	6
Bento et al 2014	1	1	0	1	0	0	0	0	0	1	1	4
Broman et al 2006	1	1	0	1	0	0	0	0	0	1	1	4
Connolly et al 2016	1	1	0	1	0	0	0	1	0	1	1	5
Costa et al 2018	0	1	1	1	0	0	1	0	1	1	1	7
Aboarrage Junior et al 2018	1	1	0	1	0	0	1	0	0	1	1	5
Junior et al 2018	1	1	1	1	0	0	0	0	0	1	1	5
Mohr et al 2015	1	1	0	1	0	0	0	1	0	1	1	5
Mohr et al 2014	1	1	0	1	0	0	0	1	0	1	1	5
Moreira et al 2014	1	1	1	1	0	0	1	0	0	1	1	6
Moreira et al 2013	1	1	1	1	0	0	1	1	0	1	1	7
Munukka et al 2019	1	1	1	1	0	0	1	1	1	1	1	8
Munukka et al 2016	1	1	1	1	0	0	1	1	1	1	1	8
Nordsborg et al 2015	1	1	1	1	0	0	0	1	0	1	1	6
Ryzkova et al 2018	1	1	0	1	0	0	0	1	0	1	1	5
Samadi et al 2019	1	1	0	1	0	0	0	1	0	1	1	5
Waller et al 2017	1	1	0	1	0	0	0	1	1	1	1	6
												Median PEDro score =5.5 (range, 4-8)

A. Eligible criteria were specified

B. Predictions were randomly allocated groups

C. Allocation was concealed

D. The groups were similar at baseline regarding the most important prognostic indicators

E. There was blinding of all assessors who measured the primary outcome
F. Therapist blinding
G. Assessor blinding
H. Follow-up > 85%
I. Intension-to-treat analysis

J. Between -group statistical comparisons

K. Point measure and measures of variability reported

9.2 Accepted Manuscript by International Journal of Environmental Research and Public Health (Chapter 4)

Effectiveness of Deep Water Running in Improving Cardiorespiratory Fitness, Physical Function and Quality of Life: A Systematic Review

Introduction

Deep water running (DWR) has gained popularity among different patient populations in recent decades for cardiovascular conditioning. DWR can be defined as running at 70% of the body immersion, submerged at the shoulder level, with or without use of a floatation device (Reilly et al., 2003). It simulates land running movement patterns performed, but is conducted in a non-weight bearing aquatic environment. With the advantage of unloading property of buoyancy, DWR eliminates vertical ground reaction forces and hence reduces joint loadings and the potential risk for injury to the musculoskeletal system. (Killgore, 2012a, 2012b; Silva, Dias, Bela, et al., 2020).

Changes in the properties of water, including temperature as well as the instructions for the training can influence the outcomes of DWR. For instance, water temperature can directly influence the physiological mechanisms brought about by DWR. A temperature of 26°C to 28°C is recommended for DWR performed at maximal intensities (Reilly et al., 2003). This can result in a reduction in maximal heart rate (HRmax), increased energy expenditure and body heat dissipation as compared with land control groups (Pendergast & Lundgren, 2009b) Speed of movement can also influence drag force and the level of resistance overload depends on the speed of the movement (Becker, 2009). At a given speed, when the surface area of the limb is increased, an additional overload can be achieved (Colado et al., 2009). Therefore a combination of changes in water temperature and drag force can promote a DWR program progression (Silva, Dias, Bela, et al., 2020).

Deep water running has emerged as an alternate exercise for athletic, healthy untrained populations as well as people with musculoskeletal conditions (Nagle et al., 2017; Dowzer et al., 1999). During DWR, the buoyancy unloading effect of the water negates the weight-bearing nature of exercise relative to that conducted on land. The lower impact force experienced in the water allows the exercise to be performed both at higher intensities and for longer durations, with a potential reduction in risk of injury (M. M. Y. Kwok, S. S. M. Ng, et al., 2022; Nagle, Sanders, & Franklin, 2017). Although DWR may induce lower physiological responses when compared with land running with lower maximal oxygen uptake (VO2 max) and HRmax (Reilly et al., 2003), the training stimulus is likely to still be sufficient for supplementary training, with a decreased orthopaedic trauma compared to land running (Dowzer et al 1999; Broamn 2006). As such, the reduction in loading on the body makes DWR a viable training alternative for athletes who are susceptible to repetitive stress injuries, as well as for elderly individuals who may be vulnerable to, or fearful of falls or joint pain.

Although it is known that DWR provides a form of unloaded, non-weight bearing exercise, the biomechanics of DWR when compared with land running remains unclear. DWR can be classified into two styles, namely, (1) the cross-country style and (2) the high knee style (Killgore et al., 2006). Deep water running instructions on style may vary, the consensus for technique follows these principles: (1) the trunk should be slightly forward in upright position, (2) the arms and legs should follow a circular leg and arm motion, (3) the swing phase leg should be brought to the horizontal position, (4) the elbows should be maintained at a 90° angle, and (5) the hands should remain either completely open or in a closed fist at all times (K. S. Chu & E. C. Rhodes, 2001). There may not be consensus on whether the DWR style should be attempting to replicate similar lower limb kinematics or whether larger ranges of movement lead to greater resistance and higher intensity.A recent review suggested that muscle activity during DWR demonstrated a higher activation of distal leg muscles than proximal thigh muscles, in comparison with land treadmill running (Silva, Dias, Bela, et al., 2020) although it is not clear what physical functional outcomes this may influence. Hence, there is an increasing interest in examining the differences in physical functional outcomes with validated measurement tools on DWR and land running.

The effect of DWR on health-related quality of life (QoL) remains unclear. It is common for people with musculoskeletal conditions to report lower levels of quality of life – including in domains related to physical function -- due to fear and pain (Luque-Suarez et al., 2019). The fear of physical activity, arising from the feeling of pain during movement or risk of reinjury, often leads to a downward spiral of negative physical and psychological consequence (Butler, 2013). Deep Water Running, a proposed low impact aerobic exercise may offer a potential solution to mitigate fear associated with pain.

Despite the fact that there is growing interest in the inclusion of DWR in cardiovascular training programs for various clinical populations, and a number of clinical trials have been conducted to evaluate the effects of DWR on numerous qualities of life domains, there has not yet been a systematic examination of the effects of DWR on cardiorespiratory fitness, physical function, and quality of life. The current systematic review was conducted to address this gap. Specifically, we sought to evaluate the effects of Deep Water Running (DWR) on cardiorespiratory fitness, physical function, and quality of life, when compared to other interventions or control groups without exercise.

Method

2.1. Study selection

Two reviewers (MK and BS) screened the articles titles and abstracts. The reviewers independently review the full texts and reach an agreement for the eligibility to include in the review. If disagreement, third reviewer (SH) resolved any discrepancies in between the two reviewers, the article will be removed from the review.

2.2. Search strategy

This systematic review was guided by the Preferred Reporting Items for Systematic reviews and Meta-analysis (PRISMA) guidelines (Moher, Liberati, Tetzlaff, Altman, et al., 2009), and was registered in PROSPERO database (CRD42020154988) prior to conducting the review. A complete search of six databases, SPORTDiscus, MEDLINE, CINAHL, AMED, Embase, and The Cochrane Library, were conducted up to December2021 with a combinations of text words listed in Appendix 1. In an effort to unveil complex evidence in obscure locations, manual searching of "reference tracking" and "citation tracking" of relevant articles were completed (Greenhalgh & Peacock, 2005). With the use of citation tracking database (i.e. "Cited by" in Google Scholar), forward tracking of selected studies was processed.

2.3. Inclusion criteria

The articles included were based on the following criteria: (1) Studies must be interventional studies, either randomised or non-randomised trials; (2) published as a full paper in English; (3) study participants were not limited to only healthy individuals (i.e., included patients with at least one health condition); (4) the interventions being evaluated were predominantly DWR (≥ 50%) based or combined with passive treatments (e.g. education, or stretching); and (5) outcomes included cardiorespiratory measures (e.g. VO_2max, maximal heart rate, blood pressure, ventilatory threshold), physical function related to running or walking (e.g. timed test, endurance test, or functional test), or health-related quality of life (validated questionnaires or assessment tools) (Appendix 2).

2.4. Exclusion criteria

The studies were excluded if (1) the study designs were non-experimental (e.g. descriptive or exploratory studies) and or cross-sectional studies; (2) participants were under 18 years of age; (3) the studies on exercises other than DWR (e.g., general aquatic exercise, shallow water running, underwater treadmill) (Appendix 2).

1.5. Data extraction

Data were extracted independently by 2 reviewers (MK and BS) and included study design, baseline demographics of study participants (age, sex, health status, body weight, height, body mass index) , sample size, intervention details (frequency, intensity, duration, and length of intervention, DWR technique, instructions, supervision of exercise intervention, equipment used, pool temperature and room temperature), outcomes of interests (measurements on cardiorespiratory outcomes, physical functional outcomes and health-related quality of life) and measures of variance of the outcomes of interest. If there were missing data or data potentially included in error, the study authors were contacted via emails and asked to provide further information.

Quality and risk of bias assessment

Each included study was critically appraised by two independent reviewers (MK and BS) using selected items from a checklist based on Downs and Black (Appendix 3) (Downs & Black, 1998). In preliminary search of articles, relevant studies of multiple study designs were drawn. The Downs and Black quality assessment tool designed for randomized and non-randomized trials was chosen to assess the quality. Despite there were limitations, it has been shown to be an effective tool irrespective to the tool development and extensive domains covered (Jarde et al., 2012). Five subscales, including *reporting*, *external validity*, *internal validity in bias, confounding*, and *power* were assessed according to the characteristics of reviewed studies. Scores for this tool can be interpreted as follows: excellent (26-28); good (20-25); fair (15-19); and poor (≤14) (Hooper, Jutai, Strong, & Russell-Minda, 2008).

1.6. Data analysis

The outcomes of interest and program parameters in specific populations were pooled for data analysis using Cochrane Software Review Manager (RevMan software, version 7.1; The Nordic Cochrane Centre, Copenhagen, Denmark). The effects of DWR on the three domains of interest were analysed with standardized mean difference (SMD) calculations if the studies reported means and standard deviations (Cohen, 1988). SMD was significant only if the 95% CI did not include zero (Sedgwick, 2015). Effect size thresholds were classified as small effect (0.2), medium effect (0.5), or large effect (0.8) (Cohen, 1988). SMD and 95% confidence intervals (CI) were reported to demonstrate effects of DWR in comparison with a control group (without exercise or other interventions such as land-based training). Meta-analyses were not completed due to the clinical diversity in methodology and risk of bias in individual studies. Therefore, results are presented as effect sizes only.

Results

1.1. Selection of studies

Following an initial search identification of 1416 articles, 11 articles were included in the review (Figure 1) for quantitative synthesis.

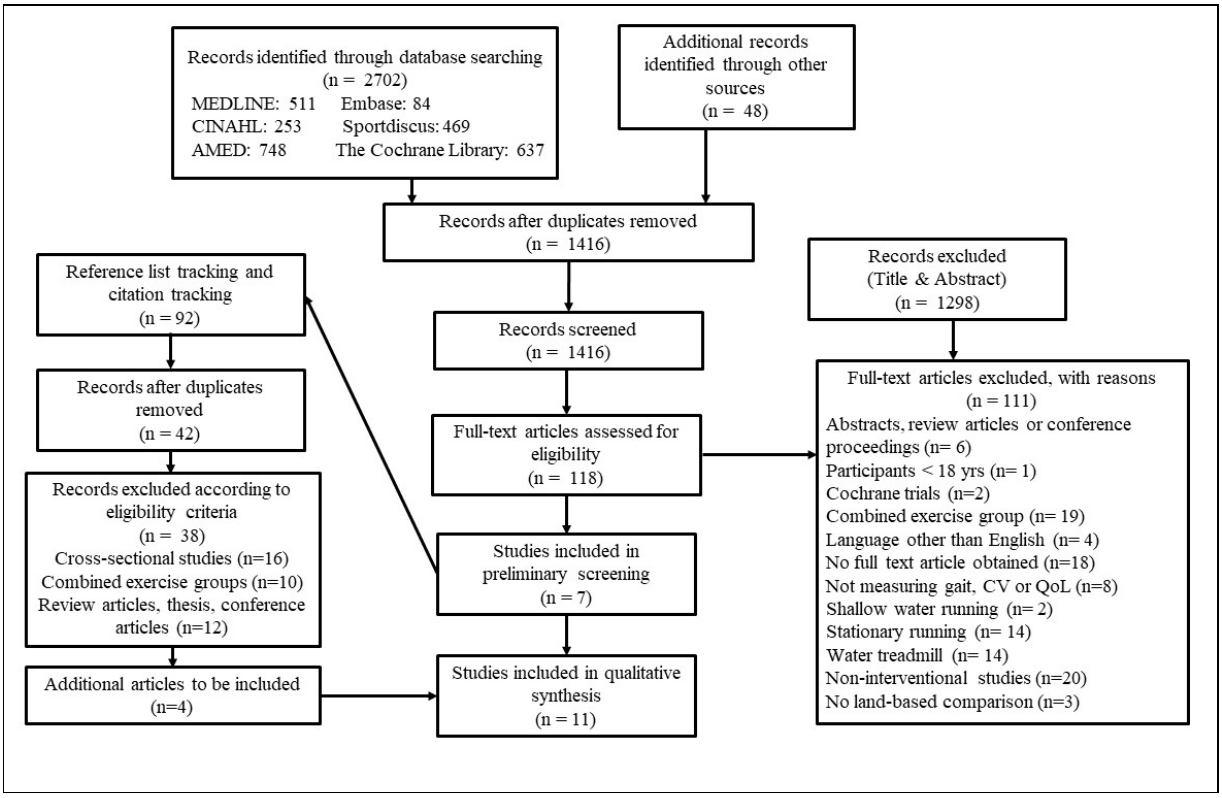


Figure 1. PRISMA flow diagram of selection process

1.2. Study Quality

Of the 11 included studies, seven studies were randomized controlled trials (RCT)s (Alberti et al., 2017; Assis et al., 2006; G. Broman et al., 2006; Colato et al., 2017; Cuesta-Vargas et al., 2012; K. Davidson & L. McNaughton, 2000; Eyestone et al., 1993; Kanitz et al., 2019; McKenzie & McLuckie, 1991; Michaud et al., 1995; Wilber et al., 1996), two were longitudinal studies (Colato et al., 2017; K. Davidson & L. McNaughton, 2000), and two were quasi-experiment studies (Michaud et al., 1995; Wilber et al., 1996).

Four studies investigated physically active or trained adults (Eyestone et al., 1993; Kanitz et al., 2019; McKenzie & McLuckie, 1991; Wilber et al., 1996), three studies investigated physically inactive or obese women (G. Broman et al., 2006; Colato et al., 2017; K. Davidson & L. McNaughton, 2000), two studies investigated healthy or community dwelling elderly (Alberti et al., 2017; Broman et al., 2006), and one study investigated populations with low-back pain (Kanitz et al., 2019). Study participant numbers varied from 10 to 60, with a total of 287 participants in the review (Table 1).

As for the comparison group, five studies included a control group that did not participate in any exercise (Alberti et al., 2017; G. Broman et al., 2006; Colato et al., 2017; Cuesta-Vargas et al., 2012; Michaud et al., 1995), and six studies included a land-based exercise group (Assis et al., 2006; K. Davidson & L. McNaughton, 2000; Eyestone et al., 1993; Kanitz et al., 2019; McKenzie & McLuckie, 1991; Wilber et al., 1996). Among them, two studies matched land exercise time to the DWR, having identical total 45 sessions over the three weeks period (Assis et al., 2006; Cuesta-Vargas et al., 2012). The rest of the studies varied in exercise intensity and sessions (Alberti et al., 2017; G. Broman et al., 2006; Ana Carolina Kanitz et al., 2019; Colato et al., 2017; Davidson & McNaughton, 2000; Eyestone et al., 1993; Michaud et al., 1995; McKenzie & McLuckie, 1991; Wilber et al., 1996).

3.3 ***Deep Water Running Intervention characteristics***

The duration of DWR interventions varied from 30 to 70 minutes, 2 to 5 sessions per week, lasted between 3 to 18 weeks and compliance rate ranged from 80% to 100% (Table 2). Of the 11 studies, 10 studies had participants performing DWR with the assistance of flotation devices (Alberti et al., 2017; Assis et al., 2006; G. Broman et al., 2006; Colato et al., 2017; Cuesta-Vargas et al., 2012; K. Davidson & L. McNaughton, 2000; Eyestone et al., 1993; Kanitz et al., 2019; McKenzie & McLuckie, 1991; Michaud et al., 1995; Wilber et al., 1996). Only in the study of McKenzie & Mcluckie (1991), participants were asked not to touch the bottom of the swimming pool without wearing flotation devices during DWR.

All the studies instructed participants to perform running in the water, there were variations in body positions during DWR. Four studies required participants to maintain a vertical body position during DWR (Assis et al., 2006; Eyestone et al., 1993; Kanitz et al., 2019; McKenzie & McLuckie, 1991). Davidson & McNaughton (2000) allowed participants to perform DWR in a vertical or slightly forward learning position, while Broman et al., (2006) and Michaud et al. (1995) instructed participants to run in a slightly forward bending position. Three studies did not specify the running positions adopted (Alberti, et al., 2017; Cuesta-Vargas et al., 2012; Colato et al., 2017, Wilber et al., 1996).

	Davidson, K., & McNaughton,	Kanitz et al.	Colato et al.	Assis et al.	Broman et al.	Cuesta-Vargas et al.	Alberti et al.	Michaud, T. J. et al.	Mckenzie et al.	Wilbur et al.	Eyestone et al.

Table 1. Study Design of included studies

	L.										
Number of subjects (M/F)	10 (0/10)	14 (7/7)	20 (0/20)	60 (0/60)	29 (0/29)	58 (25/33)	19 (0/19)	17 (2/15)	12 (12/0)	16 (16/0)	32 (32/0)
Age (years old) of respective groups	22.6 ± 3.4	DWR: 39 (95% CI: 31-47) LWR: 40 (95% CI: 36-50)	DWR: 48.81 ± 12.87 Control: 49.9±10.5	DWR: 43.96 ± 10.28 LBE: 44.04 ± 8.87	DWR: 69.0 ± 4.0 Control: 69.8 ± 3.5	DWR: 38.6 ± 12.2 Control: 37.8 ± 13.2	DWR: 64.33±4.24 Control: 64.40±4.22	DWR: 32.6 ± 6.8 Control: N/A	23.9	32.5 ± 5.4	18-26
Subjects' characteristics	Untrained women	Physically active patients of both sexes with chronic low back pain	Overweight obese women	Sedentary women with fibromyalgia	Healthy elderly women	Non-specific low back pain	Community dwelling elderly	Healthy sedentary	Competitive runners	Aerobically-trained male distance runners	Finished a 1.5 mile run in less than 10'45
Outcome measure of Physical Functions	/	/	/	/	/	/	4MWT, 6MWT, 10MWST FTSST TUGT	/	Time to fatigue	/	2 mile run time
Outcome measure of Cardiovascular	VO_2max	VO_2peak, VO_2Vt2	VO_2max	HR, VO_2max	HR (rest+test) BP (rest+test) Peak VO_2	/	/	VO_2max	VO_2max	VO_2max, Ventilator threshold	VO_2max
Outcome measure of Quality of life	/		/	SF-36	/	SF-12	/	/	/	/	/
Study Design	L	R	L	R	R	R	R	Q	R	Q	R

Note: 4MWT: 4-meter walk test; 6MWT: 6-meter walk test; 10MWST: 10-meter walking speed test; FTSST: 5 times sit to stand test; TUGT: Timed up and go test; L: Longitudinal; Q: Quasi-experimental; R: Randomized Controlled Trial

Table 2. Intervention Design of included studies

	Davidson, K., & McNaughton, L. 2000		Kanitz et al. 2019		Colato et al. 2016		Assis et al. 2006		Broman et al. 2006		Cuesta-Vargas et al. 2012		Alberti et al. 2017		Michaud et al. 1995		Mckenzie et al. 1991		Wilbur et al. 1996		Eyestone et al. 1993		
Nature of groups in respective studies	DWR	RR	DWR	LWR	DWR	Control	DWR	LBE	DWR	Control	DWR+GP	GP	DWR	Control	DWR	Control	DWR	LWT	DWR	TR	DWR	C	RR
Number of subjects	5	5	7	7	11	9	26	26	18	11	29	29	16	14	10	7	6	6	8	8	10	11	11
Supervision	✓		N/A		N/A		✓	2 PT	N/A		✓		✓		N/A		N/A		N/A		N/A		
Adverse effects %	N/A		N/A		N/A		10	16	✗		N/A		N/A		N/A		N/A		N/A		N/A		
Drop-outs	0	0	3 (30%)	3	N/A		4	4	3	2	3	4	7	4	6		N/A		1	1	N/A		
Pool temperature	22 - 25ºC		N/A		28ºC		28-31°C		27°C		N/A		28-30°C		27-29ºC		N/A		27ºC		N/A		
Water depth	N/A		N/A		1.7m		N/A		N/A		2.15m		1.35m		Diving pool		Deep end of summing pool		N/A		Diving pool		
Floating device	✓		✓		✓		✓		✓		✓		✓		✓		✗		✓		✓		
Compliance %	96	94	83	80	N/A		Excellent (no stats)		100		N/A		N/A		100		N/A		96	98	N/A		
Program time (weeks)	4		12		12		15		8		15		18		8		3		6		6		
Session time (mins)	50		45		70		60		48		30		50		40 - 70		30		30 - 60		20 - 30		
Sessions per week	3		2		3		3		2		3		2		3		5		5		Week 1: 3 Week 2: 4 Week 3-6: 5		
Total number of sessions	12		24		36		45		16		45		36		24		15		30		27		
Warm up	N/A		✓		✓		✓		✓		N/A		✓		✗		N/A		N/A		N/A		
Cool down	N/A		✓		✓		✓		✓		N/A		✓		✗		N/A		N/A		N/A		

Note: C: Cycling; DWR: Deep Water Running; RR: Road Running;; LWR: Land Walking/Running; LBE: Land-based Exercises ; GP: General Practice Consisting of Advice and Education about Exercise ; TR: Treadmill Run; WT: Water Training; ✓: Included; ✗: Not Included; N/A: Not Available.

3.4. Quality assessment

Of the 11 articles, all studies clearly described the objectives and main outcomes which were reliable and valid (Table). Nine out of 11 studies failed to report concealment of allocation (Alberti et al., 2017; Assis et al., 2006; G. Broman et al., 2006; Colato et al., 2017; K. Davidson & L. McNaughton, 2000; Eyestone et al., 1993; McKenzie & McLuckie, 1991; Michaud et al., 1995; Wilber et al., 1996). Only two studies reported on blinding of outcome assessors or intention-to-treat analysis (Assis et al., 2006; Kanitz et al., 2019).

Table 3. Quality Assessment

Subscale	Items	Davidson, K., & McNaughton, L	Kanitz et al.	Colato et al.	Assis et al.	Broman et al.	Cuesta-Vargas et al.	Alberti et al.	Michaud et al.	Mckenzie et al.	Wilbur et al.	Eyestone et al.
Reporting	*1. Hypothesis/aim/objective clearly described*	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	2. Main outcomes clearly described	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	3. Characteristics of the patients clearly described	N	Y	Y	Y	Y	Y	Y	N	N	Y	N
	4. Intervention and comparison group clearly described	Y	Y	N	Y	N	N	N	Y	Y	Y	Y
	5. Distributions of principal confounders in each group of subjects clearly described	N	Y	Y	Y	Y	Y	Y	N	Y	N	N
	6. Main findings clearly described	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y
	7. Estimates of the random variability for the main outcomes provided	N	Y	Y	Y	Y	Y	Y	N	Y	Y	Y
	10. Actual p values reported for main outcomes	Y	N	Y	Y	N	Y	Y	N	N	N	Y
External validity	*11. Subjects asked to participate represented the population*	N	N	Y	Y	Y	Y	N	N	N	N	N
	12. Subjects prepared to participate represented the population	N	N	N	N	N	N	N	N	N	N	N
Internal validity - Bias	*15. Blinded outcome assessment*	N	Y	N	Y	N	N	N	N	N	N	N
	18. Appropriate statistical tests performed	Y	Y	Y	Y	Y	Y	Y	N	N	Y	Y
	20. Accurate outcome measure used (reliable and valid)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Internal validity - Confounding	*23. Subjects randomized to intervention groups*	N	Y	N	Y	Y	Y	N	N	Y	N	Y
	24. Concealed allocation from subjects and investigators	N	Y	N	N	N	Y	N	N	N	N	N
	26. Losses to follow-up taken into account	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N
Power	*27. Power calculation*	N	Y	Y	Y	N	Y	Y	N	N	N	N

Outcomes

Among 11 included studies, three studies investigated physical functional outcomes (functional mobility and balance tests) or walking (Alberti et al., 2017; Eyestone et al., 1993; McKenzie & McLuckie, 1991), nine studies investigated cardiorespiratory fitness (Assis et al., 2006; G. Broman et al., 2006; Colato et al., 2017; Davidson & McNaughton, 2000; Eyestone et al., 1993; Ana Carolina Kanitz et al., 2019; McKenzie & McLuckie, 1991; Michaud et al., 1995; Wilber et al., 1996), and three studies investigated the quality of life (Assis et al., 2006; Cuesta-Vargas et al., 2012; Ana Carolina Kanitz et al., 2019).

3.5.1. Cardiorespiratory fitness

Peak Heart rate (HR peak)

Three studies evaluated the changes in HR peak (Broman et al., 2006; Michaud et al., 1995; Wilbur et al., 2017) but did not report means and SD therefore SMDs were unable to be calculated.

Maximal Aerobic Capacity (VO_2max)

Deep Water Running Versus Control (no active exercise)

One study, Broman et al. (2006) found a significant large effect (SMD= -1.28) of DWR in improving VO_2 max. In contrast, in the other study, Michaud et al. (1995) observed no significant difference in effect of DWR for improving VO_2 max when compared to no exercise. (Figure2)

	DWR			No active exercise			Std. Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	IV, Fixed, 95% CI
2.2.1 Untrained healthy population							
Broman 2006 (Cycle ergometer, mL/kg/min)	−27.2	2.1	15	−23.3	4	9	−1.28 [−2.20, −0.37]
Michaud 1995 (Treadmill test, L/min)	−2.49	0.68	10	−1.99	0.36	7	−0.83 [−1.84, 0.19]

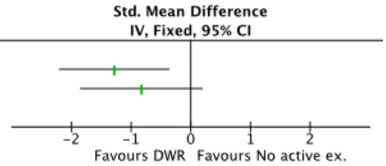


Figure 2. Standardized mean difference (95% CI) for the effect of DWR compared with No active exercises on VO_2 max

Deep Water Running Versus Land Training

Similar effects were found from five individual studies between DWR and land-based training in improving VO_2max (Davidson & McNaughton, 2000; Eyestone et al., 1993; Kanitz et al., 2019; McKenzie & McLuckie, 1991; Wilber et al., 1996) (Figure 3).

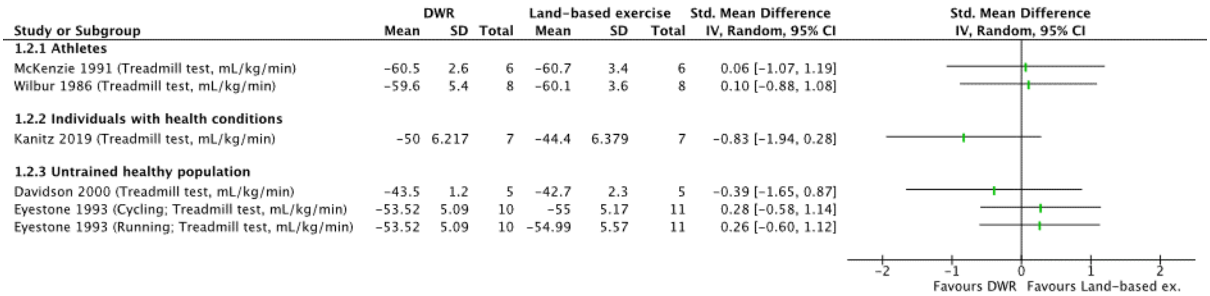


Figure 3. Standardized mean difference (95% CI) for the effect of DWR compared with land trainings on VO_2 max

3.5.2. Physical function

Deep Water Running Versus Control (no active exercise)

One study, Alberti et al. (2017) evaluated physical function between DWR group and control group with three walking tests (4MWT, 6MWT, 10MWST) and two functional mobility and dynamic balance tests Five Times Sit to Stand Test (FTSST) and Timed Up and Go Test (TUGT). Large effect sizes of DWR were observed in the three walking tests (SMD = −2.39 to -1.33). Effect sizes from revealed no significant effect of DWR compared to no active exercise for FTSST and TUGT, there were mean improvements favouring the DWR group in this study (Figure 4).

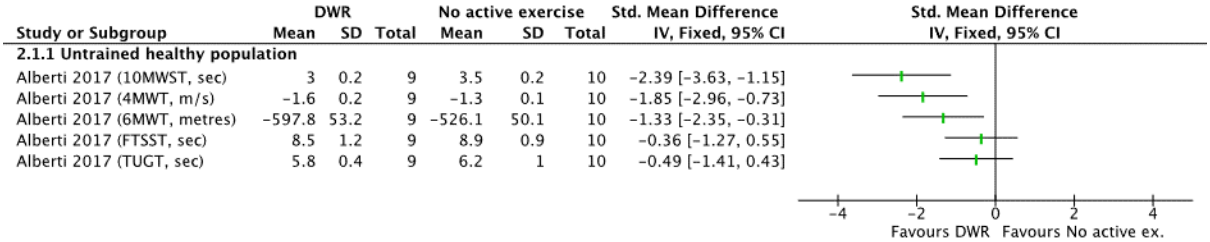


Figure 4. Standardized mean difference (95% CI) for the effect of DWR compared with No active exercises on physical function outcomes

Deep Water Running Versus Land Training

Two studies reported on running outcomes with similar effects on physical function between DWR and land training groups (Figure 5). Mckenzie et al. (1991) found a similar effect for the time to fatigue running on the treadmill between DWR and land running groups. Eyestone et al. (1993) found a similar effect of DWR and both land-running and cycling for two mile run

time.

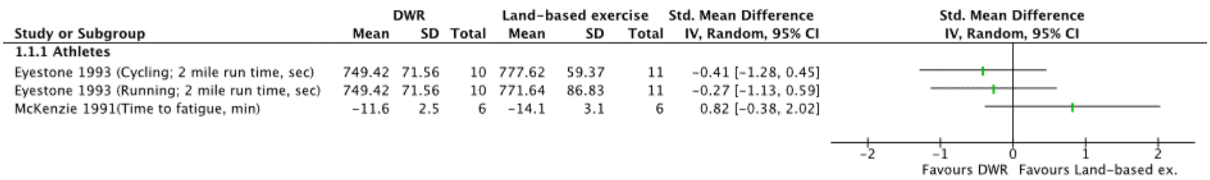


Figure 5. Standardized mean difference (95% CI) for the effect of DWR compared with Land trainings on physical function outcomes

3.5.3. Quality of Life

Deep Water Running Versus Control (no active exercise)

One study measuring quality of life, Cuesta-Vargas et al., (2012) found a large effect of DWR (SMD 2.01-3.23) in both mental and physical subsets of data in improving QoL compared with the no exercise control group (Figure 6)

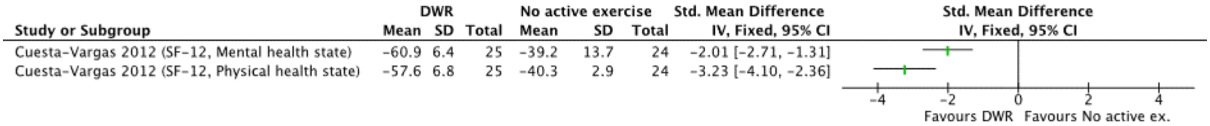


Figure 6. Standardized mean difference (95% CI) for the effect of DWR compared with No active exercises on quality of life

Deep Water Running Versus Land Training

Two studies found similar effects between DWR and land training in improving QoL (Assis et al., 2006; Kanitz et al., 2019). (Figure 7)

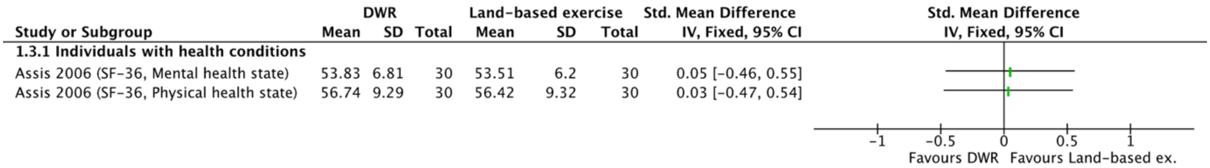


Figure 7. Standardized mean difference (95% CI) for the effect of DWR compared with land trainings on quality of life

Discussion

This systematic review found similar effects of DWR and land-training in improving VO_2max in active individuals, sedentary participants and people with chronic health conditions. Similar effects were also found in physical function and QoL outcome measures for water-based and land-based training, the conclusions for these domains are weaker due to the smaller number of studies. There were few studies reporting the effect of DWR to no-exercise across all three domains of interest with mixed results. Heterogeneity and reporting of interventions as well as varied types of participants limits stronger conclusions.

The results of the review reflect that DWR can be an equally effective form of exercise to maintain or improve cardiorespiratory fitness when compared with land-based training. VO_2 max is considered to be the highest value of VO_2 attained upon an exercise stress test (Whipp et al., 1990). The test of VO_2 max is designed to bring the subject to the limit of exhaustion, a gold standard measure of cardiorespiratory fitness (Keteyian et al., 2008). VO_2 max can be influenced by a restriction in chest expansion when inspiratory muscle contractions are unable to equal or overcome the force of hydrostatic pressure under immersion (Carter et al., 2003) . It is suggested that central hypervolaemia or the shift of blood into the chest cavity reduces lung volumes (Becker 2009) as well as reducing lung compliance and promoting gas trapping, which narrows the airways (Lau et al., 2019) Compression of both the abdomen (in turn pushing up the diaphragm) (Hall 1990) and compression of the chest wall itself by water also increases the work of breathing (Hall 1990, Becker 2009).With such unique physiological adaptations during aquatic training, minute ventilation and breathing frequency could potentially be increased when compared with land-based training with an equivalent exercise intensity (Haynes et al., 2020a). Additionally, it is suggested that by performing movements in DWR, the physiological effects of water immersion contribute to a reduction of joint loading while the tactile, thermal stimulation and drag force may enhance the joint proprioception, body balance and muscle strength (Assis et al., 2006).Since aquatic based maintenance of cardiovascular conditioning offers a number of additional advantages over land-based training, It may be ideal for populations unable to exercise on land, or those who exclusively train on land, and desire to cross-train in water for rehabilitative purposes.

This review found that DWR is more responsive in untrained, sedentary healthy elderly populations (Broman et al., 2006; Michaud et al., 1995) than trained or physical active subjects (Davidson & McNaughton, 2000; Eyestone et al., 1993; Kanitz et al., 2019; McKenzie & McLuckie, 1991; Wilber et al., 1996). One possible explanation of DWR improved aerobic capacity in sedentary individuals and elderly population for whom were of low-initial fitness (T. Reilly, C. N. Dowzer, & N. Cable, 2003a). Of note, such a magnitude of improvement likely has significant clinical relevance in sedentary healthy elderly populations than trained or physical active subjects. Given that cardiovascular function decreases with primary ageing and that cardiorespiratory fitness declines steadily in sedentary individuals at a rate of approximately 1% per year after the third decade of life (Posner et al., 1995).Such finding is in agreement with previous study done by Reilly et al. (2003) (Reilly et al., 2003b), and other systematic reviews done by Chu and Rhodes (2001) (Chu & Rhodes, 2001) as well as Jorgic et al. (2012) (Jorgic, Milanovic, Aleksandrovic, Pantelic, & Daly, 2012). As such, cardiorespiratory fitness training, for instance DWR favours improvement of cardiorespiratory fitness have shown important clinical implications for the sedentary populations, particularly in individuals with advanced age.

A key component of aquatic-based training is carryover to land-function as well as broader quality of life improvements. One of the aims of this review was to analyze the effect of DWR to land-based walking and running. This review found a large effect for DWR in walking tests (4MWT, 6MWT and 10MWT) when compared with the no active exercise control groups but only one study reported this (Alberti et al., 2017). Similarly, little data on the effect of DWR on quality was found. When DWR was compared with no active exercise control group, DWR showed significant effect in physical health and mental health in SF-12 (Cuesta-Vargas et al., 2012) but in only one study. There is potential for QoL to be influenced via changes in physical function or to improve well-being by promoting relaxation, vasodilation, reduction in joint loading, and produce an analgesic effect. (Assis et al., 2006). DWR may also release cortisol and adrenaline into bloodstream, thus increasing pain threshold for those subjects who suffered from pain (Cuesta-Vargas et al., 2012) along with removal of metabolic waste and reduce nociceptors activation (Hall et al., 2008). More evidence is required to understand if DWR has enough of an effect to improve both land-based function or quality of life.

1.1. Study limitations

Although the synthesized evidence in this review is encouraging, this study has several limitations. Firstly, majority of the included studies had small sample sizes and recruitment of participants were from convenience sampling. This may increase the chance of committing a type II error and affect the results of efficacy regarding subjects' representativeness and generalizability. Secondly, heterogeneity of participants' characteristics also makes it difficult to draw definitive conclusions about clinical outcomes. Furthermore, meta-analysis was not completed due to the clinical diversity in study interventions, variations in methodology, and risk of bias in individual studies. For instance, there were wide range of DWR protocols and methods of measurements for main outcomes, that is, measurements used or methods in measuring VO_2max were distinct across some studies. It limits the ability of this review to conclude a recommended dosage of DWR for participants. As a result, limited numbers of studies on homogeneous groups of subjects have been conducted in this review. Lastly, the lack of a meta-analysis hinders a precise estimation of effect and a statistical analysis of DWR. More clinical trials in specific populations with larger sample sizes could help yielding a more solid conclusion and consistency on the effectiveness of DWR.

1.2. Future directions

Conducting correlational studies could have been useful with an application of biopsychosocial approaches to investigate relationships between measured outcomes and DWR with a larger and more homogenous group of participants. This could further justify clinical significance of DWR in specific group of populations. Additionally, the studies measured short-term effects immediately after DWR sessions. However, long-term effects should be considered after completion of intervention to evaluate the carry over effects. Currently, there are lack of evidence in participants' experience and perceptions towards DWR programs. Qualitative researches are therefore suggested to explore the attitudes, behaviours, beliefs, and satisfaction towards effects of DWR to further consider their exercise adherence in addition to quantitative data on both land-based function and QoL. It is also difficult to conclude an optimal dosages of training programme, therefore future investigations on programme intensity are warranted.

Conclusion

DWR appears to be comparable to land-based training with mixed results for effect compared to no exercise for improving cardiorespiratory fitness, physical functions and quality of life. The small number of studies and quality of the evidence limits further conclusions. To further understand the potential benefits of DWR, future research is needed in developing an effective prescription for the targeted populations.

Conflicts of interest

None declared.

Appendix 1. Search Strategy

Databases	Main search terms
SPORTDiscus, MEDLINE, CINAHL, AMED, Embase, and The Cochrane Library	'deep water run*' or 'deep water jog*' or 'aqua jog*' or 'aqua run*' or 'running under water' or 'running in water' or 'jogging under water' or 'jogging in water'.

Appendix 2. Eligibility Criteria

Inclusion Criteria

Study Characteristics

- Adults (aged above 18)
- Longitudinal studies (either randomised or non-randomised trials)
- Studies must include comparison group(s)
- Measurable outcomes in either cardiovascular capacity, gait performance or quality of life

Report Characteristics

- Written in English
- In full text in peer-reviewed journals

Exclusion Criteria

Study Characteristics

- Non-experimental studies
- Cross-sectional studies
- Not DWR exercise
- Combined exercise or treatments in intervention group
- Measurable outcomes on biomechanics of gait, strength, balance, or anthropometric parameters

Report Characteristics

- Any reviews, unpublished articles

Appendix 3. Selected components from Downs and Black's Checklist for measuring study quality

Subscale	Items
Reporting	1. Is the hypothesis/aim/objective of the study clearly described?
	2. Are the main outcomes to be measured clearly described in the intro or methods section?
	3. Are the characteristics of the patients included in the study clearly described?
	4. Are the interventions of interest clearly described?
	5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?
	6. Are the main findings of the study clearly described?
	7. Does the study provide estimates of the random variability in the data for the main outcomes?
	10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?
External validity	11. Do the subjects asked to participate represent the population?
	12. Do the subjects in the study represent the populations?
Internal validity -Bias	15. Was an attempt made to blind those measuring the main outcomes of the intervention?
	18. Were the statistical tests used to assess the main outcomes appropriate?
	20. Were the main outcome measures used accurate (valid and reliable)?
Internal validity -Confounding	23. Were study subjects randomised to intervention groups?
	24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?
	26. Were losses of patients to follow-up taken into account?
Power	27. Did the study have a power calculation?

Appendix 4 Benefits of DWR among three target populations

Population	Purpose	Benefits of DWR	Evidence
Aerobically trained athletes			
- Injured runners (Eyestone et al., 1993; Wilber et al., 1996)	Rehabilitation	- Reduces deconditioning - Accelerates rehabilitation while maintaining high aerobic conditioning - Maintains aerobic capacity while reducing mechanical load on lower limbs	***Findings from included studies*** - Runners maintained similar VO_2max and 2-mile run time with a 6-week DWR training for rehabilitation(Eyestone et al., 1993). ***Findings from previous publications*** - Simulates running in water without incurring possible harmful effects due to weightbearing - Reduces mechanical load on lower limbs(García Tenorio & Arriaza Loureda, 2001)
- Uninjured athletes (Eyestone et al., 1993; Michaud et al., 1995; Wilber et al., 1996)	Supplementary training Alternative training Strength and conditioning training	- Minimizes likelihood of getting injured - As light recovering workout following high intensity trainings - As recovery from delayed-onset muscle soreness brought by land-based training - Prevents detraining **Application:** - Cross training	***Findings from included studies*** - Non-injured athletes showed no significant difference in VO_2max following 6-week DWR in comparison with treadmill running and land-based running(Wilber et al., 1996) - DWR workout served as an effective alternative training(Eyestone et al., 1993; Michaud et al., 1995; Wilber et al., 1996) ***Findings from previous publications*** - DWR serves as light recovering workout following high intensity trainings,(Eyestone et al., 1993; Michaud et al., 1995; Wilber et al., 1996) - DWR as cross training for runners(K. Davidson & L. McNaughton, 2000; Michaud et al., 1995)
Individuals with health conditions			
- Obesity (American College of Sports Medicine, 2014; Colato et al., 2017;	Aerobic training	- Improves body composition, blood pressure, and fitness status - Weight reduction	***Findings from included studies*** - Increases energy expenditure for weight reduction(Colato et al., 2017) - Reduces mechanical loads on joints(Colato et al., 2017) ***Findings from previous publications***

Population	Purpose	Benefits of DWR	Evidence
Gobbi et al., 2020)			- ACSM suggested aquatic aerobic exercise like DWR as favourable choice of aerobic training: (American College of Sports Medicine, 2014; Colato et al., 2017) - Enhances functional capacity with lower injury risk - Increases adherence to exercise training for inactive individuals
Other health conditions: - Chronic low back pain - Fibromyalgia	Non-pharmacologic pain management Safe therapeutic exercise	- Activates different trunk muscles - Stabilization in lumbopelvic complex - Reduces pain - Reduces kinesiophobia - Affects quality of life - Provides safer environment - Gains in cardiovascular fitness ***Application:*** - Suitable for improving dynamic trunk stability and pain reduction	***Findings from included studies*** - DWR significantly reduce VAS in subjects with fibromyalgia and low back pain with moderate to high effect size(Assis et al., 2006; Cuesta-Vargas et al., 2012; Kanitz et al., 2019). - Properties in warm aquatic environment promotes relaxation, vasodilation and analgesic effects for fibromyalgia patients(Assis et al., 2006). ***Findings from previous publications*** - Buoyancy lessens axial load on spine, while unstable aquatic environment acts as a challenging component to maintain trunk stability(Bayraktar et al., 2016). - Feet non-contacting floor during DWR fosters coordination and stabilization in lumbopelvic complex(Bayraktar et al., 2016). - Exercise helps to release cortisol and adrenaline into bloodstream and therefore increase pain threshold(Cuesta-Vargas et al., 2012). - Hydrostatic pressure exerted on skin triggers mechanoreceptors that helps blocking nociceptors(Mooventhan & Nivethitha, 2014). - Immersion in warm water speeds up body metabolism, for faster

Population	Purpose	Benefits of DWR	Evidence
			removal of metabolic waste and activates the nociceptors(Hall et al., 2008).
Untrained population			
- Community dwelling elderly	Aerobic training Strength training	- Increases cardiac output and stroke volume by aquatic immersion - Allows interval aerobic exercise at high loads with low risk of injury	***Findings from included studies*** - Elderly subjects showed increased power in lower extremity and better gait performance and function(Alberti et al., 2017). - improvements in submaximal and maximal aerobic power among elderly women after 8-week high intensity interval DWR with vest(G. Broman et al., 2006). ***Findings from previous publications*** - Gains in aerobic fitness for elderly(B. Jorgic et al., 2012). - Aquatic immersion facilitates central shift of blood volume due to hydrostatic pressure(Kaneda et al., 2008; Reilly et al., 2003). - Flotation vest keeps the body in upright position and avoid contact with the floor(A. C. Kanitz et al., 2015).

- Sedentary healthy population	Aerobic training	- Gains in cardiovascular fitness
		
		Application:
		- Suitable for low impact and less thermally stressful training
			Findings from included studies
			- Improvement in VO2max in DWR almost twice as much as treadmill running(Michaud et al., 1995)
			- DWR as non-weightbearing activity adopted different muscle recruitment pattern: (Michaud et al., 1995)
			  - exhausting less work on large muscle groups in lower extremity
			  - more work for upper extremity during arm and shower movements

ACSM: American College Sports Medicine

Figure and Table Legends

Figures

Figure 1 PRISMA flow diagram of selection process

Figure 2 Standardized mean difference (95% CI) for the effect of DWR compared with No active exercises on VO_2 max

Figure 3 Standardized mean difference (95% CI) for the effect of DWR compared with land trainings on VO_2 max

Figure 4 Standardized mean difference (95% CI) for the effect of DWR compared with No active exercises on physical function outcomes

Figure 5 Standardized mean difference (95% CI) for the effect of DWR compared with Land trainings on physical function outcomes

Figure 6 Standardized mean difference (95% CI) for the effect of DWR compared with No active exercises on quality of life

Figure 7 Standardized mean difference (95% CI) for the effect of DWR compared with land trainings on quality of life

Tables

Table 1 Study Design of included studies

Table 2 Intervention Design of included studies

Table 3 Quality Assessment

9.3 Information sheet, consent form of Chapter 5

THE HONG KONG POLYTECHNIC UNIVERSITY
香港理工大學

INFORMATION SHEET

Validity and Reliability of the Portable Metabolic Analyzer PNOE for cardio-metabolic measurement in healthy adults

Research Team Members:

Professor Shamay Ng	Professor	Department of Rehabilitation Sciences, PolyU
Dr. Billy So	Assistant Professor	Department of Rehabilitation Sciences, PolyU
Ms Manny Kwok	PhD student/ Musculoskeletal Physiotherapist	Department of Rehabilitation Sciences, PolyU
Mr. Matthew Lai	Research Assistant	Department of Rehabilitation Sciences, PolyU

Study Background:

Cardio-metabolic measurement can be assessed by metabolic carts and used in early detection of cardio-metabolic risk. In general, cardio-metabolic variables are assessed and evaluated by metabolic carts limited to a clinical or laboratory setting, however the process can be cumbersome, labor intensive and expensive. This prevents the widespread application of the assessment and highlight the necessity to design a valid and reliable cardio-metabolic measurement unit which will be portable and practical to use.

A portable metabolic system PNOE allows measurement to be done without confining to laboratory settings. It enables a measurement of metabolic costs, for instance, oxygen consumption (VO2), energy expenditure (EE), respiratory ratio (RR) and metabolic equivalent (MET). However, the validity and reliability in evaluating cardio-metabolic variables when compared to reference standard COSMED-K5 is still unknown. Therefore, it is crucial in order to address the accuracy and reliability of the PNOE in measuring cardio-metabolic outcomes (VO2, RR, MET, EE) in the expired air of healthy individuals.

If you are healthy and aged between 18-35, you are cordially invited to participate in this study.

Study procedure:

The study will be conducted at gait and motion laboratory (ST004) located at the Hong Kong Polytechnic University, Hung Hom. The session will last for 45 minutes. Participants are required to perform an interview for screening and familiarization before signing off an informed consent to the study. Subjects will be screened by the International Physical Activity

Questionnaire, measuring their resting heart rate, Blood pressure (BP), body mass and height. All subjects will perform and complete a familiarization session at the lab a week before the study. The usage of mouthpiece and Hans Rudolph valve will also be practiced. They are instructed to wear PNOE and COSMED K5 with facemask covering the nose and mouth which offers freedom of movement during testing. All participants receive feedback and instruction during the trials.

Participants will follow a 5-stages treadmill walking incremental protocol during assessment. Each stage lasts for three minutes, starting from 2.7km/h inclines at 10% and increasing by 0.8-1.4 km/h and 2 % inclination respectively in according to the protocol until exhaustion, as will be estimated by the visual inspection of the heart rate response and the Borg Scale score. Cardio-metabolic variables of VO2, RR, MET and EE will be calculated using values from the final minute of each three-min stage. Measurements will be made in-line with the COSMED – K5 and PNOE while COSMED-K5 system acts as a reference standard, The reliability of the PNOE device will be assessed by measuring cardio-metabolic variables on the selected subjects with the same walking protocol, on separate days. The same experimental setup will be followed in both visits. The test will be performed at the same time of day and subjects are instructed to wear similar clothing and the same walking shoes for the two trials.

<u>Study benefit:</u>

It is hoped that the findings of the proposed study can advance the understanding of the validity and reliability of PNOE when compared with portable metabolic cart COSMED-K5. This knowledge will aid to provide an alternative portable device in measuring cardio-metabolic variables in an accurate and reliable manner.

<u>Possible side effect, risk or discomfort in the study</u>

The test should not result in any serious discomfort, but participants may experience muscle fatigue or post-exercise soreness at 1-2 days after the training sessions. Adequate rest after the training can achieve a full recovery.

<u>Automaticity and confidentiality:</u>

Your participation in this study is completely voluntary. You have every right to withdraw from the study before or during the study without penalty of any kind. All information related to the participants will remain confidential, and will be identified by codes only known to the research team.

If you would like to get more information about this study, please contact Ms Manny Kwok on 9206 . If you have any complaints about the conduct of this research study, please do not hesitate to contact Ms Vangie Chung, Secretary of Department Research Committee on 27664329.

Thank you for your interest in participating in this study.

Dr. Billy So

Principal Investigator

香港理工大學
康復治療科學系
研究資料

研究項目: 便携式代谢分析儀器 PNOE 對健康成人在心臟代謝測量度中的有效性和可靠性比較

研究人員:

伍尚美教授	物理治療教授	香港理工大學康復治療科學系
蘇俊龍博士	物理治療助理教授	香港理工大學康復治療科學系
郭文瑩女士	博士二年級生/ 物理治療師	香港理工大學康復治療科學系
黎倬堯先生	研究助理	香港理工大學康復治療科學系

研究項目簡介:

近年的科研建議可以通過代謝車進行及評估心臟代謝，這樣可以及早檢測心臟代謝風險。一般來說，心臟代謝大多數局限於臨床或實驗室環境進行評估，但是該過程可能造成很多麻煩，而且十分昂貴，這樣更阻礙了評估的廣泛應用，所以設計一個有效可靠，便攜式和實用的心臟代謝測量儀器卻是十分必要。

便攜式代謝分析儀器 PNOE 允許在不局限於實驗室環境的情況下進行測量。它可以測量心臟代謝，例如，氧氣消耗（VO2），能量消耗（EE），呼吸比率（RR）和代謝當量（MET）。然而，與參考標準 COSMED-K5 相比，便攜式代謝分析儀器 PNOE 評估心臟代謝的有效性和可靠性仍然未清楚。因此，此項研究針對 PNOE 對健康成人在心臟代謝測量（VO2，RR，MET，EE）中的有效性和可靠性作出重要的比較。

若你是年齡介乎18-35歲的健康人士，我們誠意邀請你參與此項研究。

研究方法和程序:

測試將在香港理工大學步態分析實驗室進行，整個程序和評估將持續 45 分鐘。參與者在簽署研究的同意書之前，必須進行面談篩選和熟習該項測試。受試者將通過 International

Physical Activity Questionnaire 問卷進行篩查，並測量他們的靜止心率，血壓（BP），體重和身高。
所有受訪者將在研究前一周在實驗室進行並熟悉測試過程和練習使用的呼吸面罩。研究人員將為你配戴 PNOE 和 COSMED- K5，配備呼吸面罩以收集你呼出的氣體。

在評估期間，參與者將遵循 5 個階段的跑步機增量步行來進行測試。每個階段持續三分鐘，從 2.7 公里/小時開始，傾斜度為 10%，按步增加 0.8-1.4 公里/小時和 2%傾斜度，直到筋疲力盡為止。在測試中，我們將監視並記錄你的 RPE Borg 6-20 勞累率的主觀評分。我們會為你量度 VO2，RR，MET 和 EE 等心臟代謝作準確測試，測試將與 COSMED-K5 和 PNOE 一致進行，而 COSMED-K5 系統則作為參考標準。PNOE 設備的可靠性將通過在不同日期，使用相同的跑步機增量步行方案，用心臟代謝來作測試。兩次的測試中，實驗設置會是完全相同。測試將在一天中的同一時間進行，請你在兩次試驗中穿著相似的衣服和相同的步行鞋。

本研究項目的益處:

此項研究有助我們更深入促進對攜帶型PNOE與COSMED-K5的有效性和可靠性的理解。這些知識將有助於提供多一種便攜式代谢分析儀器的選擇，以準確又可靠的方式測量心臟代謝。

研究可能產生的副作用、危險、不適、處理方法:
有機會導致延遲性肌肉酸痛或疲倦乏力；訓練後作充足的休息可達到全面舒緩效果，酸痛一般在1-2日內消失。

自願參與性質:
本研究全屬自願參與性質，閣下有權利於任何階段退出或中止參與是次研究。閣下之任何決定將不會受到任何懲罰或不公平對待。

若你對是項研究有任何查詢或疑問，可致電 9206 ，與郭文瑩女士聯絡。如你對是項研究或研究員有任何投訴或建議，可致電 27664329，與部門研究委員會秘書鍾女士聯絡。

蘇俊龍博士 (首席研究員)

香港理工大學康復治療科學系

THE HONG KONG POLYTECHNIC UNIVERSITY
香港理工大學

CONSENT TO PARTICIPATE IN RESEARCH

Validity and Reliability of the Portable Metabolic Analyzer PNOE for cardio-metabolic measurement in healthy adults

Research Team Members:

Professor Shamay Ng	Professor	Department of Rehabilitation Sciences, PolyU
Dr. Billy So	Assistant Professor	Department of Rehabilitation Sciences, PolyU
Ms Manny Kwok	PhD student / Musculoskeletal Physiotherapist	Department of Rehabilitation Sciences, PolyU
Mr Matthew Lai	Research Assistant	Department of Rehabilitation Sciences, PolyU

I ________________________ hereby consent to participate in the captioned research conducted by Dr. Billy So Chun Lung.

I understand that information obtained from this research may be used in future research and published. However, my right to privacy will be retained, i.e. my personal details will not be revealed. This study abides the Declaration of Helsinki.

The procedure as set out in the attached information sheet has been fully explained. I understand the benefit and risks involved. My participation in the project is voluntary.

I acknowledge that I have the right to question any part of the procedure and can withdraw at any time without penalty of any kind.

If I would like to get more information about this study, I can contact Ms Manny Kwok at 9206 . If I have any complaints about the conduct of this research study, I can contact Ms Vangie Chung, Secretary of Department Research Committee at 27664329.

Name of Participant : ________________________ Date : ________________________

Signature of Participant : ________________________

____

Name of Witness : ____________________ Date : ____________________

Signature of Witness : ____________________

Name of Researcher : ____________________ Date : ____________________

Signature of Researcher : ____________________

THE HONG KONG POLYTECHNIC UNIVERSITY
香港理工大學

香港理工大學
康復治療科學系
研究資料

研究項目: 便携式代谢分析儀器PNOE 對健康成人在心臟代謝測量度中的有效性和可靠性比較

研究人員:

伍尚美教授	物理治療教授	香港理工大學康復治療科學系
蘇俊龍博士	物理治療助理教授	香港理工大學康復治療科學系
郭文瑩女士	博士二年級生/ 物理治療師	香港理工大學康復治療科學系
黎倬堯先生	研究助理	香港理工大學康復治療科學系

本人 ____________________明白是項研究的詳細情況，並願意參與由蘇俊龍博士開展的是項研究。本人的參與，是屬於自願性質。

本人明白在任何時間可以放棄及退出測試，而毋須給予任何理由。本人亦不會因為退出測試而受到任何處罰或不公平對待。

本人得悉並且明白參與是次研究所帶來的潛在危險。除有關研究之研究員，本人的個人資料不會展示給予與任何人。如未經本人的同意，本人的名字及照片並不會刊登於是項研究的任何發表佈告之中。這項研究是符合赫爾辛基宣言的原則。

本人若果對是項研究有任何查詢或疑問，可致電9206　　　，與郭女士聯絡。如對是項研究或研究員有任何投訴或建議，可致電27664329，與部門研究委員會秘書鍾女士聯絡。本人簽署後表示本人已收到此同意書副本乙份。

簽署(參與者)	:	______________________	日期	:	______________________
名稱(參與者)	:	______________________			
簽署(見證人)	:	______________________	日期	:	______________________
名稱(見證人)	:	______________________			
簽名(研究員)	:	______________________	日期	:	______________________
名稱(研究員)	:	______________________			

THE HONG KONG
POLYTECHNIC UNIVERSITY
香港理工大學

To	So Chun Lung (Department of Rehabilitation Sciences)		
From	Yee Kay Yan Benjamin, Delegate, Departmental Research Committee		
Email	benjamin.yee@	Date	28-May-2021

Application for Ethical Review for Teaching/Research Involving Human Subjects

I write to inform you that approval has been given to your application for human subjects ethics review of the following project for a period from 01-Jul-2021 to 30-Jun-2022:

Project Title:	Comparing Aquatic and Land Environment of High Intensity Interval Training (HIIT) on Oxygen Consumption, Heart Rate and perceived effort in healthy women
Department:	Department of Rehabilitation Sciences
Principal Investigator:	So Chun Lung
Project Start Date:	01-Jul-2021
Project type:	Human subjects (non-clinical)
Reference Number:	HSEARS20210522001

You will be held responsible for the ethical approval granted for the project and the ethical conduct of the personnel involved in the project. In case the Co-PI, if any, has also obtained ethical approval for the project, the Co-PI will also assume the responsibility in respect of the ethical approval (in relation to the areas of expertise of respective Co-PI in accordance with the stipulations given by the approving authority).

You are responsible for informing the PolyU Institutional Review Board in advance of any changes in the proposal or procedures which may affect the validity of this ethical approval.

Yee Kay Yan Benjamin

Delegate

Departmental Research Committee (on behalf of PolyU Institutional Review Board)

Page 1 of 1

9.4 Information sheet, consent form of Chapter 6

THE HONG KONG POLYTECHNIC UNIVERSITY
香港理工大學

INFORMATION SHEET

Effects of aquatic high intensity interval deep water training and land based high intensity interval training on cardio-metabolic health parameters and perceptual responses in women

Research Team Members:

Professor Shamay Ng	Professor	Department of Rehabilitation Sciences, PolyU
Dr. Billy So	Assistant Professor	Department of Rehabilitation Sciences, PolyU
Ms Manny Kwok	PhD student/ Musculoskeletal Physiotherapist	Department of Rehabilitation Sciences, PolyU

Study Background:

Physical inactivity associates with a reduction in cardio-metabolic health. Thus, identifying and reducing physical inactivity will have an essential effect on cardio-metabolic diseases prevention. Older women populations have higher cardio-metabolic risks and hence it has been recommended that exercise training at a higher intensity is beneficial to prevent associated ageing cardio-metabolic diseases.

Land High Intensity Interval Training (L-HIIT) can be defined as repeated bouts of vigorous but submaximal exercise that elicits ≥80% maximum heart rate [HRmax], interspersed with short periods of recovery. Although L-HIIT has been shown to improve cardio-metabolic health, some characteristics of this training performed on hard surface. are considered less appropriate for elderly populations due to barriers to exercise such as pain and movement difficulties. An aquatic environment is considered to be safe and efficient for physical training because of the physical properties of water. Aquatic High Intensity Interval Training (AHIIT) provides a lower weight bearing aerobic alternative to L-HIIT. Among aquatic exercises, Deep water running (DWR) has gained prominence in the scientific literature. DWR is performed with the aid of a floatation vest, which serves to keep the body upright and prevent feet from touching the bottom of the pool. This characteristic allows AHIIT to be applied in DWR by eliminating any impact with a reduced risk of injury (AHIIT-DWR).

In view of a growing application of AHIIT-DWR, the intention of this proposed study are 1. to design an AHIIT- DWR exercise protocol to optimize cardio-metabolic outcomes and perceptual responses 2.to investigate the effects of 8-week AHIIT-DWR or L-HIIT intervention performed at matched intensity on cardio-metabolic health outcomes and perceptual responses in older women

If you are female, healthy and aged ≥60 years, you are cordially invited to participate in this study.

Study procedure:

Participants will be randomly assigned to either the AHIIT-DWR or L-HIIT group. An incremental test in water or land will be performed prior the exercise interventions to confirm an individualized cadence required at a matched level of exercise intensity in each condition. The incremental test in water and on land will be carried out by DWR and treadmill running respectively. During the incremental test, gas exchange data will be obtained by a portable metabolic device PNOE.

For the AHIIT-DWR program, participants will be asked to wear a flotation vest to keep the feet without touching the pool floor. They will be given a cardiac frequency meter (Polar®, Electro, Oi, Finland) for heart rate control and monitoring. Ten 2-min bout of AHIIT-DWR at 80-90%HR max with 1-min active recovery at 50%HR max in between bouts will be performed. Participants will complete the session in 30-minute, twice a week for 8 weeks (a total of 16 sessions)

On the other hand, the L-HIIT exercise will be performed in a room of ambient temperature and relative humidity of 23°C and 50% respectively. The participants are instructed to perform ten 2-min bout of treadmill running at 80-90%HR max with 1-min active recovery at 50%HR max in between bouts. Participants will complete the session in 30-minute, twice a week for 8 weeks (a total of 16 sessions),

Both AHIIT-DWR and L-HIIT programs will be performed at the Spotlight Recreation Club, Hung Hum and the Gait and Motion Analysis Lab at the Hong Kong Polytechnic University (HKPU) respectively. The assessments will comprise a measurement of cardiorespiratory responses (oxygen capacity, oxygen pulse), metabolic outcomes (lipid profile, glucose and cholesterol) and perceptive responses. Of note, fingerstick blood sampling and small sample size (40μL) will be collected with a capillary tube for metabolic markers measurement. The assessment session will last for 30 minutes.

Study benefit:

It is hoped that this information will help us to understand the difference in cardio-metabolic and perceptive responses of AHIIT-DWR and L-HIIT in women. At the completion of the trial, the transferability of the movements between aquatic and land environments can provide clinical practitioners with a clear understanding of the roles of implications of water immersion for HIIT and optimize the clinical practice guidelines for practitioners.

Possible side effect, risk or discomfort in the study

The test should not result in any serious discomfort, but participants may experience muscle fatigue or post-exercise soreness at 1-2 days after the training sessions. Adequate rest after the training can achieve a full recovery.

Automaticity and confidentiality:

Your participation in this study is completely voluntary. You have every right to withdraw from the study before or during the study without penalty of any kind. All information related to the participants will remain confidential, and will be identified by codes only known to the research team.

If you would like to get more information about this study, please contact Ms Manny Kwok on 9206 . If you have any complaints about the conduct of this research study, please do not hesitate to contact Ms Vangie Chung, Secretary of Department Research Committee on 27664329.

Thank you for your interest in participating in this study.

Dr. Billy So

Principal Investigator

香港理工大學
康復治療科學系
研究資料

研究項目: 針對婦女在水上進行的高強度間歇性深水跑步訓練和陸地進行的高強度間歇性跑步訓練的心臟代謝和知覺反應之實驗

研究人員:
伍尚美教授　物理治療教授　香港理工大學康復治療科學系
蘇俊龍博士　物理治療助理教授　香港理工大學康復治療科學系
郭文瑩女士　博士三年級生/ 物理治療師　香港理工大學康復治療科學系

研究項目簡介:
缺乏運動與降低心臟代謝健康有關。 因此，能識別和減少身體運動將對預防心臟代謝疾病產生重要影響。 老年女性的心臟代謝風險較高，有研究指出，進行高強度的運動訓練有利於預防相關的老化心臟代謝疾病。

陸地進行的高強度間歇訓練 (L-HIIT) 可定義為反復進行的高強度運動，並引發≥80% 的最高心率 [HRmax]，當中包括短時間的恢復。儘管 L-HIIT 已證明可以改善心臟代謝健康，但這種訓練是在地面上進行的。由於疼痛和其他運動困難等障礙，普遍被認為不太適合老年人群。但由於水的物理特性，在水的環境運動被認為是安全而有效的體育訓練。水上進行的高強度間歇性訓練 (AHIIT) 可提供一種替代 L-HIIT 的低負重有氧運動。在水上運動中，深水跑步 (DWR) 在科學文獻中佔有重要位置。 DWR 是在漂浮背心的幫助下進行，該背心用於保持身體直立並防止腳板接觸池底。AHIIT-DWR 通過此特性降低受傷風險。

鑑於 AHIIT-DWR 的應用越來越廣泛，這項研究的目的是 1. 設計 AHIIT-DWR 運動以加強心臟代謝結果和知覺反應 2. 研究 8 週 AHIIT-DWR 或 L-HIIT 的效果，並以相對的強度對老年女性的心臟代謝健康結果和知覺反應進行研究

若你是年齡≥60歲的健康女性，我們誠意邀請你參與此項研究。

研究方法和程序:
參與者將被隨機分配到 AHIIT-DWR 或 L-HIIT 組。在進行運動之前，將在水中或陸地進行增量測試，以確認在每種情況下以相對的運動強度所需作個人化節奏調整。水上增量試

驗和陸上增量試驗將分別通過DWR和跑步機進行。在增量測試期間，氣體交換數據將通過便攜式代謝設備 PNOE 獲得。

對於 AHIIT-DWR，參與者將穿著漂浮背心，以保持雙腳不接觸池底。他們將獲得用於心率控制和監測的心臟頻率計（Polar®、Electro、Oi、芬蘭）。進行 10 次 2 分鐘的 AHIIT-DWR ，最大心率達到 80-90%，在兩次回合之間有1 分鐘的恢復，而最大心率為 50%。參與者將在 30 分鐘內完成每週兩次，持續 8 週（共 16 節) 的訓練。

另一方面，L-HIIT 練習會在環境溫度和相對濕度分別為 23˚C 和 50% 的房間內進行。參與者被指示在跑步機上以 80-90% 的最大心率進行 10 次 2 分鐘的跑步，跑步之間以 50% 的最大心率進行 1 分鐘的恢復。參與者將在 30 分鐘內完成每週兩次持續 8 週（共 16 節）的訓練。

AHIIT-DWR 和 L-HIIT 將分別在香港理工大學 (HKPU) 的步態分析實驗室和博藝會進行。評估將包括心肺反應（氧容量、氧脈博）、代謝結果（血脂、葡萄糖和膽固醇指數）和感知反應的測量。代謝指數會在測量手指採血，小樣本量（40μL）將用毛細管收集，每次評估將在 30 分鐘內完成。

<u>本研究項目的益處:</u>
希望這研究項目能幫助我們了解女性 AHIIT-DWR 和 L-HIIT 在心臟代謝和感知反應方面的差異。研究結果可以讓臨床從業者清楚地了解水的環境對 HIIT 的影響，並可以指引從業者的臨床實踐。

<u>自願參與性質:</u>
本研究全屬自願參與性質，閣下有權利於任何階段退出或中止參與是次研究。閣下之任何決定將不會受到任何懲罰或不公平對待。

若你對是項研究有任何查詢或疑問，可致電 9206 ，與郭文瑩女士聯絡。如你對是項研究或研究員有任何投訴或建議，可致電 27664329，與部門研究委員會秘書鍾女士聯絡。

蘇俊龍博士 (首席研究員)

香港理工大學康復治療科學系

THE HONG KONG
POLYTECHNIC UNIVERSITY
香港理工大學

CONSENT TO PARTICIPATE IN RESEARCH

Title: Effects of aquatic high intensity interval deep water training and land based high intensity interval training on cardio-metabolic health parameters and perceptual responses in women

Research Team Members:

Professor Shamay Ng	Professor	Department of Rehabilitation Sciences, PolyU
Dr. Billy So	Assistant Professor	Department of Rehabilitation Sciences, PolyU
Ms Manny Kwok	PhD student / Musculoskeletal Physiotherapist	Department of Rehabilitation Sciences, PolyU

I ________________________ hereby consent to participate in the captioned research conducted by Dr. Billy So Chun Lung.

I understand that information obtained from this research may be used in future research and published. However, my right to privacy will be retained, i.e. my personal details will not be revealed. This study abides the Declaration of Helsinki.

The procedure as set out in the attached information sheet has been fully explained. I understand the benefit and risks involved. My participation in the project is voluntary.

I acknowledge that I have the right to question any part of the procedure and can withdraw at any time without penalty of any kind.

If I would like to get more information about this study, I can contact Ms Manny Kwok at 9206 . If I have any complaints about the conduct of this research study, I can contact Ms Vangie Chung, Secretary of Department Research Committee at 27664329.

Name of Participant : ______________________ Date : ______________________

Signature of Participant : ______________________

Name of Witness : ______________________ Date : ______________________

Signature of Witness : ______________________ ______________________
____

Name of Researcher : ______________________ Date : ______________________

Signature of Researcher : ______________________

THE HONG KONG POLYTECHNIC UNIVERSITY
香港理工大學

香港理工大學
康復治療科學系
研究資料

研究項目: 針對比較健康婦女的高強度間歇深水跑步訓練和陸地進行的高強度間歇訓練的心臟代謝和知覺反應之實驗

研究人員:

伍尚美教授	物理治療教授	香港理工大學康復治療科學系
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郭文瑩女士	博士三年級生/ 物理治療師	香港理工大學康復治療科學系

本人 ______________________明白是項研究的詳細情況，並願意參與由蘇俊龍博士開展的是項研究。本人的參與，是屬於自願性質。

本人明白在任何時間可以放棄及退出測試，而毋須給予任何理由。本人亦不會因為退出測試而受到任何處罰或不公平對待。

本人得悉並且明白參與是次研究所帶來的潛在危險。除有關研究之研究員，本人的個人資料不會展示給予與任何人。如未經本人的同意，本人的名字及照片並不會刊登於是項研究的任何發表佈告之中。這項研究是符合赫爾辛基宣言的原則。

本人若果對是項研究有任何查詢或疑問，可致電9206 ，與郭女士聯絡。如對是項研究或研究員有任何投訴或建議，可致電27664329，與部門研究委員會秘書鍾女士聯絡。本人簽署後表示本人已收到此同意書副本乙份。

簽署(參與者) : ______________________ 日期 : ______________________

名稱(參與者) ： ____________________ ____________________

簽署(見證人) ： ____________________ 日期 ： ____________________
名稱(見證人) ： ____________________

簽名(研究員) ： ____________________ 日期 ： ____________________
名稱(研究員) ： ____________________

9.4.1 Accepted Manuscript by the International Journal of Environmental Research and Public Health (Chapter 6)

Effects of Aquatic High Intensity Interval Training and Land High Intensity Interval training on cardio-metabolic fitness and affective responses in women

Introduction

Physical exercises are beneficial to health. There were well documented evidence support physical exercises in preventing cardio-metabolic diseases including cardiovascular diseases, cancer and diabetes leading to premature mortality (Chestnov, 2013) (Warburton & Bredin, 2017b). The WHO recommended that all adults should undertake 150–300min of moderate-intensity, or 75–150min of vigorous-intensity physical activity, or some equivalent combination of both per week (Bull et al., 2020). However, it was found that 46.7% of adults aged 18 and over failed to meet the Physical Activity Guidelines for aerobic physical activity in the United States (U.S. Bureau of Labor Statistics 2017). Particularly, women were less physically active than men. Among adults aged 18 years or older, 31.7% of women and 23.4% of men failed to achieve physical activities up to or exceeding the WHO recommended level (Guthold et al., 2018). While in Europe, only 36% of women exercise and 52% never exercise (European Commission 2020).One of the most common exercise barriers faced by women was lack of time, probably due to career commitment or family responsibilities(El Ansari & Lovell, 2009). Therefore, an effective exercise with reduced time commitment can be advocated to encourage women in exercises participation, in order to prevent the harmful consequences led by physical inactivity.

High Intensity Interval Training (HIIT) can be defined as repeated short bouts of high levels of exercise (≥ 80% maximal heart rate) intersperses with periods of rest or low to moderate levels of exercise, which only lasts for less than 30 minutes per training (Martin J. Gibala et al., 2012). It has been proven to be a more time-efficient and effective option for physical activity than continuous training in improving cardio-metabolic health, weight management, insulin and blood glucose regulation (Batacan et al., 2017; M. Wewege et al., 2017).

Based on the gaining popularity of aquatic exercises, predominantly among women populations, performing HIIT in an aquatic environment (AHIIT) may be of interest and can be an alternative to land-based HIIT (L-HIIT)(Nagle et al., 2007). AHIIT has emerged for individuals who are more likely to incorporate HIIT into their lifestyles while immersed in an aquatic environment(Nagle, Sanders, & Franklin, 2017). AHIIT is considered to be effective because many physiological benefits are associated with the physical properties of water(Becker et al., 2009). Water buoyancy reduces discomfort and stress placed on the joints while the hydrodynamic nature of water acts as a form of resistance to movements, as well as optimising the development of muscle strength(S. M. Heywood et al., 2016; Rana et al., 2007). With these water properties, can further challenge the cardio-metabolic fitness. There were evidence proven that AHIIT improved cardio-metabolic health, aerobic capacity (VO_2) significantly in both non -athletic populations and women when compared to without exercise training(Depiazzi et al., 2018; M. M. Y. Kwok, S. S. M. Ng, et al., 2022). Hence, AHIIT can offer a great option for women to minimise some of the hindrances to exercise associated with land-based training, such as physical inactivity and consequently lead to reduced cardio-metabolic fitness.

In addition to the potential physiological effects brought by AHIIT, it is suggested that the affective responses are congruent to exercise compliance(Ekkekakis et al., 2012). Affective responses can lead to behavioural changes in physical activity and thus including affective response is an important factor for decision-making in exercise prescription(Oliveira et al., 2013). There were studies reported HIIT interspersed with intervals of rest period encouraged development of positive feelings, resulted in a higher exercise compliance(Thum et al., 2017). For instance, it is suggested that an increase in perceived affect is associated with an additional 38 minutes of physical activity per week (Williams et al., 2008). Affective responses play an utmost essential role in improving exercise adherence for women.

As to optimize training outcomes arise from AHIIT and land HIIT (L-HIIT), a correct exercise prescription of high intensity was crucial. Although the physiological parameters of heart rate (HR) and oxygen capacity (VO_2)responses are commonly adopted to monitor the exercise intensity, with water immersion, the use of these parameters in the aquatic environment remains debatable(Benelli et al., 2004). As a matter of fact that the physiological responses in aquatic and land exercises may differ and thus a matched optimal intensity is putting forward to ensure an equivalent intensity is provided for adequate exercise prescription in both environments (Alberton et al., 2009).

To the best of our knowledge, evidence have shown conflicting results on the cardio-metabolic responses of land and water-based exercises with exercises performed at maximal exertion. For instance, several studies used the same cycling or treadmill walking exercise performed in water and land and demonstrated a similar maximal aerobic capacity (VO_2 max)(Alberton et al., 2009; L. S. Andrade, S. S. Pinto, et al., 2020b; Costa et al., 2019).

Other studies reported a lower cardio-metabolic response when compared aquatic running to land running programs(Bojan Jorgic et al., 2012; Ana Carolina Kanitz et al., 2015; Kruel et al., 2013b; Ogonowska-Slodownik et al., 2020). Hence, to date, little evidence has been found associating AHIIT with L-HIIT on cardio-metabolic responses difference. Similarly, the affective responses of AHIIT remains yet to be determined when compared to land HIIT. This line of inquiry is necessary to identify the effects of AHIIT compared with L-HIIT to impact women with better participation in physical activity. Profound evidence are able to further extend the applications of interval trainings while AHIIT can be an alternative exercise option to optimize physical activities which potentially draw an impact in physical health among women.

The objectives of this study were to (1) establish a matched intensity between aquatic and land incremental test and (2) to evaluate the cardio-metabolic and affective responses of AHIIT and L-HIIT in women. It was hypothesized that AHIIT would improve the cardio-metabolic acute responses with a higher positive affective response than L-HIIT.

Methods

Participants

A total of twenty young active female subjects were recruited through local poster advertisement from the university community. The inclusion criteria were women who were non-pregnant, clinically healthy, physically active and between 20-35 years of age. The exclusion criteria included subjects with chronic medical and health conditions, not limited to orthopaedic, cardiovascular diseases, cancers, autoimmune diseases, neurological disorders, fear of water and skin diseases. All subjects were advised not to involve in strenuous exercise for 3 days prior the experiment and maintain their daily routines. All women were informed of the experimental risks by an investigator and signed an informed consent form prior data collection. Then they complete the International Physical Activity Questionnaire to evaluate their physical activity levels(Helmerhorst et al., 2012). The power calculation was based on the primary outcome of a previous study comparing VO_2 max between land and water stationary running(Kruel et al., 2013b). Using the G*power software and based on the effect size 0.64 obtained, assuming power of 0.8 at an alpha level of 0.05 (G*Power version 3.0.10), the sample size computed was 16 or more subjects per group. Considering an estimated 20% attrition rate, the anticipated sample size of 20 subjects was adequate to detect difference between groups on our primary outcome (i.e. VO_2 max). The study was approved by office of research ethical committee, the Hong Kong Polytechnic University (HSEARS20210522001). This study conforms to the Declaration of Helsinki for study involving humans.

Study design and procedures

A randomized crossover design was used in this study. The 20 participants were assigned to complete AHIIT and L-HIIT groups. Participants were assessed by performing a stationary running exercise at high intensity intervals determined by either an aquatic or land incremental test done prior the AHIIT or L-HIIT intervention irrespectively. Participants were asked not to complete any aerobic trainings for 48 hours prior to any testing sessions to limit the influence of prior exercise on pain, fatigue and performance. Each participant attended two sessions at the pool and 2 sessions on land at the laboratory of our institution. Incremental testing was completed first either at the pool or on land in a randomised order, followed by the AHIIT and L-HIIT respectively. The immersion was at chest depth (xiphoid-sternal depth or up to five centimetres deeper) in a swimming pool (temperature 29 °C) while the room temperature of ambient air was maintained at 23°C at the laboratory.

Aquatic and Land Incremental tests

An incremental test in water and on land was performed prior the exercise interventions to confirm a matched exercise intensity. We measured participants anthropometric data including 1) body weight in kg and 2) body height in cm with an electronic scale (BC-730b, Tanita, Japan) and a stadiometer (BRAND) respectively. The incremental test was carried out by stationary running. Instructions for stationary running directed participants to flex the hip and knee to as close to 90° as comfort and control allowed and then push to straighten up hip and knee. Prior to testing, all exercises were demonstrated first then practiced once. Participants was continuously monitored and recorded at a frequency of 1Hz by a HR sensor (Polar OH1, Kempele, Finland). It has been validated previously and worn by participants to monitor heart rate(Bergamin et al., 2015a). During the incremental test, gas exchange data were also obtained by a portable metabolic device PNOE. The PNOE device was operated by a breath-by-breath mode which continuously measures volume and determines expired gas concentrations simultaneously. It was calibrated prior to each session according to manufacturer's specifications. PNOE has been validated in previous study when compared to a validated stationary metabolic cart (COSMED QUARK-CPET). It was suggested that strong correlation and no significant differences found in oxygen capacity (VO_2)(Tsekouras et al., 2019a). The incremental protocol increased the exercise load from 85 beats per minute (bpm) and increased the cadence by 15 bpm every 2 minutes for each progression(Ana Carolina Kanitz et al., 2015). A metronome (Intelli IMT 300, Japan) was used to monitor the speed of movements throughout the trial. The heart rate (HR), VO_2, rate of perceived exertion (RPE) per minute were recorded. VO_2max was considered to be attained when the following standardized criteria were met: (1) a respiratory exchange ratio of greater than or equal to 1.10; (2) failure of heart rate to increase with increases in workload; (3) post-exercise blood lactate ≥8.0 mmol•L^{-1}(American College of Sports & Medicine, 2018); (4) clear signs of exhaustion (facial flushing, unsteady gait) and (5) refusal to carry on despite strong verbal encouragement. Blood lactate was measured via capillary blood sampling from the fingertips with a portable analyser (Lactate Plus, Nova Biomedical, Waltham, Massachusetts). Data collected from the incremental test was used to determine the intensity required for the exercise interventions.

AHIIT and L-HIIT Exercise Interventions

Participants warmed up for 2 minutes before exercises. Instructor asked the participants to do stationary running followed the intensity determined from previous incremental trials. AHIIT or L-HIIT training will then be performed under the desired cadence with audible metronome. A metronome provided audible feedback for the timing of the exercise. The AHIIT and L-HIIT protocol consisted of 10 bouts of 1-min stationary run at 90% HRmax separated by 1-min active recovery at 70% HRmax. The total exercise trial time was 20-minutes in duration. We used a randomised counterbalanced sequence to determine the order of AHIIT or L-HIIT on subsequent visits. The two tests were separated by at least 48 hours and a maximum of 72 hours intervals. All the sessions were held at the same time of the day to avoid variations related by circadian rhythms. No external stimuli such as music and verbal encouragement were given in all trials.

Cardio-metabolic fitness

Cardiorespiratory outcomes

Maximal oxygen capacity (VO_2max) was determined by both aquatic and land incremental tests with stationary running to test to volitional exhaustion based on the protocol demonstrated previously(L. S. Andrade, A. C. Kanitz, M. S. Häfele, et al., 2020). The percentage of VO2 max (%VO2 max), percentage of HR max (% HRmax), percentage of VO2 reserve (%VO2R), percentage of HR reserve (%HRR), cadence and RPE will be matched between the two environments. Upon AHIIT and L-HIIT trainings, VO_2, oxygen pulse, respiratory exchange ratio (RER), minute ventilation (VE) and HR were measured by PNOE device. HR (bpm) was recorded continuously using a Polar heart rate monitor during the test and intervention.

Energy expenditure (EE)

Measures of participants' EEs , cumulative EEs and metabolic equivalent (MET) were used by both estimation procedures and measurement via indirect calorimetry by PNOE(Campbell et al., 2003).

Blood lactate

Blood lactate concentrations were recorded immediately before and after AHIIT or L-HIIT in all trials. Capillary blood samples (approximately 25 μL) were acquired from the fingertips using a portable analyser (Lactate Plus, Nova Biomedical, Waltham, MA, USA).

Affective responses

Enjoyment

Enjoyment and self-efficacy were assessed after the AHIIT and L-HIIT interventions. Enjoyment can be described as a positive effective state that reflects feelings such as pleasure, liking and fun(Wankel, 1993) It associated with physical activity participation and adherence(Motl et al., 2000). Participants were asked to complete the 18- item, 7-point bipolar Physical Activity Enjoyment Scale (PACES)scale immediately after the intervention to determine perceived enjoyment to the exercise condition they were randomized to. A higher score (out of 126) indicates a higher enjoyment level. PACES is reported to be a valid and reliable tool in measuring enjoyment in physical activity(Teques et al., 2020).

Self-efficacy

Self-efficacy is conceptualized as beliefs relative to one's capabilities to successfully execute necessary courses of action(Hu et al., 2007). Participants' self-efficacy was assessed via a 5-item questionnaire designed to determine participants' confidence to repeat either AHIIT or L-HIIT(McAuley et al., 1999). The self- efficacy scale has been demonstrated with good internal consistency (α's = 0.9)(Poon et al., 2020). Responses were scored at a percentage of 0% (Not at all) to 100% (Extremely confident) with 10% increments, and then averaged for the five items. Participants were asked to complete the scale immediate after each intervention.

Muscle soreness

Muscle soreness is often measured with the use of a 7-point Likert scale of muscle soreness for lower limbs, which combines verbal to numeric cues. Indeed, it is suggested that Likert based scales are easier to use, require shorter time to explain to patients, and do not require anchoring procedures that may be influenced by the experience of the subjects. The construct validity of the Likert scale as a measure of lower limb muscle soreness is well supported(Impellizzeri & Maffiuletti, 2007).

Statistical analysis

Data were presented with mean ± SD and analysed by IBM SPSS Version 25.0 (IBM Corporation, Armonk, NY) software. Shapiro-Wilk's test was used to verify the normal distribution of the data. A two-factor (medium vs training zones) repeated- measures ANOVA, as well as their interactions were used to compare the effect of aquatic incremental test with land incremental test (ie. “medium”) on %VO_2 max, %VO_2R, %HRR, RPE and cadence under a matched intensity (i.e. training zones) recommended by ACSM (2022) on the approximate classification of exercise intensity commonly used in practice. A paired- t test was used to compare the with-in group difference on cardio-metabolic and affective variables on AHIIT and L-HIIT. All continuous data was used for statistical analysis with a significance level of P<0.05.

Results

The mean age of the participants was 21.95±2.35, height 160.95±5.76 and weight 53.95±8.08. The physical activity levels deduced from IPAQ was 85% of level 2 and 15% of level 3. There were no adverse events during the incremental testing and HIIT interventions in both environments.

Insert Table 1

Effects of increased exercise intensity on %VO2 max, %HRR and %VO2R, RPE and cadence in aquatic and land incremental tests

An increasing in HR work zones created an increase in the physiological responses. As illustrated in figure 1a-1e, there was a significant main effect of work zones (Zone 1 to 3) (P<0.01) on the physiological variables (%VO2 max, %HRR, %VO2R), as well as the subjective RPE and mechanical cadence. The training zones were expressed in percentage of HR max according to the ACSM intensity guidelines (2009). The three training zones, namely are 64-76%, 77-95% and ≥ 96% of HR max. However, there were no significant interactions between different mediums on %VO2 max, % HRR and %VO2R, subjective

RPE and mechanical cadence. The matched intensity between aquatic and land incremental test was shown in table 2.

Insert Figure 1

Insert Table 2

Effects of AHIIT and L-HIIT on cardiorespiratory outcomes

AHIIT group showed a significant decrease in heart rate in both work and recovery intervals ($P<0.01$). The AHIIT HR was significantly lower when compared to the L-HIIT. The AHIIT oxygen pulse was significantly higher than that from L-HIIT ($P=0.038$). There was no significant difference in other cardiorespiratory parameters between AHIIT and L-HIIT. The VO2, %VO2 max, RER, VE ($P>0.05$) (Table 3).

Energy expenditure and lactate

The EE, MET and cumulative EE did not show a significant change ($P>0.05$). There was also no significant changes in blood lactate concentration changes between AHIIT (6.08 ±2.86mmol/L) and L-HIIT (5.84±2.42 mmol/L). The overall energy expenditure and post lactate concentrations were equivalent ($P>0.05$) (Table 3).

Insert Table 3

Effects of AHIIT and L-HIIT on psychological response

There were no significant differences found for the rate of perceived exertion in work ($P=0.6$) and recovery intervals ($P=0.948$) in AHIIT and L-HIIT. Both AHIIT and L-HIIT responded similarly in enjoyment ($p= 0.875$), self-efficacy score ($P=0.072$) and muscle soreness index ($P = 0.873$) (Table 4).

Insert Table 4

Discussion

To the best of the authors knowledge, this is the first study to determine the effect of AHIIT and L-HIIT on cardio-metabolic fitness and affective responses after matched incremental tests performed in both environments among women.

One major objective of this study was to match the exercise intensity between aquatic and land incremental test. From our findings of the incremental tests performed in both environments, as the exercise intensity increased, the variables (%VO_2 max, %HRR, %VO_2R, RPE and cadence) were increased. This is in consensus with previous studies which highlighted as speed increased in both aquatic and land environment, cadence, HR and VO_2 were increased(Hall et al., 2004a). Despite there were no significant interactions between the medium and training zones on the variables (%VO2 max, %HRR, %VO_2R, RPE and cadence), there were intersections between %VO_2 max, %HRR, %VO_2R and RPE. Such findings agree with Andrade et al (2020), that also described the relative intensity in VO_2, HR, RPE and cadence in older women with stationary running with an aquatic incremental test (L. S. Andrade, A. C. Kanitz, M. S. Hafele, et al., 2020).

Although it is common to use subjective (RPE) or mechanical (Cadence) or physiological outcomes (HR, VO2max) to monitor exercise intensity, the application can be limited to land environment. The training zones 1 to 3 in our study were categorized according to the gold standard of relative intensity guidelines recommended by ACSM exercise prescription (moderate to high intensity) performed in dry land. Such guidelines are commonly adopted since they are easy to use, quick reference points and easily applied in the group exercise setting. According to the ACSM guidelines on maintaining cardiorespiratory capacity, values of 64-76% HR max, 77-95% HR max and ≥ 96% max HR are categorized as moderate, vigorous and near maximal-to-maximal intensity which fits in our defined HIIT training zones. These recommendations were based on incremental maximal tests performed on dry land while the usage for aquatic exercises were limited. The use of land-based values would underestimate metabolic demand in water. Hence, we conducted aquatic incremental test to match the results of land incremental tests with a comparable intensity of moderate to maximal. Based on the present findings, instructors and coaches may use the matched intensity revealed between aquatic and land incremental tests to efficiently and precisely prescribe HIIT training sessions. Thus, when it is not possible to directly measure the aforementioned variables with incremental tests, as in the practical situations at gyms and clubs, the matched intensity may be used to individualize the aerobic loads prescription. Consequently, a more individualized and precise measurement of matched incremental test was used to monitor the actual equivalent intensity prescribed between the two environments. This can add as a guideline and supports the use of comparable relative intensity at different environment. It can also add valuable values to clinical practitioners in providing a matched intensity of aquatic and land incremental in order to optimize the training outcomes.

This study confirms a reduction in work and recovery HR during aquatic AHIIT compared to L-HIIT which supports our hypothesis. It also provides more evidence in the accuracy of exercise prescription when intensity was monitored solely by HR. Our findings were similar to previous aquatic and land-based studies, where the heart rate decreased with water immersion(Alberton et al., 2013). Hall et al (2004) highlighted HR was lower when running in water than on land(Hall et al., 2004b). It is known that with water immersion, hydrostatic pressure causes blood to be displaced from the peripherals to the central, resulting a significant increase in venous return and volume of heart(Plotnick et al., 1986). This mechanism promotes a reduction in HR because of stimulation of the carotid and aortic receptors which is likely to be directly proportional to the immersion depth(Becker et al., 2009). Therefore extra cautions should be taken when used the HR values obtained from land to regulate exercise in water since exercise intensity was one of the variables for aquatic exercise prescription(Ogonowska-Slodownik et al., 2020). Mounting evidence indicates that reduced heartrate is associated with a reduction in cardio-metabolic morbidity and mortality, for instance, high blood pressure, hypertension, atherosclerosis and increased cardiovascular diseases(Weston et al., 2014). In this respect, a reduced HR resulted in AHIIT can be considered as a marker for decreasing cardio-metabolic risk factors(Chatterjee et al., 2012).

A significant improvement in oxygen pulse was demonstrated in AHIIT than L-HIIT in work phase. Although there was insignificant change in the recovery phase, the P value was marginal and approaching significance (P= 0.078). This perhaps can be improved by increasing the sample size and power of the study. The oxygen pulse provides a reflection of oxygen taken up by the pulmonary blood during the period of a heartbeat, the combined product of stroke volume and the difference between the arterial and mixed venous blood oxygen content(Provost-Craig, 2005). An increase in the oxygen pulse can be attributed to an increase in arteriovenous oxygen difference. This could be explained by a more effective ventilation at peak exercise in an aquatic environment as a result of decreases in physiological dead space which, in turn, causes an increased in cardiorespiratory efficiency during progressively increasing exercise work rate under immersion(Kelly S. Chu & Edward C. Rhodes, 2001). Another possible reason for the higher oxygen pulse resulted in AHIIT in our study could be explained by increased breathing frequency that occurs while submerged in water at the chest level(Silvers et al., 2007). A comparatively greater ventilatory drive may have been required to overcome the effects of hydrostatic pressure on the thoracic cavity, causing an increased residual volume, and decreased tidal volume and vital capacity. As a result, a higher oxygen pulse is revealed in AHIIT than L-HIIT in our study, which could potentially challenge the cardiorespiratory fitness of individuals further.

The rest of the cardiorespiratory outcomes (VO_2, %VO_2max, RER, VE) were insignificant between the AHIIT and L-HIIT groups. The VO_2 max appeared as no different and this was also observed in Masumoto et al (2007) and Greene et al (2011) who compared maximal tests on a treadmill on both environments without significant differences shown on VO_2 max(Greene et al., 2011a; Masumoto et al., 2007). Similarly Silver et al (2007) demonstrated no significant difference between VO_2 max in incremental treadmill test in both environments(Silvers et al., 2007). Even Alberton et al (2009) suggested stationary running did not produce a significant change in VO_2 max, proposed that the VO_2 max depends on the muscle mass involved(Alberton et al., 2009). Only jumping jack of a smaller range of motion and slower speed resulted at a higher VO_2 max when compared with other aquatic exercises. Water environment might have reduced VO_2 max by reducing vital capacity, total lung capacity, pulmonary elasticity which caused VO_2 to be consumed by the respiratory muscles and reduced the VO_2 availability to other muscles and hence reduce the overall contribution to VO_2 max. This finding suggests that the VO2 adjustments during dynamic HIIT were not changed by water immersion, despite the assumed increase in cardiac output. This aligns with the previous review findings with stationary water running and stationary running on land and with water cycling and bicycle ergometer(Barker et al., 2011; Ana Carolina Kanitz et al., 2015). Therefore, a determining factor for the VO2max pattern is the mode of exercise performed rather than inherent physical properties of water.

In our study, the total EE for a 20-minute AHIIT or L-HIIT intervention was between 610-667 kcals (7-9 kcals/min; 7-9 METs). Our findings indicated that the EE of the HIIT programs was similar to the reported in previous studies involving aquatic interval trainings(Nagle et al., 2013). The comparatively high EE achieved could be explained by an increased speed and intensity optimized water resistance causing a higher EE while maintained the same range of movements(Colado et al., 2009). Our results were in lined with previous aquatic and land-based studies, where high-intensity interval training elicited a comparatively greater EE when compared with constant intensity or continuous exercises regimes(Kruel et al., 2013b). The energy expenditure recommendation proposed by ACSM for moderate vigorous exercise was above 1000 kcal/ week. It seemed AHIIT could potentially generate a higher energy expenditure due to water resistance as elucidated by the results.

The mean values of blood lactate in AHIIT were similar during the work period and active recovery performed in immersed running compared to the values observed in L-HIIT. The blood lactate level was the net lactate difference between lactate production and elimination. The blood lactate level was positively associated with post exercise fatigue(Fiorenza et al., 2019). Although there were studies suggested that post exercise lactate levels in water was lower than on land when the exercise intensity was matched in comparison, it was proposed that an increased in blood flow caused by the hydrostatic pressure increased the lactate removal rate(Masi et al., 2007). Another possible reason for our result showing no significant difference between two mediums was we adopted an interval exercise, with active recovery consisting of 60-second. The active recovery period could compensate the energy consumed and facilitate the removal of metabolites. Therefore, it helps to decrease the post-exercise lactate concentration and offset the influences of environment on the production of lactate. This is also supported by the study conducted by Chien et al (2020), when AHIIT and L-HIIT were compared, there was no difference in the lactate level between the two environments immediately post exercise(Chien et al., 2020).

As for the affective responses, no significant group difference for RPE, enjoyment, self-efficacy and muscle soreness was found. HIIT combines time efficiency, diversity and fun. The physiological benefits brought by HIIT were vastly published and aligned while the affective responses in HIIT reached no consensus among the authors(MacInnis & Gibala, 2017). Our result is in agreement with Ma et al (2017) which suggested that both aquatic and land interval trainings elicited similar changes in RPE in a group of women(Ma et al., 2017). It was also suggested that AHIIT was perceived as more affective and enjoyable than L-HIIT in men with obesity(Sriton et al., 2022b). These results vary may be related to variability in water depth, subject characteristics, genders, exercise intensity, intervals, depending on the modalities performed in AHIIT.

This study has several strengths. The novelty of an incremental test performed prior the HIIT intervention allowed a correct, optimal and matched intensity of %VO_2 max, RPE and cadence monitoring in AHIIT and L-HIIT for comparison. In most of the land and water comparison studies, the expressed intensity was based on incremental maximum tests performed on land(Raffaelli et al., 2012). In this study, the intensity was based on the values obtained from an aquatic incremental test which has been shown as a most accurate and appropriate methodological option for exercise prescription in the aquatic environment because of the previously aforementioned characteristics and properties of water(Nagle, Sanders, Gibbs, et al., 2017).The matched intensity provides a precise guideline for determining an individual's baseline level of fitness for AHIIT prescriptive purposes and will serve as a method of outcomes assessment of AHIIT program. This is practical in clinical applications, for instance, among athletes, implementation of standardized incremental tests methodology for cardio-metabolic evaluation can be important to the development of a training needs and serves as a baseline measure from which AHIIT adaptations may be monitored. Furthermore, such testing protocols will serve as motivational tools to monitor training and promote physical activity.

Despite these strengths, major limitations of the present study included that only young female participants were recruited and hence caution should be taken when generalizing to older women, as well as men. Some other limitations in this study were subjected to healthy populations and might not be able to generalize to the clinical populations. In addition, it is acknowledged that the relatively small sample size might fail to impose a power sufficient enough to detect a significance in other measured outcomes. Although running movement is a basic and natural exercise for humans, it may not be representative to all the exercises. Also, the design of this study was a cross sectional study which examined the acute effect of HIIT interventions only, it was not representative of the long-term effect. Nevertheless, our results provide practical guidelines in applying matched intensity of aquatic and land incremental tests followed by the HIIT corresponding interventions. From a practical point of view, AHIIT can be an adjunct or alternative to land based HIIT on improving cardio-metabolic and enhancing affective responses in women. This may be ideal for women unable to exercise on land, or those who exclusively train on land, and desire to cross-train in water for assessment or rehabilitative purposes. By evaluating the cardio-metabolic outcomes allow therapists or exercise professionals to better promote the unique benefits and physiologic advantages of AHIIT and L-HIIT. This may lead to the engagement of women to participate in AHIIT or L-HIIT as a more widely practiced form of physical activity. A randomized control study can be adopted to study a long-term effect of the interventions on physiological or affective outcomes among different environments. Therefore, future studies examine the efficacy of AHIIT using clinical populations, athletes, and those unable to perform L-HIIT are also warranted.

Conclusion

In sum, our findings suggested that AHIIT can offer cardiorespiratory benefits and affective response as comparable to L-HIIT. Also, the comparable results demonstrated by the incremental tests in both environments had matched in intensity for HIIT prescriptions and allows participants precisely perform exercise at the same domain. It suggests that AHIIT has distinct difference on heart rate and oxygen pulse despite no distinct difference from L-HIIT on few cardio-metabolic and affective response, at least immediate after interventions. Future researches with randomised controlled trails of a longer duration are suggested in comparing the causal effects AHIIT and L-HIIT on cardio-metabolic and affective outcomes.

Acknowledgement

The authors wish to express their gratitude to Dr Raymond Chung, PhD, for his contributions as scientific advisor of this manuscript.

Figures and Tables Legends

Figures

Figure 1. Aquatic VS land incremental tests on (A)HR, (B)% max HR, (C)%HRR, (D)VO_2, (E) %VO_2max, (F) %VO_2R, (G)RPE at the different incremental test stages.

Tables:

Table 1 Descriptive characteristics of participants: (mean± SD).

Table 2 Number of participants who reached each stage of the aquatic and land incremental tests.

Table 3 Descriptive statistics (mean ±SD) of the HR, %HR max, %HRR, VO_2, %VO_2 max, %VO_2R and RPE at each stage of the aquatic and land incremental tests.

Table 4 Cardio-metabolic outcomes in the AHIIT and L-HIIT interventions (mean ±SD).

Table 5 Perception outcomes in the AHIIT and L-HIIT (mean ±SD).

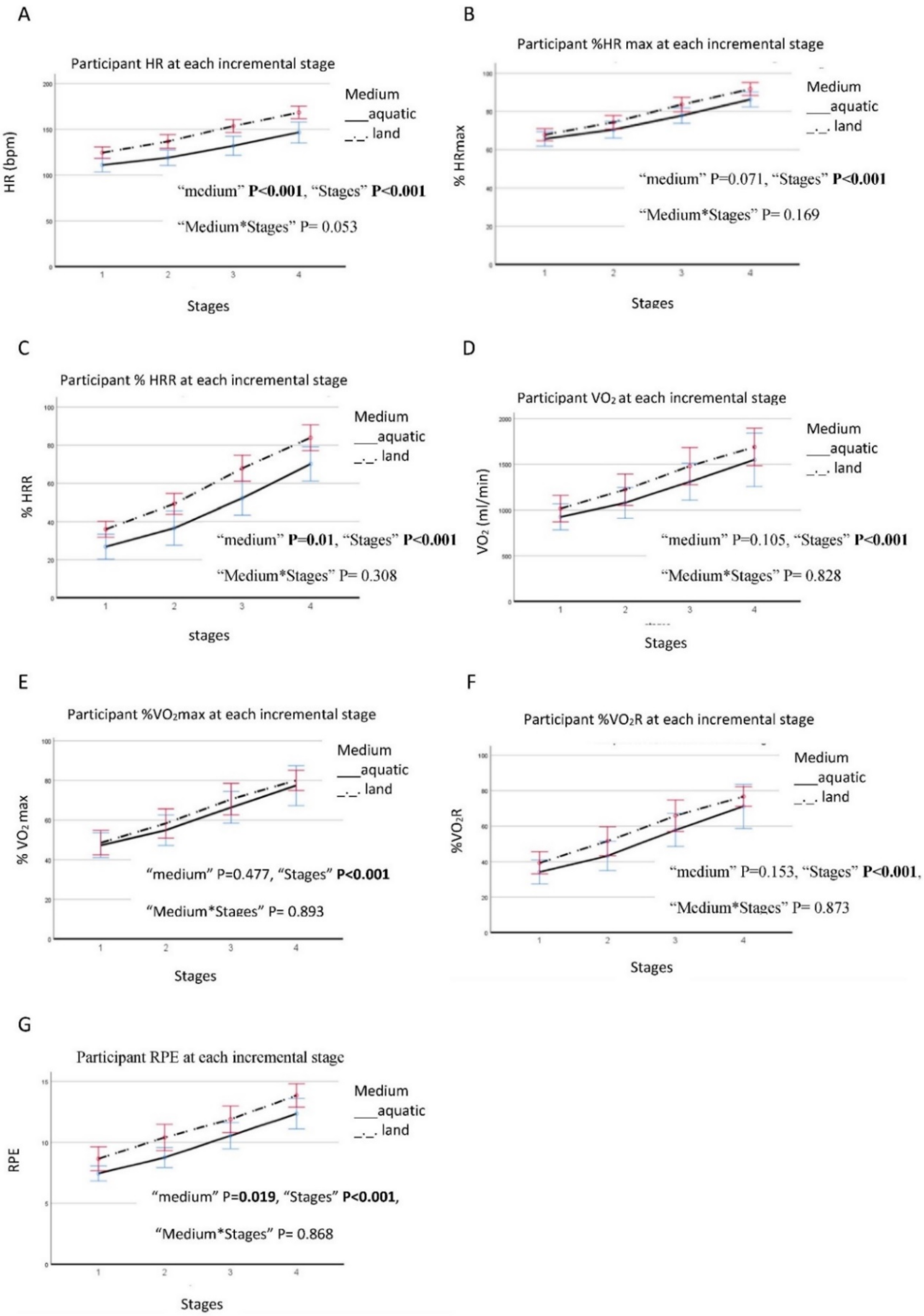
A
Participant HR at each incremental stage
HR (bpm)
Medium
___aquatic
_._. land
"medium" P<0.001, "Stages" P<0.001
"Medium*Stages" P= 0.053
Stages
B
Participant %HR max at each incremental stage
% HRmax
Medium
___aquatic
_._. land
"medium" P=0.071, "Stages" P<0.001
"Medium*Stages" P= 0.169
Stages
C
Participant % HRR at each incremental stage
% HRR
Medium
___aquatic
_._. land
"medium" P=0.01, "Stages" P<0.001
"Medium*Stages" P= 0.308
stages
D
Participant VO2 at each incremental stage
VO2 (ml/min)
Medium
___aquatic
_._. land
"medium" P=0.105, "Stages" P<0.001
"Medium*Stages" P= 0.828
Stages
E
Participant %VO2max at each incremental stage
% VO2 max
Medium
___aquatic
_._. land
"medium" P=0.477, "Stages" P<0.001
"Medium*Stages" P= 0.893
Stages
F
Participant %VO2R at each incremental stage
%VO2R
Medium
___aquatic
_._. land
"medium" P=0.153, "Stages" P<0.001,
"Medium*Stages" P= 0.873
Stages
G
Participant RPE at each incremental stage
RPE
Medium
___aquatic
_._. land
"medium" P=0.019, "Stages" P<0.001,
"Medium*Stages" P= 0.868
Stages

Figure 1 Aquatic VS land incremental tests on (A)HR, (B)% max HR, (C)%HRR, (D)VO2, (E) %VO2max, (F) %VO2R, (G)RPE at the different incremental test stages.

Table 1 Descriptive characteristics of participants: (mean± SD)

	Participants
N	20
Sex	F
Age (in years)	21.95±2.35
Height (cm)	160.95±5.76
Weight (kg)	53.95±8.08
International Physical activity levels (%)	
Level 1 (inactive)	0%
Level 2 (minimally active)	85%
Level 3 (active)	15%

Table 2 Number of participants who reached each stage of the aquatic and land incremental tests.

Stage number	Cadence (bpm)	Aquatic incremental: N (%)	Land incremental: N (%)
1	85	20 (100%)	20 (100%)
2	100	20 (100%)	20 (100%)
3	115	20 (100%)	20 (100%)
4	130	20 (100%)	20 (100%)
5	145	19 (95%)	16 (80%)
6	160	11 (55%)	12 (60%)
7	175	9 (45%)	8 (40%)
8	190	8 (40%)	7 (35%)
9	205	6 (30%)	3 (15%)

10	220	4 (20%)	0 (0%)

Table 3 Descriptive statistics (mean ±SD) of the HR, %HRmax, %HRR, VO2, %VO2 max, %VO2R and RPE at each stage of the aquatic and land incremental tests.

Stages	Medium	HR	%HRmax	%HRR	VO_2	$\%VO_2max$	$\%VO_2R$	RPE
1	Aquatic	110.9±16.2	65.7±8.0	26.8±14.1	926.0±303.8	47.4±13.4	34.1±14.7	7.5±1.3
	Land	136.7±15.8	67.8±6.7	36.1±8.9	1016.7±305.1	49.6±13.2	39.3±13.3	8.7±2.1
2	Aquatic	119.0±18.2	70.5±9.4	36.6±19.3	1078.3±357.3	54.9±16.4	43.2±17.6	8.8±1.8
	Land	153.6±15.3	74.3±7.5	49.2±11.7	1221.4±366.7	58.3±15.8	51.5±17.5	10.4±2.3
3	Aquatic	132.0±21.9	77.8±8.6	52.2±19.0	1309.2±431.7	65.5±17.1	57.8±20.0	10.6±2.3
	Land	168.5±14.5	83.6±8.1	67..9±14.5	1479.8±436.3	70.6±17.0	65.9±18.9	11.9±2.3
4	Aquatic	146.6±24.5	86.3±8.4	70.2±19.2	1548.6±625.3	77.4±21.6	71.1±26.5	12.4±2.7
	Land	168.5±14.5	91.7±7.2	83.9±14.5	1689.7±438.7	80.0±11.0	76.7±11.9	13.9±2.0

Note: HR = heart rate; VO_2 max = maximal oxygen uptake; HRmax = maximal heart rate; RPE = rate of perceived exertion; HRR = heart rate reserve; VO_2R = VO_2 reserve

Table 4 Cardio-metabolic outcomes in the AHIIT and L-HIIT interventions (mean ±SD)

Measure	AHIIT	L-HIIT	*P*-value
HRmax (bpm)	162±19.1	179.1±14.3	<0.01*
HR (bpm)	W149.62±18.88	W166.75±16.41	<0.01*
	R 139.26±17.90	R158.07±15.78	<0.01*
%HRmax (%)	W92.71±4.25	W93.26±3.98	0.693
	R 86.40±6.60	R 88.75±3.47	0.177
VO_2max ($mL\cdot kg^{-1}\cdot min^{-1}$)	35.78±6.58	36.14±7.24	0.819
VO_2 ($mL\cdot min^{-1}$)	W1758.57±348.76	W1829.18±287.67	0.293
	R1383.19±280.37	R1462.29±318.55	0.273
$\%VO_2$ max (%)	W91.4±5.98	W95.38±11.29	0.091
	R72.18±10.15	R76.66±11.02	0.173
Oxygen pulse (ml/beat)	W11.81±2.05	W11.05±1.78	0.038*

	R9.92±1.55	R9.27±1.78	0.078
RER	W0.93±0.09	W0.94±0.06	0.460
	R1.05±0.09	R1.01±0.08	0.178
VE (L/min)	W61.31±15.31	W62.93±11.22	0.621
	R52.40±13.63	R51.35±8.81	0.704
EE (kcal/min)	W8.64±1.70	W9.02±1.44	0.257
	R6.99±1.44	R7.33±1.57	0.340
MET	W9.22±1.50	W9.79±1.52	0.122
	R7.28±1.48	R7.77±1.35	0.189
Cumulative EE (kcal)	W609.81±235.53	W618.70±246.65	0.897
	R663.98±255.72	R667.07±265.39	0.967
Lactate changes (mmol/L)	6.08±2.86	5.84±2.42	0.572

Notes: W = work period, R = active recovery period, VO_2 = oxygen uptake, VO_2max = maximal oxygen uptake, RER = respiratory exchange ratio, VE = minute ventilation, EE = energy expenditure, MET = metabolic equivalent. * ($P < 0.05$)

Table 5 Perception outcomes in the AHIIT and L-HIIT (mean ±SD)

Measure	AHIIT	L-HIIT	*P* value
RPE (6-20)	W13.18±2.0	W12.86±1.84	0.60
	R11.66±2.18	R11.71±1.89	0.948
Enjoyment (Score of 126)	68.55±7.53	68.55±9.24	1.00
Self -efficacy (Score of 100)	37.8±27.48	45.75±20.91	0.072
Muscle soreness index (Score 0-6)	5.3±2.07	5.4±1.7	0.873

Note: RPE, rate of perceived exertion

9.4.2 Information sheet, consent form of Chapter 6

THE HONG KONG POLYTECHNIC UNIVERSITY
香港理工大學

INFORMATION SHEET

The effect of single bout aquatic high-intensity interval training (AHIIT) and resistive aquatic high-intensity interval training (R-HIIT) on resting energy expenditure and respiratory ratio in healthy adult

Research Team Members:

Dr. Billy So	Assistant Professor	Department of Rehabilitation Sciences, PolyU
Ms Manny Kwok	PhD student/ Musculoskeletal Physiotherapist	Department of Rehabilitation Sciences, PolyU
	MPT Student	
Mr. Daniel Pang	MPT Student	Department of Rehabilitation Sciences, PolyU
Mr. Gordon Tan	MPT Student	Department of Rehabilitation Sciences, PolyU
Mr. Daniel Tang		Department of Rehabilitation Sciences, PolyU

Study Background:

Tiredness, physical discomfort, stress and time constraints are commonly cited reasons for adults who fail to meet World Health Organisation's recommended physical activity level. Sedentary lifestyle increases risk factors of non-communicable diseases, such as hypertension and cancer, sufficient and optimal dosage of physical exercises is suggested.
High Intensity Interval Training (HIIT) can burn more calories per unit time and provide greater enjoyment than traditional moderate-intensity physical activity. Thus, HIIT can be effective in optimising the benefits of physical activity.

Training in an aquatic environment is gaining popularity as it reduces risk of injury and joint pathologies related pain. Physical properties of water, for example water buoyancy and hydrostatic pressure, can reduce loading over joints and promote blood circulation. An aquatic environment is therefore a safe and beneficial medium to encourage participation in physical activity.

In view of a growing application of Aquatic HIIT (AHIIT), the purpose of this proposed study is to find out the differences between performing AHIIT and resistive AHIIT (R-AHIIT) in metabolic responses and to suggest an AHIIT prescription.

If you are a healthy adult aged between 18-35, you are cordially invited to participate in this study.

Study procedure:

A stationary running HIIT will be performed in an aquatic environment twice, one with and one without aquatic fins at the same match intensity as measured by cadence set by a digital metronome (MA-30, KORG; Tokyo, Japan).

For the AHIIT program, participants will be immersed up to the chest depth in a heated pool of water temperature of 29°C. First, they will be given a cardiac frequency meter (Polar®, Electro, Oi, Finland) for heart rate control and monitoring. Next, participants will be outfitted with the aqua-trainer by wearing a tube and nose clips which allows breath by breath gas exchange parameters to be calculated. The portable gas analyser will be waterproofed and suspended near the participant by means of a stainless-steel support to protect the device from splashing water. A PNOE wearable metabolic system will be used to measure oxygen capacity. During each HIIT session, the subjective Ratings of Perceived Exertion RPE Borg's 6-20 scale for breathing (BORG 1982) will be monitored and recorded. On the other hand, the R-AHIIT exercise will be performed in the same setting as the AHIIT program with the aquatic fins attached to ankles. The Participants are instructed to wear PNOE with facemask covering the nose and mouth which offers freedom of movement during testing. It measures gas exchange with breath-by-breath techniques.

Both AHIIT and R-AHIIT exercises will be performed at the Hong Kong Polytechnic University Block X swimming pool.

The AHIIT and R-AHIIT each will be completed in 30 mins. The assessments will comprise a measurement of metabolic rate, oxygen capacity, heart rate and RPE. There will be a minimum period of 72 hours and a maximum period of 10 days between the AHIIT and R-AHIIT sessions to maximize performance on each protocol. For each session, the exercise lasts for 30 mins, with 5 mins warm up and 5 mins cool down. Each exercise consists of 10 sets of stationary running (1 min 90% maximal HR followed by 1 min dynamic rest at 70% maximal HR per exercise).

Study benefit:

It is hoped that this information will help us to understand the difference in metabolic responses of aquatic HIIT performed with and without resistive devices. At the completion of the trial, it can provide clinical practitioners with a clear understanding of the roles of implications of water immersion for HIIT and optimize the clinical practice guidelines for practitioners.

Possible side effect, risk or discomfort in the study

The test should not result in any serious discomfort, but participants may experience muscle fatigue or post-exercise soreness 1-2 days after the training sessions. Adequate rest after the training can achieve a full recovery.

Automaticity and confidentiality:

Your participation in this study is completely voluntary. You have every right to withdraw from the study before or during the study without penalty of any kind. All information related to the participants will remain confidential, and will be identified by codes only known to the research team.

If you would like to get more information about this study, please contact Ms Manny Kwok on 9206 . If you have any complaints about the conduct of this research study, please do not hesitate to contact Ms Vangie Chung, Secretary of the Department Research Committee on 27664329.

Thank you for your interest in participating in this study.

Dr. Billy So

Principal Investigator

THE HONG KONG POLYTECHNIC UNIVERSITY
香港理工大學

香港理工大學

康復治療科學系

研究資料

研究項目: 針對比較健康成年人在單回合水中高強度間歇帶氧運動(AHIIT)和阻力性高強度間歇帶氧運動(R-AHIIT)的靜息能量消耗和呼吸比之實驗

研究人員:

蘇俊龍博士	物理治療助理教授	香港理工大學康復治療科學系
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鄧卓翹先生	物理治療學碩士學生	香港理工大學康復治療科學系

研究項目簡介:

普遍認為疲倦，身體不適，壓力大和時間不足是成年人無法達致世界衛生組織對成年人所定立的運動量的原因。久坐的生活方式會增加高血壓和癌症等非傳染性疾病的危險因素，故此建議成年人進行充足和最佳的體育鍛煉劑量。高強度間歇訓練（HIIT）比傳統的中等強度的體育活動可以在相等的時間內燃燒更多的卡路里，並提供更大的喜悅。因此，HIIT 可以有效地優化運動的益處。

在水中進行訓練在近年越來越受歡迎，因為它可以降低受傷風險和與關節病相關的疼痛。水的物理特性，例如水的浮力和靜液壓，可以減少關節的負荷並促進血液循環。因此，水生環境是鼓勵參與體育活動的安全和有益的媒介。

鑑於水生 HIIT (AHIIT) 的應用越來越廣泛，本研究的目的是找出執行 AHIIT 和抵抗性 AHIIT (R-AHIIT) 在代謝反應方面的差異，並提出對 AHIIT 的運動劑量的建議。

如果您是 18-65 歲的健康成年人，我們誠邀您參與本研究。

研究方法和程序:
此研究針對 HIIT 練習。運動將在水中環境中在不同阻力以數字節拍器（MA-30，KORG；日本東京）設定的節奏測量的相同強度進行。

就 AHIIT 訓練，你會浸在水深及胸，溫度為 28°C 的水池中。首先，研究人員將為你配戴一個心臟頻率計（Polar®，Electro，Oi，Finland）來進行心率控制和監測。接下來，我們將為你配戴 (PNOE portable metabolic system)，配備呼吸面罩以收集你呼出的氣體。便攜式氣體分析儀將被放置在防水箱內，通過不鏽鋼支撐懸掛在參與者附近，以保護設備免受濺水。 我們會使用一個流量傳感器的套件將呼出的空氣作耗氧量分析。在每個 HIIT 練習中，我們將監視並記錄你的 RPE Borg 6-20 勞累率的主觀評分（BORG 1982）。你須聽從治療師的指示，進行指定的運動。

AHIIT 和 R-AHIIT 鍛煉將在香港理工大學進行，地址為九龍紅磡 11 育才路 X 座地下泳池。整個程序和評估將持續 45 分鐘，包括測量氧氣容量、心率和 RPE。評估將在 HIIT 訓練進行期間在水中進行。AHIIT 和 R-AHIIT 鍛煉之間的最短時限為 72 小時，最長期限為 10 天。每次練習持續 30 分鐘，練習前有 5 分鐘熱身，練習後有 5 分鐘降溫。一次訓練由十組組成（主動訓練 1 分鐘、主動恢復 1 分鐘，一共 20 分鐘），步速由運動增量壓力測試斷定。

本研究項目的益處:
此項研究有助我們更深入了解在水中不同阻力的 HIIT 的心肺反應的差異，為臨床提供水環境對 HIIT 的影響作清晰理解，並可以優化臨床實踐指南。

研究可能產生的副作用、危險、不適、處理方法:
高強度間歇訓練（HIIT）有機會導致延遲性肌肉酸痛或疲倦乏力；訓練後作充足的休息可達到全面舒緩效果，酸痛一般在 1-2 日內消失。

自願參與性質:
本研究全屬自願參與性質，閣下有權利於任何階段退出或中止參與是次研究。閣下之任何決定將不會受到任何懲罰或不公平對待。若你對是項研究有任何查詢或疑問，可致電

9206 ，與郭文瑩女士聯絡。如你對是項研究或研究員有任何投訴或建議，可致電 27664329，與部門研究委員會秘書鍾女士聯絡。

蘇俊龍博士 (首席研究員)
香港理工大學康復治療科學系

THE HONG KONG
POLYTECHNIC UNIVERSITY
香港理工大學

<u>CONSENT TO PARTICIPATE IN RESEARCH</u>

The effect of single bout aquatic high-intensity interval training (AHIIT) and resistive aquatic high-intensity interval training (R-AHIIT) on resting energy expenditure and respiratory ratio in healthy adult

-
<u>Research Team Members:</u>

Professor Shamay Ng	Professor	Department of Rehabilitation Sciences, PolyU	P
Dr. Billy So	Assistant Professor	Department of Rehabilitation Sciences, PolyU	D
Ms Manny Kwok	PhD student/ Musculoskeletal Physiotherapist	Department of Rehabilitation Sciences, PolyU	M
Mr. Gordon Tan	MPT student	Department of Rehabilitation Sciences, PolyU	M
Mr. Daniel Pang	MPT student	Department of Rehabilitation Sciences, PolyU	
Mr. Daniel Tang	MPT student	Department of Rehabilitation Sciences, PolyU	

I ________________________ hereby consent to participate in the captioned research conducted by Dr. Billy So Chun Lung.

I understand that information obtained from this research may be used in future research and published. However, my right to privacy will be retained, i.e. my personal details will not be revealed. This study abides by the Declaration of Helsinki.

The procedure as set out in the attached information sheet has been fully explained. I understand the benefits and risks involved. My participation in the project is voluntary.

I acknowledge that I have the right to question any part of the procedure and can withdraw at any time without penalty of any kind.

If I would like to get more information about this study, I can contact Ms Manny Kwok at 9206 . If I have any complaints about the conduct of this research study, I can contact Ms Vangie Chung, Secretary of the Department Research Committee at 27664329.

Name of Participant	:	____________________	Date	:	____________________
Signature of Participant	:	____________________			
Name of Witness	:	____________________	Date	:	____________________
Signature of Witness	:	____________________			
Name of Researcher	:	____________________	Date	:	____________________
Signature of Researcher	:	____________________			

香港理工大學
康復治療科學系
研究資料

研究項目: 針對比較健康成年人在單回合水中高強度間歇帶氧運動(AHIIT)和阻力性高強度間歇帶氧運動(R-AHIIT)的靜息能量消耗和呼吸比之實驗

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本人 ____________________明白是項研究的詳細情況，並願意參與由蘇俊龍博士開展的是項研究。本人的參與，是屬於自願性質。

本人明白在任何時間可以放棄及退出測試，而毋須給予任何理由。本人亦不會因為退出測試而受到任何處罰或不公平對待。

本人得悉並且明白參與是次研究所帶來的潛在危險。除有關研究之研究員，本人的個人資料不會展示給予與任何人。如未經本人的同意，本人的名字及照片並不會刊登於是項研究的任何發表佈告之中。這項研究是符合赫爾辛基宣言的原則。

本人若果對是項研究有任何查詢或疑問，可致電 9206　　，與郭女士聯絡。如對是項研究或研究員有任何投訴或建議，可致電　27664329，與部門研究委員會秘書鍾女士聯絡。本人簽署後表示本人已收到此同意書副本乙份。

簽署(參與者)　:　______________________　日期　:　______________________
名稱(參與者)　:　______________________

簽署(見證人)　:　______________________　日期　:　______________________
名稱(見證人)　:　______________________

簽名(研究員)　:　______________________　日期　:　______________________
名稱(研究員)　:　______________________

9.4.3 Accepted Manuscript by the Frontiers in Sports and Active Living (Chapter 6)

Acute effect of resistive aquatic high-intensity interval training on metabolic costs in adults

Introduction

The sedentary lifestyle has been proven to be associated with body mass changes and obesity, increasing the risk of deaths caused by comorbidities such as Type 2 diabetes mellitus, dyslipidemia, hypertension, and obstructive sleep apnea(Park et al., 2020). Adults often face barriers to participating in exercise, such as tiredness, physical discomfort, stress, and time constraints (Martin J. Gibala et al., 2012). To address these challenges, high-intensity interval training (HIIT) is promoted as a way to enhance basal metabolism, exercise enjoyment, and time efficiency (Bartlett et al., 2011).

HIIT involves alternating between high-intensity exercise and rest or moderate-intensity active recovery periods. The exercise intensity during HIIT reaches 85-90% of maximal heart rate (HR) or over 90% of maximal oxygen consumption (VO_2) during the work phase (Gibala & Jones, 2013). HIIT can also be performed in a water-based environment, and the properties of water may lead to more favorable training effects compared to land HIIT (LHIIT)(Nagle, Sanders, & Franklin, 2017).

Water possesses several properties that contribute to its training benefits, including buoyancy, hydrostatic pressure, drag force, and thermodynamics(Becker, 2009). Buoyancy effectively reduces a person's body mass when immersed in water, with 40% of weight offloaded when the umbilicus is immersed and 60% when the xiphoid is immersed(Becker et al., 2009). Hydrostatic pressure displaces blood from the venous and lymphatic system back to the heart, increasing stroke volume (Plotnick et al., 1986). Drag force refers to the resistance experienced by an object in water due to its shape and size, and it is directly proportional to the amount of resistance encountered (Colado & Triplett, 2009). Water is also an efficient heat conductor, transferring heat 25 times faster than air (Pendergast et al., 2011) . It can easily deliver temperature to immersed body parts, with typical hydrotherapy pools operating within the range of 33.5-35.5 degrees Celsius (Bergamin et al., 2015a).

Evidence suggests that aquatic HIIT (AHIIT) elicits more desirable training effects than LHIIT. Studies have shown that, at the same exercise intensity, AHIIT is associated with lower perceived exertion during and after training compared to LHIIT, as measured by the Borg Rating of Perceived Exertion Scale (Andrade et al., 2022). AHIIT has also demonstrated faster recovery than LHIIT, as indicated by a more than 10% reduction in post-exercise heart rate reserve (Faíl et al., 2022). Moreover, AHIIT has favorable effects on oxygen consumption and energy expenditure(Depiazzi et al., 2018). In a study conducted by Kwok et al. (2022), AHIIT was found to result in a decrease in maximal heart rate (HR max) and work and recovery heart rates compared to LHIIT (M. M. Y. Kwok, E. T. C. Poon, et al., 2022). These findings suggest that a water-based environment offers more favorable training outcomes for HIIT.

However, to the best of our knowledge, no current study has investigated the variation of AHIIT and compared which variation yields the best training effects on basal metabolism. Basal metabolism refers to the energy expenditure of a person at rest and reflects their health status and physical fitness to some extent(Schaun et al., 2018). Previous research has shown that high-intensity interval resistance training increases post-exercise resting energy expenditure (REE) and respiratory ratio, indicating an improvement in basal metabolism and fat oxidation(Paoli et al., 2012). Aquatic resistive training has been widely examined in recent year, which resulted in increased in maximal dynamic strength of the lower limbs(Barbosa et al., 2009). Furthermore, resistive exercises increase power and, as a consequence, improve physical fitness, health, and functional autonomy (Bayles & Swank, 2018). The density of water generates increased muscle strength because water generates resistance 900 times greater than in air(McGinnis, 2020). An aquatic resistance device may be deployed to ride along the resistance caused by the drag force when moved in water. The use of resistive devices generates muscular tension when moved in opposition to the water. Hence, the drag force is responsible for the resulting resistance during the use of the resistive device and can be defined as a resistant force opposite to the direction of movement of an object, which can occur both in front of and behind the object that is moved(Pöyhönen et al., 2000). The magnitude of the drag force depends mainly on the surface area and the shape of the device but is also determined by the velocity of movement (cadence) such that an increase in the velocity of movement exponentially increases the drag force. Drag force imposed by water enables a greater recruitment of motor units with higher excitation thresholds activated. The effects of resistance training on resting metabolic rate (RMR) are less clear and has the potential to increase RMR and daily energy expenditure (Kirk et al., 2009). This suggests that performing resistive AHIIT may potentially have a greater impact on resting metabolic rate than AHIIT. In the context of aquatic exercise, resistance can be applied by increasing cadence and limb surface area. In this study, resistive AHIIT is performed by wearing resistance boots to increase the surface area of the lower limbs.

Hence, AHIIT has demonstrated superior improvements in cardio-metabolism compared to LHIIT, and resistive AHIIT represents a variation of AHIIT that potentially yields greater training effects on basal metabolism. However, no studies have been conducted to examine the training effects of resistive AHIIT on basal metabolism or to compare the metabolic effects of

AHIIT and resistive AHIIT. Therefore, the research question for this study is twofold: 1) What is the exercise effect of resistive AHIIT on basal metabolism? and 2) What are the differences in basal metabolism between AHIIT and resistive AHIIT in healthy adults? The objective of this study is to investigate metabolic responses after a single bout of resistive AHIIT and to compare the differences between AHIIT and resistive AHIIT. It is hypothesized that resistive AHIIT will result in comparable or superior basal metabolism compared to AHIIT.

Materials & Methods

Study design and Procedure

This study is a randomized crossover study aimed at comparing the cardiorespiratory and metabolic responses of young, healthy adults performing AHIIT and resistive AHIIT, with a focus on matching intensities between aerobic and interval resistance training.

Prior to participating in the study, subjects underwent a screening interview and a familiarization period. Informed consent was obtained from all subjects. The screening process involved a standard health questionnaire (International Physical Activity Questionnaire), as well as measurements of resting heart rate, blood pressure, body mass, and height, in order to assess the subjects' habitual physical activity levels (Puthoff et al., 2006). All subjects completed a familiarization session before the study, during which the details of the exercises, including the range of movements and the use of a mouthpiece and Hans Rudolph valve, were explained. A registered aquatic physiotherapist provided feedback and instruction to the subjects during the trials and sessions.

The HIIT exercise, specifically stationary running, was performed at an intensity set at 90% of the subjects' maximum heart rate (HRmax), as determined by an incremental test. This intensity was used to establish individualized cadence for later high-intensity aerobic interval training. Both AHIIT and resistive AHIIT exercises were performed at the same matched intensity, measured by cadence (130-150 beats per minute), set by a digital metronome (MA-30, KORG; Tokyo, Japan). The exercises took place in a heated pool with a water temperature of 34°C and a depth of 0.95-1.40m at a hydrotherapy Pool. Each exercise session lasted for 10 minutes and was preceded by a 5-minute warm-up and followed by a 5-minute cool-down. During the 10-minute exercise period, there were 5 sets of stationary running, with each set consisting of 1 minute at 90% HRmax followed by 1 minute of dynamic rest at 70% HRmax. The AHIIT and resistive AHIIT protocols were identical in setup, except for the use of resistance boots in the resistive AHIIT protocol (Figure 1) and the cadence for the resistive AHIIT exercises.

Insert Figure 1 here

To measure the subjects' resting energy expenditure (REE) and respiratory exchange ratio (RER), a portable metabolic analyzer (PNOE Cardio-metabolic Analyzer, Yale Street, USA) was used (Figure 2). The analyzer collected breath-by-breath data by means of an aqua trainer adapter covering the flow sensor, which measured inhaled and exhaled air. The RER and REE (breath-by-breath oxygen consumption) were calculated using the device. The gas analysis system was calibrated before each test using standard reference gases and a 3-L syringe (Model 5530, Hans Rudolf, Kansas City, MO). During each session, subjects wore a breathing mask for the collection of expiratory gas. The set included a facemask covering the nose and mouth, a cap, and a flow sensor, which directed the exhaled air to the PNOE device. Heart rate was continuously monitored and recorded at a frequency of 1Hz using a heart rate sensor (Polar OH1, Kempele, Finland), (Figure 3). Perceived exertion was assessed during the intervention using the

Borg 15-point rate of perceived exertion (RPE) scale (Barbosa et al., 2009). Subjects were shown an A3-format plastic panel (297mm x 420 mm) representing the Borg's scale for this purpose.

Insert Figure 2 here

Insert Figure 3 here

Sampling and sample size calculation

Subjects were recruited through convenience sampling at the Hong Kong Polytechnic University. The sample size was determined based on the primary outcome of a previous study that compared REE and RER in aquatic aerobic and resistive training (Heisz et al., 2016). Effect sizes (ES) were calculated by Cohen's d. Using the G*power software and based on the effect size 0.28 obtained, the primary outcome (REE) assuming a 5% type I error and 80% power, the sample size of 17 or more subjects per group was calculated for the primary outcome, assuming a 5% type I error and 80% power. Considering an estimated attrition rate of 20%, a total enrolled sample size of 20 was determined to ensure sufficient statistical power.

Ethics approval and informed consent

Prior to data collection, both the ethical approval and informed consent sheets were obtained.

Outcome measures

The primary outcomes of this study were REE and RER. REE accounts for 60-75% of daily energy expenditure, and was estimated by the gas analyzer PNOE. It measured resting energy expenditure via indirect calorimetry. It measured the amount of oxygen inhaled (VO_2) and the amount of carbon dioxide exhaled (VCO_2). (Paoli et al., 2012). RER is the ratio of carbon dioxide production to oxygen uptake, directly measured using VCO_2 and VO_2 helps determine the proportion of carbohydrates and fats used for energy consumption at rest (Patel et al., 2018). Values of 0.7, 0.8, and 1.0 represent respiratory quotient values for fat, protein, and carbohydrates, respectively (Fonseca et al., 2018; Patel et al., 2018)

The secondary outcomes included VO_2 peak, HR max, HR mean, TEE, and RPE. For male subjects, a good level of VO_2 peak is typically in the range of 42-46 ml/kg/min, while for female subjects, it falls within 33-37 ml/kg/min (Scribbans et al., 2016). VO_2peak, HR max, HR mean, and TEE were selected to reflect the cardiometabolic aspects of the subjects, while RPE was monitored to observe their perceived exertion.

Inclusion and exclusion criteria

Subjects were recruited through convenience sampling. The inclusion criteria were as follows: 1) age between 18-35, 2) clinically healthy, 3) no musculoskeletal, bone and joint, cardiac, or pulmonary distress requiring medication, and 4) not pregnant. The exclusion criteria were: 1) presence of cardiovascular, respiratory, musculoskeletal, orthopedic, or metabolic problems, neurological pathology, recent lower limb fracture or surgical intervention within the past six months, 2) hydrophobia, and 3) other pathologies that would hinder participation in aquatic exercise. A total of 20 healthy subjects (9 females, 11 males) were recruited, and all 20 subjects (9 females, 11 males) completed the study.

Data collection procedure

The data collection procedure involved subjects participating in an incremental exercise test (stationary run) while immersed in the test pool. The incremental test was performed prior to the exercise interventions to confirm an individualized cadence required at a matched level of exercise intensity (stationary running at 90% with 1-min active recovery at 70% HR max in between) in each condition. Instructions for stationary running directed participants to flex the hip and knee to as close to 90° as comfortable and control allowed and then push to straighten up hip and knee. Prior to testing, all exercises were demonstrated first, then practiced once. Participants was monitored continuously and recorded at a frequency of 1Hz by a HR sensor (Polar OH1, Kempele, Finland). The HR sensor used has been shown to provide valid and reliable HR data (Bergamin et al., 2015a, 2015b). During the incremental test, gas exchange data were obtained by a portable metabolic device PNOE. The PNOE device was operated by a breath-by-breath mode which continuously measures volume and determines expired gas concentrations simultaneously. It was calibrated prior to each session according to manufacturer's specifications. PNOE has been validated in previous research, as compared to a validated stationary metabolic cart (COSMED QUARK-CPET) (Tsekouras et al., 2019a, 2019b). The incremental protocol increased the exercise load from 85 beats per minute (bpm) and increased the cadence by 15 bpm every 2 minutes for each progression (A. C. Kanitz et al., 2015). A metronome (Intelli IMT 300, Japan) was used to monitor the speed of movements throughout the trial. The HR, VO_2, RPE per minute were recorded. VO_2max was considered to be attained when the following standardized criteria were met: (1) a respiratory exchange ratio of greater than or equal to 1.10; (2) failure of heart rate to increase with increases in workload; (3) post-exercise blood lactate ≥8.0 mmol•L^{-1}; (4) clear signs of exhaustion (facial flushing, unsteady gait) and (5) refusal to carry on despite strong verbal encouragement (Edvardsen et al., 2014). Maximal oxygen consumption (VO_2max) was determined by both aquatic and land incremental tests with stationary running to test to volitional exhaustion. HR, percentage of VO_2 max (%VO_2 max), percentage of HR max (% HR max), measured as the highest value obtained were recorded between the two environments. Blood lactate was measured via capillary blood sampling from the fingertips with a portable analyzer (Lactate Plus, Nova Biomedical, Waltham, Massachusetts). Data collected from the incremental test were used to determine the intensity required for the exercise interventions for each participant.

Experimental data were then collected during the AHIIT or resistive AHIIT exercises, which consisted of 5 work-rest cycles lasting a total of 10 minutes. REE, RER, and VO_2 peak were continuously recorded using the PNOE breath-by-breath analyzer. Maximal and average HR were recorded using the Polar OH sensor while connected to PNOE, and RPE was recorded 15 seconds before transitioning to the next work/rest stage. REE referred to the energy expenditure prior to exercise with an RPE of 6 while immersed in water, as well as 2 minutes after the 10-minute intervention. TEE represented the cumulative energy expenditure during the 10 minutes of HIIT exercise, recorded and calculated using PNOE software. VO_2 peak and mean RER were also continuously measured by PNOE. HR max and HR mean were measured by the Polar OH sensor and then recorded and analyzed using PNOE software.

Statistical analysis

Descriptive statistics were first computed for the demographic data and a series of Shapiro-Wilk's tests were conducted to evaluate the normality of the data distributions. Continuous data measures were then summarized with means and SDs. Analyses were performed using the Statistical Package for Social Sciences for Windows version 22.0 (SPSS, Inc., Chicago, IL.). Statistical significance was delimited at $P < 0.05$. All continuous variables are presented as means and standard deviation. Mean differences among groups (AHIIT and resistive AHIIT) for each primary and secondary outcome were tested by mixed model repeated measures ANOVA. Mixed effects models were applied to analyze the effects of group (AHIIT vs resistive AHIIT), time (pre and post interventions), and the interaction between group and time on both outcomes. Turkey post-hoc analysis was used to analyse within-group and between-group comparisons.

Results

Subject Characteristics

There was a total of 20 subjects with 11 males and 9 females. The mean age of the participants was 24.27±6.59 years for male and 25.44±4.22 years for females. Their average height was 174.27±6.40 cm for male and 161.44±4.50 cm for female. Male and female average weight was 66.64±9.11 kg and 57.28±9.02 kg respectively. All the participants completed 4 stages of incremental tests and training protocol.

Results of Primary outcomes

REE within group pre-post were found to be significantly different ($p<0.001$ and $p<0.001$ for AHIIT and resistive AHIIT groups respectively). However, change in REE between groups do not have significant difference ($p=0.875$ and $p=0.332$ for AHIIT and resistive AHIIT groups respectively) (Figure 4). Mean RER between AHIIT and resistive AHIIT exercise were not found to have significant between group difference [F (1,38) = =0.615) (Figure 5). Group-by-time interactions revealed an insignificant difference in REE and RER [$F(1,38) = 0.17$, $P=0.76$, $ŋ^2=0.01$] and RER [$F(1,38) = 0.02$, $P=0.88$, $ŋ^2=0.01$] between AHIIT and resistive AHIIT (Table 1).

Insert Figure 4 here

Insert Figure 5 here

Insert Table 1 here

Results of Secondary outcomes

TEE of 10 minutes of AHIIT and resistive AHIIT exercise were not found to be significantly different (p=0.782) (Figure 6). HR max and HR mean during 10 minutes of AHIIT and resistive AHIIT exercise were not found to have significant between group differences (p=0.578 and p=0.615 respectively) (Figure 7). None of the secondary outcomes exhibited a significant difference in the group-by-time interactions or between groups. TEE [F (1,38) =0.81, P=0.59, η^2=0.001], HR max [F (1,38) =0.01, P=0.50, η^2=0.012] and HR mean [F (1,38) =0.08, P=0.48, η^2=0.52]. However, there was a significant interaction shown in TEE when gender was added as covariates (ES: 0.38, p<0.01).

Insert Figure 6 here

Insert Figure 7 here

VO_2 peak during 10 minutes of AHIIT and resistive AHIIT exercise were not found to have significantly difference between groups (p=0.449) (Figure 8). RPE before and after exercise were significantly different for AHIIT and resistive AHIIT exercises (p<0.01 and p<0.01 respectively). AHIIT and resistive AHIIT exercise were not found to have significant between group difference (p=0.615). Group-by-time interactions revealed an insignificant difference in VO_2 peak [F (1,38) =0.00, P=0.81, η^2=0.06] between AHIIT and resistive AHIIT (Table 2). However, there was a significant interaction shown in VO_2 peak when gender was added as covariates (ES: 0.37, p<0.01)

Insert Table 2 here

Insert Figure 8 here

Simple linear regression was used to predict RER from RPE. Simple linear regression model was used to show the best adjustment in all analysis, with significant relationship (p= 0.013) observed between subjective and the metabolic variables between RPE and RER (Figure 9). The R (0.543) was shown to be statistically significant, the RPE is a significant predictor of RER. And r values were classified according to the recommendations from Safrit-and Wood (Safrit & Wood, 1995), i.e., 0-0.19 as no correlation, 0.2-0.39 as low correlation, 0.4-0.59 as moderate correlation, 0.6-0.79 as moderately high correlation, and 0.8-1.0 as high correlational analyses.

Insert Figure 9 here

Discussion

To the best of the author's knowledge, this study represents the first attempt to investigate the impact of AHIIT and resistive AHIIT on metabolic and cardiorespiratory responses in healthy adults. The objectives of this study were to determine whether an acute session of resistive AHIIT would produce comparable or increased metabolic and cardiorespiratory responses compared to AHIIT at the same training intensity. The key findings of this study are summarized as follows: 1. Both AHIIT and resistive AHIIT were associated with higher resting energy expenditure (REE) following a single session, but no significant differences were observed between the two groups. 2. Significant differences were observed between the two groups in terms of rating of perceived exertion (RPE), with resistive AHIIT showing a higher RPE. Additionally, there was a moderate correlation between RPE and respiratory exchange ratio

(RER). 3. Both within-group and between-group analyses revealed significant pre-post differences for various outcomes. 4.Regarding the cardiometabolic outcomes of HR max, HR mean, VO_2 peak, and TEE, no significant differences were observed between the two groups.

Resting energy expenditure

The results of this study support our initial hypothesis that a single session of resistive AHIIT would have a comparable effect on basal metabolism as AHIIT. Both AHIIT and resistive AHIIT significantly increased post-training basal metabolism, indicating that performing high-intensity interval training in an aquatic environment enhances basal metabolic rate in general. In contrast, studies conducted in a land-based environment by Hazell et al. (2012) and Skelly et al. (2014) demonstrated that a single session of LHIIT significantly increased resting energy expenditure (REE) after 1 hour and 24 hours of exercise (Hazell et al., 2012; Skelly et al., 2014). When comparing LHIIT and AHIIT, Kwok et al. (2022) found no significant difference in terms of energy expenditure (M. M. Kwok et al., 2022). This suggests that high-intensity interval training improves basal metabolic rate in general, regardless of the training environment. Therefore, the effect on basal metabolism should not be the sole consideration when deciding whether to perform high-intensity interval training on land or in water. However, AHIIT offers advantages such as lower heart rate, lower perceived exertion during exercise, and faster recovery rates compared to LHIIT. Factors such as cardiometabolic responses, perceived exertion, recovery rate, and the properties of water, such as buoyancy and hydrostatic pressure, should be primary considerations when choosing AHIIT over LHIIT (Chien et al., 2020; M. M. Kwok et al., 2022).

Although both AHIIT and resistive AHIIT resulted in a significant increase in REE, this study did not find evidence to suggest that resistive AHIIT would elicit a greater increase in post-training basal metabolism compared to AHIIT. This finding is consistent with the secondary outcomes, as no significant differences were observed between the AHIIT and resistive AHIIT groups in terms of HR max, HR mean, and VO_2 peak. Potential reasons for this lack of difference can be explained by factors highlighted by Paoli et al. (2012): the specific exercise selected (in this case, stationary run) directly influences the trained muscle groups and consequently the resulting REE. Additionally, the active recovery periods between sets, as well as the number of repetitions and sets, are crucial factors that determine whether a statistically significant difference in REE or other secondary metabolic outcomes can be observed (Paoli et al., 2012). Therefore, it is suggested that besides exercise intensity, other training details and factors, such as the specific muscle groups targeted and the overall exercise setup, should be taken into account to achieve a higher post-training REE in a single session of AHIIT.

Metabolic outcomes

Regarding another primary metabolic outcome, respiratory exchange ratio (RER), our study found no significant difference between the AHIIT and resistive AHIIT groups. This suggests that adding a resistive component to our originally designed AHIIT protocol did not result in a statistically significant difference in RER compared to non-resistive AHIIT. When considering other cardiometabolic outcomes such HR max, HR mean, VO_2 peak, and TEE, the addition of resistive components did not lead to significant changes in these outcomes. This led the researchers to question whether non-resistive HIIT, which solely relies on water as a resistance medium, is sufficient to challenge RER as a metabolic marker in healthy adults.

Due to technical limitations with the PNOE analyzer, our study was only able to measure the change in RER rather than retrieving pre-post RER values for both the AHIIT and resistive AHIIT groups. Our results showed no significant difference in RER between the AHIIT and resistive AHIIT groups ($p = 0.981$). Additionally, a study by Tang et al. (2022) demonstrated the pre-post RER difference in an AHIIT group compared to moderate intensity continuous training on land(Tang et al., 2022). In that study, thirty-one inactive adults were randomly assigned to either AHIIT or moderate intensity continuous training on land, and various parameters including central hemodynamics, endothelial function, and aerobic fitness were measured over a 6-week period. The results showed an effect size of -0.222 for the influence of AHIIT on RER, while the effect size for moderate intensity continuous training on land was 0.00. This indicates that neither training protocol, whether in a land or aquatic medium, produced a statistically significant effect on RER.

Despite the lack of evidence establishing a positive relationship between AHIIT and RER, AHIIT is still considered beneficial for other cardiometabolic outcomes such as HRmax, VO2max, and energy expenditure when compared to continuous aerobic exercise protocols on land (Becker, 2009).

Self-perceived exertion

Our study indicated that adding a resistive component to the AHIIT protocol resulted in a higher perceived exertion level, as reflected by statistically significant differences in rating of perceived exertion (RPE). However, the addition of the resistive component to aquatic HIIT did not lead to statistically significant differences in the studied cardiometabolic outcomes. In terms of RPE, there were significant differences within both the AHIIT and resistive AHIIT groups

($p<0.01$), as well as between-group differences ($p=0.007$). Extra drag force added by additional resistance becomes significant on load enforcement therefore even a small increase in resistance leads to a considerable rise in rate of perceived exertion (Hilman et al). A moderate correlation was found between RPE and RER in the resistive AHIIT group ($r=0.543$). This result aligns with other studies that highlight RPE as an indicator of exercise intensity (Andrade et al., 2022). Additionally, the RPE is a significant predictor of RER and accounted for 9.3% of the variance in RER because of maximal tests require an individual to exercise to the point of volitional fatigue or until a clinical indication to stop. Criteria have been used to confirm the maximal effort which included RPE at peak exercise > 17 on the 6-20 scale or >7 on the 0-10 scale or a peak of RER ≥ 1.10.

Regarding other cardiometabolic outcomes such as REE, RER, VO_2 peak, HR max, HR mean, and TEE, our results showed no statistically significant differences between the two groups. Since both AHIIT and resistive AHIIT demonstrated comparable effects on resting energy expenditure and cardiometabolic responses, but participants perceived a lower exertion level in AHIIT compared to resistive AHIIT at the same exercise intensity, AHIIT can be preferred as a training program to enhance participants' exercise compliance and overall health. This finding also suggests that instead of progressing subjects with a resistive component in a water-based environment, the focus could be on monitoring subjects' RPE and maintaining appropriate heart rates during AHIIT (M. M. Kwok et al., 2022). For the effect of sex as covariate that influenced VO2 peak. This is primarily due to male have a higher ventricular ejection volume, hemoglobin concentration, muscle mass and lower body fat. And since as muscle is the greatest consumer of oxygen during exercise, greater muscle mass in men is responsible in part for their greater absolute VO2 peak compared to women.

Judging by the standardized regression coefficients, RPE is an important predictor of RER. Those with higher RPE tend to have greater RER, but no causal relationship can be identified due to cross sectional design.

Beneficial effects of resistive AHIIT as reflected in other metabolic outcomes

Resistive training did not lead to statistically significant changes in resting energy expenditure (REE) and respiratory exchange ratio (RER) when compared to non-resistive HIIT. However, the researchers hypothesized that the beneficial effects of resistive training might be observed in other metabolic outcomes such as muscle fiber capillarization, muscle morphology, and succinate dehydrogenase (SDH) activity (Leuchtmann et al., 2020).

In a randomized controlled trial conducted by Leuchtmann et al. (2020), twenty older recreationally active men were recruited and assigned to either 12 weeks of habitual observation followed by 12 weeks of resistance training (RT), or 12 weeks of high-intensity interval training (HIIT) followed by 12 weeks of RT (Leuchtmann et al., 2020). The results showed that both groups were equally effective in improving capillarization and oxidative enzyme activity, as assessed through biopsies of the vastus lateralis muscle. Furthermore, the RT group was able to sustain the metabolic parameters induced by the HIIT intervention. This suggests that future research could focus on examining whether there are significant differences in the aforementioned metabolic outcomes between AHIIT and resistive AHIIT, rather than relying solely on RER and REE as metabolic indicators.

Strengths and Limitations

This study has several strengths. The novelty of an incremental test performed prior to the AHIIT and resistive AHIIT intervention allowed a correct, optimal and matched intensity of REE, RER, HR mean, %HR max, %VO_2 max, RPE and monitoring in both groups for comparison. The aquatic incremental tests were conducted in both non-resistive and resistive groups, which were considered the most accurate methodology for exercise intervention as these allowed the standardization of water as the medium as well as previously aforementioned properties (i.e. buoyancy, hydrostatic pressure, drag forces) of water compared to conducting the incremental tests on land.(Nagle, Sanders, & Franklin, 2017) By using the HR matched cadence for determining the equivalent intensity for 90% and 70% HR max, this could individualize the training intensity for healthy adults and to serve as the baseline measure for later cardiometabolic evaluation.

Despite these strengths, major limitations of the present study included small sample size and hence caution should be taken when generalizing to the older population. Moreover, the small sample size of 20 subjects might hinder the generalization to the clinical populations. Regarding the design of this study which was a cross-sectional study for examining the instantaneous effect of AHIIT and resistive AHIIT only, the long-term effect of these interventions on cardiometabolic outcomes were yet to be proven. Nevertheless, our results provide practical guidelines in applying matched intensity of aquatic incremental tests followed by the HIIT corresponding interventions. From a practical point of view, the HR matched cadence could allow healthy subjects to achieve training intensity equivalent to 90% and 70% of

HRmax in water medium. The distinctive characteristics of water like buoyancy and hemodynamic properties enabled subjects to enjoy physiological advantages of AHIIT. A randomized control study could be suggested studying the long-term effect of AHIIT on cardiometabolic outcomes which might yield different results from the single bout session.

Conclusion

In summary, our findings indicate that both AHIIT and resistive AHIIT result in significant differences in RPE, while showing no significant differences in other metabolic and cardiorespiratory responses such as HR max, HR mean, VO_2 peak, and TEE. The moderate correlation between RPE and RER in resistive AHIIT suggests that RPE can serve as an indicator for prescribing exercise intensity effectively. The addition of a resistive component to AHIIT can yield comparable results to using water's drag force as the sole medium for resistance. AHIIT still offers cardiometabolic benefits when compared to resistive AHIIT. Therefore, future research should focus on conducting randomized controlled trials to examine the long-term effects of comparing AHIIT with resistive AHIIT.

Data availability statement

The datasets analyzed in this research are available from the corresponding author upon reasonable request.

Ethics statement

The study was conducted in accordance with the Declaration of approved by the Ethics Committee for Research on Human Beings of the Hong Kong Polytechnic University approved (reference no.: HSEARS20220204005). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

MK: Conceptualization, Methodology, Project administration, Writing – original draft. GT: Data curation, Investigation, Methodology, Writing – original draft. BS: Data curation, Investigation, Methodology, Writing – original draft. PPZ: Data curation, Investigation, Methodology, Supervision: Data curation, Investigation, Project administration, Writing – original draft. YZ: Conceptualization, Formal Analysis, Methodology, Writing – original draft. SN: Conceptualization, Formal Analysis, Methodology, Writing – review & editing. MN: Conceptualization, Formal Analysis, Methodology, Writing – review & editing.

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Conflict of interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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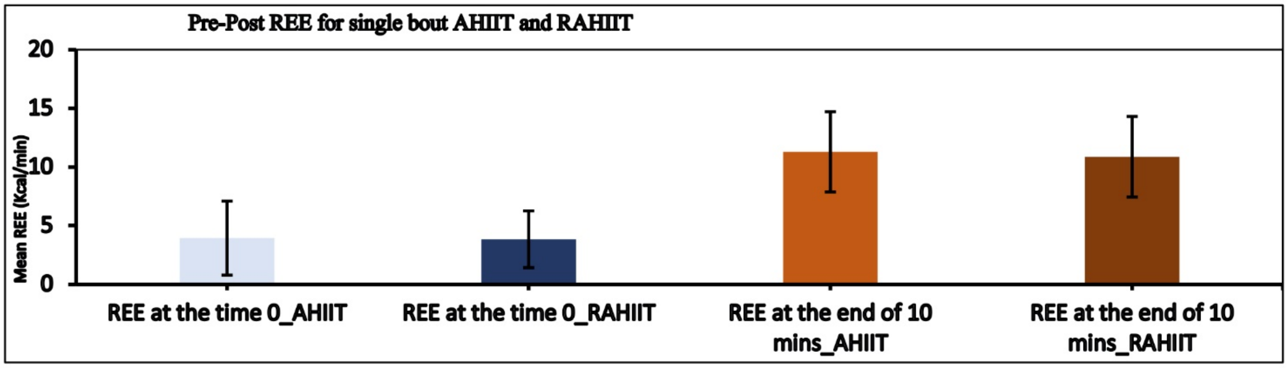
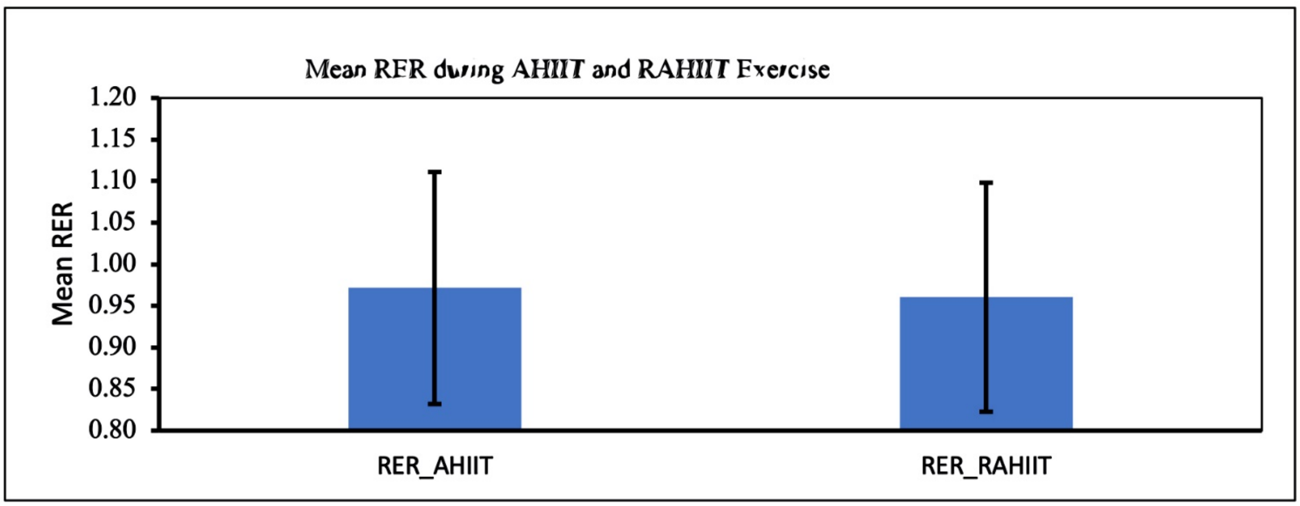


Figure 4: Pre and post resting energy expenditure (REE) difference in AHIIT and Resistive AHIIT (mean ± SD)



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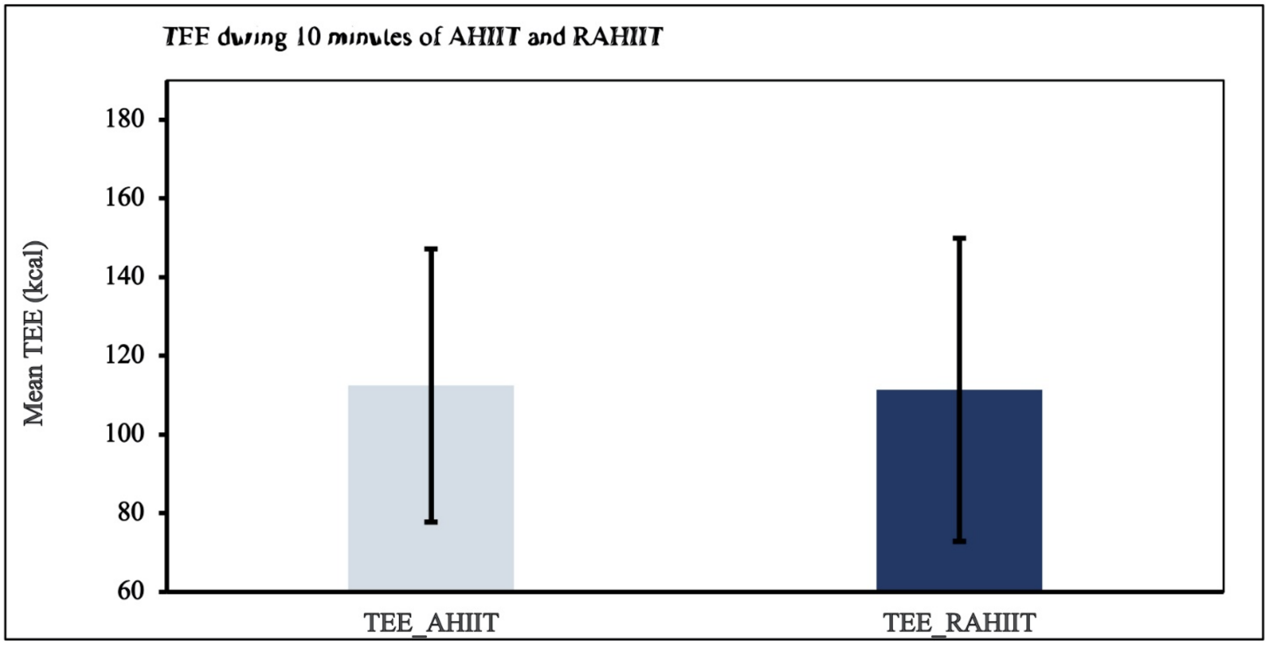

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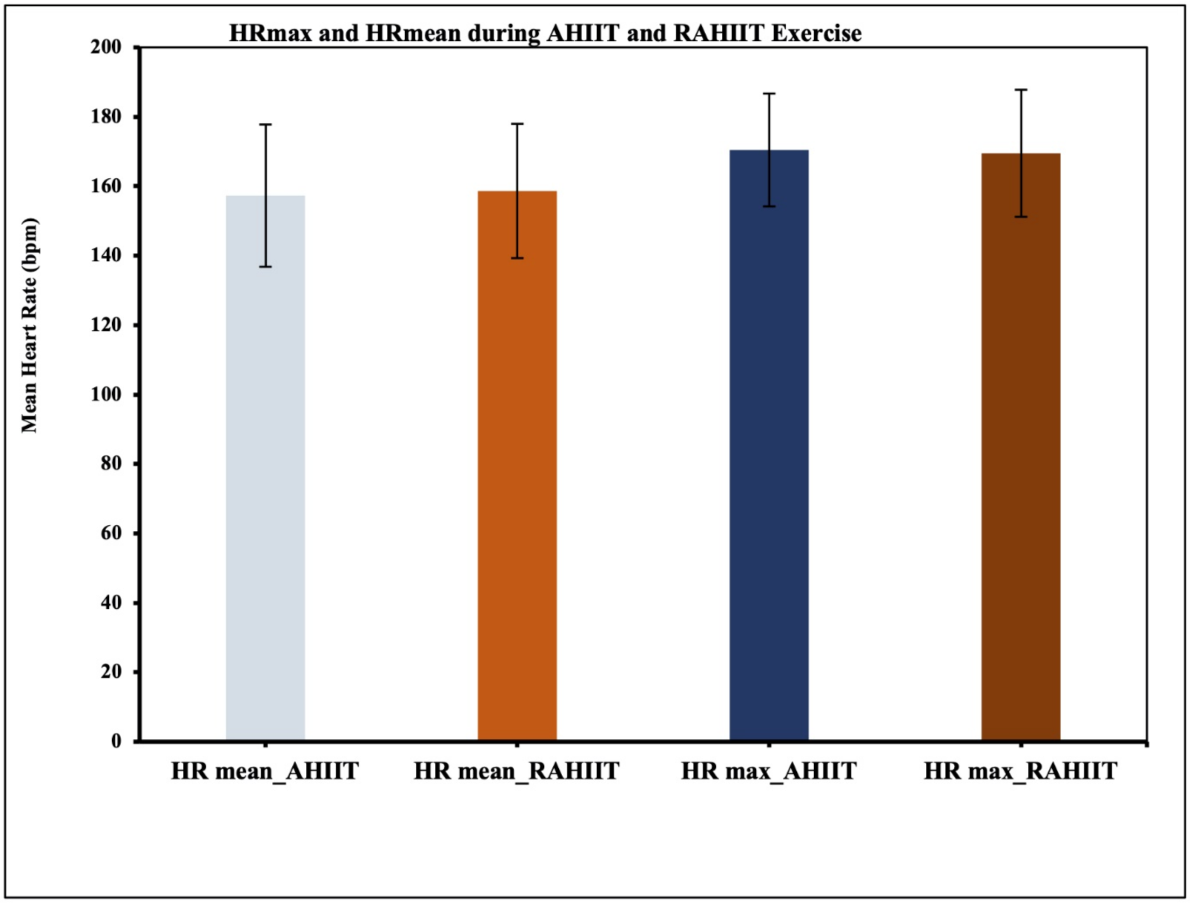

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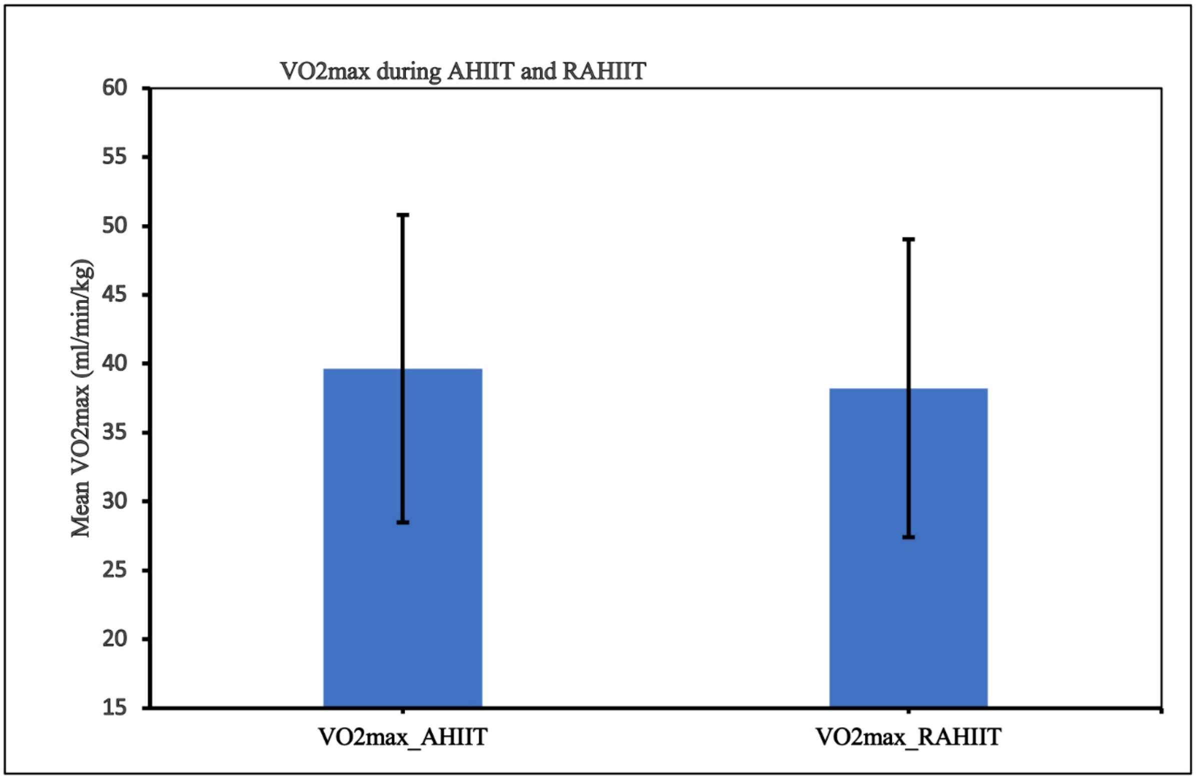

Figure 8: Peak aerobic power (VO_2 peak) during AHIIT and Resistive AHIIT exercise (mean ± SD).

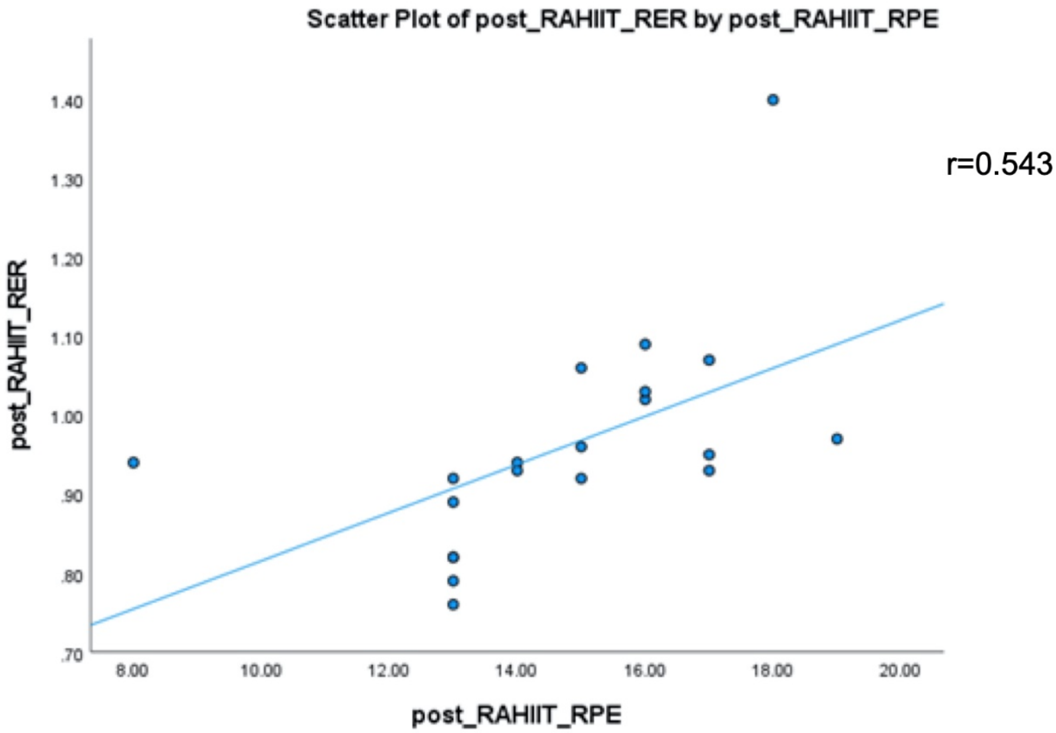

Figure 9: Correlation between the rate of perceived exertion (RPE) and respiratory exchange ratio (RER), (mean ± SD), r=0.543

Parameters	AHIIT (n=20)		Resistive AHIIT (n= 20)		Time effect			Group *time effect		
	Pre-intervention	Post-intervention	Pre-intervention	Post-intervention	F	P value	ES	F	P value	ES
REE (Kcal/min)	3.95±3.15	11.30±3.42*	3.85±2.42	10.88±3.43*	14.99	<0.01	0.29	0.17	0.76	0.01
RER	1.1±0.15	0.97±0.14*	1.1±0.16	0.97± 0.13*	7.04	<0.05	0.17	0.02	0.88	0.01

Table 1: Primary outcomes in AHIIT and resistive AHIIT (mean ± SD)

*time effect difference upon pairwise comparison (p<0.05)

Note: REE, resting energy expenditure, RER, Respiratory exchange ratio, ES, effect size

Parameters	AHIIT (n=20)		Resistive AHIIT (n= 20)		Time effect			Group *time effect		
	Pre-intervention	Post-intervention	Pre-intervention	Post-intervention	F	P value	ES	F	P value	ES
TEE (Kcal)	113.26±57.84	101.20±37.66	123.84±60.32	103.72±35.74	0.78	0.23	0.04	0.81	0.59	0.001
HR max (bpm)	178.20±14.94	170.45±16.25	173.20±15.52	169.75±18.04	2.73	0.15	0.07	0.01	0.50	0.012
HR mean (bpm)	146.3±16.43	160.25±18.19	143.70±17.39	161.70±16.53	0.52	0.20	0.05	0.08	0.48	0.52
VO_2 peak (ml/min/kg)	42.61±9.98	39.65±11.16	43.21±11.08	39.61±9.57	0.27	0.63	0.24	0.00	0.81	0.06
RPE	6.00±0.00	13.70±1.72*	6.00±0.00	14.75±2.45*	35.76	<0.01	0.49	2.63	0.11	0.07

Table 2: Secondary outcomes in AHIIT and resistive AHIIT (mean ± SD)

***time effect difference upon pairwise comparison (p<0.05).**

Note: TEE, total energy expenditure, HR max, maximal heart rate, HR mean, mean heart rate, VO_2 peak, peak oxygen consumption, RPE, rate of perceived exertion, ES, effect size

9.5 Information sheet, consent form of Chapter 7

THE HONG KONG POLYTECHNIC UNIVERSITY
香港理工大學

To	So Chun Lung (Department of Rehabilitation Sciences)		
From	Yee Kay Yan Benjamin, Delegate, Departmental Research Committee		
Email	benjamin.yee@	Date	28-Sep-2022

Application for Ethical Review for Teaching/Research Involving Human Subjects

I write to inform you that approval has been given to your application for human subjects ethics review of the following project for a period from 01-Nov-2022 to 31-Dec-2023:

Project Title:	Effects of aquatic high intensity interval deep water training and land based high intensity interval training on cardiometabolic health parameters and perceptual responses in women
Department:	Department of Rehabilitation Sciences
Principal Investigator:	So Chun Lung
Project Start Date:	01-Nov-2022
Project type:	Human subjects (non-clinical)
Reference Number:	HSEARS20220926002

You will be held responsible for the ethical approval granted for the project and the ethical conduct of the personnel involved in the project. In case the Co-PI, if any, has also obtained ethical approval for the project, the Co-PI will also assume the responsibility in respect of the ethical approval (in relation to the areas of expertise of respective Co-PI in accordance with the stipulations given by the approving authority).

You are responsible for informing the PolyU Institutional Review Board in advance of any changes in the proposal or procedures which may affect the validity of this ethical approval.

Yee Kay Yan Benjamin

Delegate

Departmental Research Committee (on behalf of PolyU Institutional Review Board)

THE HONG KONG POLYTECHNIC UNIVERSITY
香港理工大學

INFORMATION SHEET

Effects of aquatic high intensity interval deep water training and land based high intensity interval training on cardio-metabolic health parameters and perceptual responses in women

Research Team Members:

Professor Shamay Ng	Professor	Department of Rehabilitation Sciences, PolyU
Dr. Billy So	Assistant Professor	Department of Rehabilitation Sciences, PolyU
Ms Manny Kwok	PhD student/ Musculoskeletal Physiotherapist	Department of Rehabilitation Sciences, PolyU

Study Background:

Physical inactivity associates with a reduction in cardio-metabolic health. Thus, identifying and reducing physical inactivity will have an essential effect on cardio-metabolic diseases prevention. Older women populations have higher cardio-metabolic risks and hence it has been recommended that exercise training at a higher intensity is beneficial to prevent associated ageing cardio-metabolic diseases.

Land High Intensity Interval Training (L-HIIT) can be defined as repeated bouts of vigorous but submaximal exercise that elicits ≥80% maximum heart rate [HRmax], interspersed with short periods of recovery. Although L-HIIT has been shown to improve cardio-metabolic health, some characteristics of this training performed on hard surface. are considered less appropriate for elderly populations due to barriers to exercise such as pain and movement difficulties. An aquatic environment is considered to be safe and efficient for physical training because of the physical properties of water. Aquatic High Intensity Interval Training (AHIIT) provides a lower weight bearing aerobic alternative to L-HIIT. Among aquatic exercises, Deep water running (DWR) has gained prominence in the scientific literature. DWR is performed with the aid of a floatation vest, which serves to keep the body upright and prevent feet from touching the bottom of the pool. This characteristic allows AHIIT to be applied in DWR by eliminating any impact with a reduced risk of injury (AHIIT-DWR).

In view of a growing application of AHIIT-DWR, the intention of this proposed study are 1. to design an AHIIT- DWR exercise protocol to optimize cardio-metabolic outcomes and perceptual responses 2.to investigate the effects of 8-week AHIIT-DWR or L-HIIT intervention performed at matched intensity on cardio-metabolic health outcomes and perceptual responses in older women

If you are female, healthy and aged ≥60 years, you are cordially invited to participate in this study.

Study procedure:

Participants will be randomly assigned to either the AHIIT-DWR or L-HIIT group. An incremental test in water or land will be performed prior the exercise interventions to confirm an individualized cadence required at a matched level of exercise intensity in each condition. The incremental test in water and on land will be carried out by DWR and treadmill running respectively. During the incremental test, gas exchange data will be obtained by a portable metabolic device PNOE.

For the AHIIT-DWR program, participants will be asked to wear a flotation vest to keep the feet without touching the pool floor. They will be given a cardiac frequency meter (Polar®, Electro, Oi, Finland) for heart rate control and monitoring. Ten 2-min bout of AHIIT-DWR at 80-90%HR max with 1-min active recovery at 50%HR max in between bouts will be performed. Participants will complete the session in 30-minute, twice a week for 8 weeks (a total of 16 sessions)

On the other hand, the L-HIIT exercise will be performed in a room of ambient temperature and relative humidity of 23°C and 50% respectively. The participants are instructed to perform ten 2-min bout of treadmill running at 80-90%HR max with 1-min active recovery at 50%HR max in between bouts. Participants will complete the session in 30-minute, twice a week for 8 weeks (a total of 16 sessions),

Both AHIIT-DWR and L-HIIT programs will be performed at the Spotlight Recreation Club, Hung Hum and the Gait and Motion Analysis Lab at the Hong Kong Polytechnic University (HKPU) respectively. The assessments will comprise a measurement of cardiorespiratory responses (oxygen capacity, oxygen pulse), metabolic outcomes (lipid profile, glucose and cholesterol) and perceptive responses. Of note, fingerstick blood sampling and small sample size (40μL) will be collected with a capillary tube for metabolic markers measurement. The assessment session will last for 30 minutes.

Study benefit:

It is hoped that this information will help us to understand the difference in cardio-metabolic and perceptive responses of AHIIT-DWR and L-HIIT in women. At the completion of the trial, the transferability of the movements between aquatic and land environments can provide clinical practitioners with a clear understanding of the roles of implications of water immersion for HIIT and optimize the clinical practice guidelines for practitioners.

Possible side effect, risk or discomfort in the study

The test should not result in any serious discomfort, but participants may experience muscle fatigue or post-exercise soreness at 1-2 days after the training sessions. Adequate rest after the training can achieve a full recovery.

Automaticity and confidentiality:

Your participation in this study is completely voluntary. You have every right to withdraw from the study before or during the study without penalty of any kind. All information related to the participants will remain confidential, and will be identified by codes only known to the research team.

If you would like to get more information about this study, please contact Ms Manny Kwok on 9206 . If you have any complaints about the conduct of this research study, please do not hesitate to contact Ms Vangie Chung, Secretary of Department Research Committee on 27664329.

Thank you for your interest in participating in this study.

Dr. Billy So

Principal Investigator

THE HONG KONG POLYTECHNIC UNIVERSITY
香港理工大學

香港理工大學
康復治療科學系
研究資料

研究項目: 針對婦女在水上進行的高強度間歇性深水跑步訓練和陸地進行的高強度間歇性跑步訓練的心臟代謝和知覺反應之實驗

研究人員:
伍尚美教授　物理治療教授　香港理工大學康復治療科學系
蘇俊龍博士　物理治療助理教授　香港理工大學康復治療科學系
郭文瑩女士　博士三年級生/ 物理治療師　香港理工大學康復治療科學系

研究項目簡介:
缺乏運動與降低心臟代謝健康有關。 因此，能識別和減少身體運動將對預防心臟代謝疾病產生重要影響。 老年女性的心臟代謝風險較高，有研究指出，進行高強度的運動訓練有利於預防相關的老化心臟代謝疾病。

陸地進行的高強度間歇訓練 (L-HIIT) 可定義為反復進行的高強度運動，並引發≥80% 的最高心率 [HRmax]，當中包括短時間的恢復。儘管 L-HIIT 已證明可以改善心臟代謝健康，但這種訓練是在地面上進行的。由於疼痛和其他運動困難等障礙，普遍被認為不太適合老年人群。但由於水的物理特性，在水的環境運動被認為是安全而有效的體育訓練。水上進行的高強度間歇性訓練 (AHIIT) 可提供一種替代 L-HIIT 的低負重有氧運動。在水上運動中，深水跑步 (DWR) 在科學文獻中佔有重要位置。 DWR 是在漂浮背心的幫助下進行，該背心用於保持身體直立並防止腳板接觸池底。AHIIT-DWR 通過此特性降低受傷風險。

鑑於 AHIIT-DWR 的應用越來越廣泛，這項研究的目的是 1. 設計 AHIIT-DWR 運動以加強心臟代謝結果和知覺反應 2. 研究 8 週 AHIIT-DWR 或 L-HIIT 的效果，並以相對的強度對老年女性的心臟代謝健康結果和知覺反應進行研究

若你是年齡≥60歲的健康女性，我們誠意邀請你參與此項研究。

研究方法和程序:
參與者將被隨機分配到 AHIIT-DWR 或 L-HIIT 組。在進行運動之前，將在水中或陸地進行增量測試，以確認在每種情況下以相對的運動強度所需作個人化節奏調整。水上增量試

驗和陸上增量試驗將分別通過DWR和跑步機進行。在增量測試期間，氣體交換數據將通過便攜式代謝設備 PNOE 獲得。

對於 AHIIT-DWR，參與者將穿著漂浮背心，以保持雙腳不接觸池底。他們將獲得用於心率控制和監測的心臟頻率計（Polar®、Electro、Oi、芬蘭）。進行 10 次 2 分鐘的 AHIIT-DWR ，最大心率達到 80-90%，在兩次回合之間有1 分鐘的恢復，而最大心率為 50%。參與者將在 30 分鐘內完成每週兩次，持續 8 週（共 16 節) 的訓練。

另一方面，L-HIIT 練習會在環境溫度和相對濕度分別為 23˚C 和 50% 的房間內進行。參與者被指示在跑步機上以 80-90% 的最大心率進行 10 次 2 分鐘的跑步，跑步之間以 50% 的最大心率進行 1 分鐘的恢復。參與者將在 30 分鐘內完成每週兩次持續 8 週（共 16 節）的訓練。

AHIIT-DWR 和 L-HIIT 將分別在香港理工大學 (HKPU) 的步態分析實驗室和博藝會進行。評估將包括心肺反應（氧容量、氧脈博）、代謝結果（血脂、葡萄糖和膽固醇指數）和感知反應的測量。代謝指數會在測量手指採血，小樣本量（40μL）將用毛細管收集，每次評估將在 30 分鐘內完成。

<u>本研究項目的益處:</u>
希望這研究項目能幫助我們了解女性 AHIIT-DWR 和 L-HIIT 在心臟代謝和感知反應方面的差異。研究結果可以讓臨床從業者清楚地了解水的環境對 HIIT 的影響，並可以指引從業者的臨床實踐。

<u>自願參與性質:</u>
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THE HONG KONG
POLYTECHNIC UNIVERSITY
香港理工大學

CONSENT TO PARTICIPATE IN RESEARCH

Title: Effects of aquatic high intensity interval deep water training and land based high intensity interval training on cardio-metabolic health parameters and perceptual responses in women

Research Team Members:

Professor Shamay Ng	Professor	Department of Rehabilitation Sciences, PolyU
Dr. Billy So	Assistant Professor	Department of Rehabilitation Sciences, PolyU
Ms Manny Kwok	PhD student / Musculoskeletal Physiotherapist	Department of Rehabilitation Sciences, PolyU

I ________________________ hereby consent to participate in the captioned research conducted by Dr. Billy So Chun Lung.

I understand that information obtained from this research may be used in future research and published. However, my right to privacy will be retained, i.e. my personal details will not be revealed. This study abides the Declaration of Helsinki.

The procedure as set out in the attached information sheet has been fully explained. I understand the benefit and risks involved. My participation in the project is voluntary.

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Signature of Participant : ________________________

Name of Witness : ____________________ Date : ____________________

Signature of Witness : ____________________

Name of Researcher : ____________________ Date : ____________________

Signature of Researcher : ____________________

香港理工大學
康復治療科學系
研究資料

研究項目:針對比較健康婦女的高強度間歇深水跑步訓練和陸地進行的高強度間歇訓練的心臟代謝和知覺反應之實驗

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Aquatic High Intensity Interval Deep Water Running Influence on Cardio-metabolic Health and Cognitive Psychological Responses in Women

INTRODUCTION

Recent public health guidelines have promoted land based High Intensity Interval training (L-HIIT) as an efficacious exercise strategy that may offer some time savings (1). L-HIIT is generally defined as repeated bouts of vigorous but submaximal exercise that elicits ≥80% maximum heart rate (HRmax), interspersed with short periods of recovery (2, 3). Although L-HIIT has generally been shown to improve cardio-metabolic health and cardiovascular function(4), some characteristics of this training performed on hard surfaces are considered less appropriate for elderly populations due to barriers such as deconditioning, arthritis and other joint degenerative processes, and movement difficulties compounded by the land environment (5).Therefore, an aquatic environment may harness a valuable alternative for L-HIIT because of the unique hydrodynamic properties. For example, Aquatic High Intensity Interval Training (AHIIT) provides a reduction in lower extremity joint weight bearing aerobic alternative to L-HIIT. Previous evidence of aquatic exercise using interval training have reported significant aerobic and cardiorespiratory benefits (6). Among these aquatic exercises, Deep Water Running (DWR) has gained prominence in the scientific literature. DWR is performed with the aid of a floatation vest, which serves to keep the body upright and prevent the feet from touching the bottom of the pool (7, 8). This characteristic allows AHIIT to be applied in DWR by reducing weight bearing, studies have shown that

DWR has shown greater exercise compliance, potentially because of this (9, 10). Hence, it appears that the use of AHIIT-DWR can be an effective strategy for older adults to train at higher intensities than on land. It has been recommended that exercise training at a higher intensity is beneficial to middleaged and older adults to prevent cardio-metabolic diseases associated with ageing (11). For instance, older women populations are vulnerable in terms of higher cardio-metabolic risk, and hence are the main target population of aquatic exercise programs. It is known that aerobic capacity declines 10% per decade of life beyond 20 years of age (2). This is relevant to cardio-metabolic function and related to functional capacity which is an important predictor of mortality (12). Therefore, stimulating cardio-metabolic function becomes fundamental to help decrease mortality in such populations. The perceptions of exercise will influence whether a participant continues with an exercise program, tries a different program instead, or stops exercising completely. This is a definite concern when considering inactive elderly populations. It is crucial that their perceptions for exercise are positive for participants to adhere to a program. It was observed that the perception of exercise variety is an important factor for adherence (13). It was also determined that the perception of health benefits and competence of facility staff resulted in improved adherence in older adults (14). It has been suggested that self-efficacy and enjoyment of exercise is positively correlated with exercise adherence and that lack of time was the most frequently reported barrier for physical activity (15). AHIIT-DWR may potentially provide further enjoyment or affective levels with a modification in the exercise environment. (16) The American Psychological Association (APA) defined cognition as all forms of knowing and awareness, for instance, perceiving, conceiving, remembering, reasoning, judging, imagining and problem solving (17). These high-levels of mental processing have critical implications in synthesizing and integrating of thoughts and experiences (18). These cognitive abilities vary greater among older populations. Although overall cognitive function tends to diminish with age, a number of studies have shown that cognitive performance can improve at any age through physical exercise (19). It was reported that a global trend for positive improvements in cognitive function (i.e., through MMSE instrument) occurred as a result of intervention with aquatic exercise groups versus control groups (20). Aquatic exercise programs are potentially beneficial to older individual's cognition levels. Despite the growing popularity of AHIIT, research investigating the cardio-metabolic health benefits and the cognitive psychological responses comparing AHIIT-DWR and L-HIIT have not been investigated to date. There is well-established evidence about the effects of L-HIIT on cardio-metabolic or physical health, while similar evidence regarding AHIIT -DWR is lacking (4). In addition to cardio-metabolic health, cognitive psychological responses can have a significant behavioural impact on exercise compliance (21). Given this knowledge gap alongside the growing application of AHIIT, the purpose of this study was therefore to investigate the effects of 8-weeks of AHIIT-DWR and L-HIIT on cardio-metabolic health and cognitive psychological outcomes among inactive elderly women. We hypothesized that 8-weeks of AHIIT-DWR maybe similarly effective as L-HIIT in improving cardio-metabolic health and cognitive psychological responses in inactive elderlywomen.

METHODS

Participants

Seventy inactive elderly women with a stable medical history were recruited for this study. After providing their written informed consent, all participants declared that they were free of any cardiorespiratory, neurological pathology and/or had an orthopedic fracture or any surgical intervention done to the lower extremities in the six months prior the study. None of the participants were taking any medications.

Study design

A parallel two-group randomized controlled trial (RCT) design was used according to the Consolidated Standards for Reporting of Trials (CONSORT) (22). Selected participants who fulfilled the selection criteria were randomly assigned to either the AHIIT-DWR program or the L-HIIT, using the computer software Research Randomizer (Figure 1).

Experimental Procedures

Participants were required to perform an interview for screening and familiarization before signing an informed consent to the study. The experimental procedures completed comprise the basis for the baseline data. Subjects were screened by a standard health questionnaire (International Physical Activity Questionnaire), measuring their resting heart rate (HR), blood pressure (BP), body mass and height. All participants performed and completed a familiarization session at the pool a week before the study. An incremental test in water and on land were performed prior the exercise interventions to confirm an individualized exercise intensity in each condition (23). The incremental test in water and on land were carried out by DWR and treadmill running respectively. DWR trained groups performed DWR maximal test while treadmill running subjects performed max tests during treadmill running maximal test. Prior to testing, all exercises were demonstrated first, then practiced once. Participants were monitored continuously and HRs were recorded at a frequency of 1Hz by a HR sensor (Polar OH1, Kempele, Finland). During the incremental test, gas exchange data were obtained by a PNOE portable metabolic device. The PNOE device acquired data breath-by-breath and continuously measured ventilatory volume and determined expired gas concentrations simultaneously. The DWR incremental protocol increased the exercise load from 85 beats per minute (bpm) and increased the cadence by 15 bpm every 2 minutes for each progression (23) while the land treadmill incremental followed the s Bruce protocol(24). A metronome (Intelli IMT 300, Japan) was used to provide the target cadence during DWR protocol. The HR, VO2, and rate of perceived exertion (RPE) each minute were recorded. 'A metronome was used to provide the target cadence during the DWR protocol.

Interventions

All intervention groups performed a standardized 3-min warm-up and 3-min cool down that included pool walking, jogging, and pool wall stretches at 50% heart rate reserve (HRR). HRR is obtained by using the maximal heart rate (HR max) determined from the incremental tests to subtract resting heart rate (HR rest) (25).

AHIIT-DWR

The AHIIT-DWR training program consisted of ten 2-min bouts of DWR at 80% HRR with 1-min active recovery at 60% HRR in between bouts. The classes were held in a sports club pool with water depth ranging from 1.4 to 2 m, water temperature at 28 °C, and air (room) on 06/30/2024 temperature at 26 °C. Participants participated in 30-minute sessions twice a week for 8 weeks (a total of 16 sessions). AHIIT-DWR was conducted by an experienced aquatic fitness instructor. Participants were asked to wear a flotation vest to prevent the feet from touching the pool floor, and to keep the trunk straight with the chest out. The body angle was adjusted so it was slightly leaning forward in the sagittal plane. The arms were swung in a relaxed and slightly flexed position. The elbow was flexed at 90 degrees while keeping the thumbs below the water level. The shoulder was flexed and extended to bring the elbow back and forth to complete the running cycle. The running stride began by flexing the hip to 70-80 degrees while maintaining the knee at right angle (about 90 degrees). Both legs performed a cyclic movement, alternating between hip flexion and hip extension to complete the running cycle, while allowing for forward progress (Figure 2). The quality of the movement was closely monitored by the aquatic fitness instructor.

L-HIIT

The L-HIIT training program consisted of ten 2-min bout of treadmill running were performed at 80% HRR with 1-min active recovery at 50%HRR between bouts. Participants participated in 30-minute sessions twice a week for 8 weeks (a total of 16 sessions). The quality of the movement was closely monitored by an experienced exercise instructor.

Exercise Progression

During the first 2 weeks, the participants performed 10 bouts of 2 min at 80–85% HRR (weeks 1–4), with 1 min of active recovery at 50% HRR between bouts. During weeks 3-5, the participants performed 10 bouts of 2 min at 85–90% HRR of the AHIIT-DWR, with 1 min of active recovery 50% HRR between bouts. During the last 6-8 weeks, the participants performed 10 bouts of 2 min at 90–95% of the HRR, also with 1 min of active recovery at 50% of HRR between bouts. In the L-HIIT protocol, for the first two weeks, participants performed the HIIT treadmill run using 10 bouts of 2 min at 80–85% HRR (weeks 1–4), with 1 min of active recovery at 50% HRR between bouts. During weeks 3-5, the participants performed 10 bouts of 2 min at 85–90% HRR of the AHIIT-DWR, with 1 min of active recovery at 50% HR max between bouts. During the last 6-8 weeks, the participants performed 10 bouts of 2 min at 90–95% of the HRR, also with 1 min of active recovery at 50% of HRR between bouts. The AHIIT- DWR and L-HIIT training periodization is shown in Table 1.

Outcomes

Cardio-metabolic markers were measured at baseline (pre -ex) and at least 48 hours following but within 5 days after the final session of the 8-week intervention. For each pre and post exercise assessment, the session lasted for 30 minutes.

Cardiorespiratory fitness

Gas exchange data were obtained by a portable metabolic device PNOE after incremental tests. A PNOE device was used to assess participants cardiorespiratory fitness level (i.e. VO2 max, oxygen pulse, VCO2, RER, MET) and HR max pre and post AHIIT-DWR and L-HIIT. VO2max was considered to be attained when the following standardized criteria were met: (1) a respiratory exchange ratio of greater than or equal to 1.10; (2) failure of heart rate to increase with increases in workload; (3) post-exercise blood lactate $\geq$8.0 mmol•L-1 (24); (4) clear signs of exhaustion (facial flushing, unsteady gait); and (5) refusal to carry on despite strong verbal encouragement. Blood lactate was measured via capillary blood sampling from the fingertips with a portable analyzer (Lactate Plus, Nova Biomedical, Waltham, Massachusetts) (24). Data collected from the incremental test was used to determine the intensity required for the exercise interventions for each participant.

Blood metabolic markers analysis

A qualified nurse performed a venous blood sampling of 20 mL after a 12-hour fasting period. Glucose levels were measured using the enzymatic-amperometric method with a coefficient of variation (CV) of 0.5% (Biosen-C; EKF Diagnostics, Germany). The lipid profile was assessed using commercially available kits (RX Monza; Randox Biosciences, UK). Total cholesterol levels were determined using the cholesterol oxidase, esterase, and peroxidase colorimetric method, with an intra-assay CV of 1.3%. High-density lipoprotein (HDL) cholesterol levels were measured using the polyethylene glycol direct method, while low-density lipoprotein (LDL) cholesterol levels were assessed using the direct method, with respective intra-assay CVs of 0.7% and 1.3%. Triglyceride levels were measured using the enzymatic method without glycerol blanking, with an intra-assay CV of 1.3%. Insulin levels were determined using an enzyme-linked immunosorbent assay (ELISA) (Insulin ELISA; Mercodia AB, Sweden). Duplicate measures were taken for each marker, and the average value was reported. Insulin resistance was estimated using the homeostasis assessment model for insulin resistance (HOMA-IR) (26).

Cognitive Psychological responses

Cognition was be assessed before and after the 8-week exercise intervention using cognitive batteries including MMSE and MOCA. Both are validated instruments suitable for use as a mediator variable for devising interventions for promoting cognition. The perceptions of exercise will influence whether a participant continues with an exercise program, tries a different program instead, or stops exercising completely. It was observed that the perception of exercise variety is an important factor for adherence (13).

Statistical Analysis

Analyses were performed using the Statistical Package for Social Sciences for Windows version 22.0 (SPSS, Inc., Chicago, IL.). Statistical significance was delimited at $P<0.05$. All continuous variables are presented as means and standard deviation. Mean differences among groups (AHIIT-DWR and L-HIIT) for each cardio-metabolic and cognitive psychological variable were tested by mixed model repeated measures ANOVA. Mixed effects models were applied to analyze the effects of group (AHIIT-DWR vs L-HIIT), time (0 vs 8 weeks), and the interaction between group and time on cardio-metabolic outcomes and cognitive responses. Turkey post-hoc analysis was used to analyze within-group and between-group comparisons.

Effect sizes (ES) were calculated by Cohen's d. The sample size was calculated based on the primary outcome of a previous study comparing the effects of interval DWR and land trainings on aerobic fitness (27). Using the G*power software and based on the effect size 0.28 obtained, the primary outcome (VO2 max) assuming a 5% type I error and 80% power, the sample size computed was 30 or more subjects per group. Considering an estimated 20% attrition rate, the total enrolled sample size for each group required to ensure adequate statistical power was 36.

RESULTS

The mean ages of participants in the AHIIT-DWR and L-HIIT groups were 66.33 ± 4.99 and 65.68 ± 6.19 years, respectively. Eight participants (10.1%) were excluded from the statistical analysis, with one dropout from the AHIIT group and nine from the L-HIIT group. The anthropometric parameters in terms of height, body weight and BMI are shown in Table 2.

Effects of AHIIT-DWR and L-HIIT on cardiovascular fitness

Group-by-time interactions revealed a significant difference in relative VO2 (ES:0.06, $p=0.04$); VE (ES: 0.11, $p< 0.01$), relative VO2 ($p< 0.01$), peak O2 pulse ($p < 0.01$), RER ($p< 0.01$) were detected after both AHIIT-DWR and L-HIIT.

Effects of AHIIT-DWR and L-HIIT on metabolic blood markers

None of the metabolic blood markers exhibited a significant difference in the group-by time interactions or between groups despite nearly significant interactions shown in fasting glucose level (p=0.05, ES=0.07) (Table 4). However, simple effects testing revealed both AHIIT- DWR and L-HIIT significantly decreased triglycerides ($p < 0.05$, ES= 0.07). Eight weeks of AHIIT-DWR and L-HIIT did not change total cholesterol ($p > 0.05$), high density lipoprotein (HDL) or low-density lipoprotein (LDL), despite the fact that both interventions decreased TC, HDL and LDL.

Effects of AHIIT- DWR and L-HIIT on cognitive tests

There were no significant differences or interactions shown in either cognitive tests between the AHIIT-DWR and L-HIIT groups ($p>0.05$). However, there was significant improvement in MOCA score ($p < 0.01$, ES=0.22) after AHIIT-DWR versus L-HIIT (Table 5). Moreover, MMSE remained similar after AHIIT-DWR and L-HIIT ($p >0.05$).

Effects of AHIIT-DWR and L-HIIT on psychological response and exercise adherence

There were no significant differences for enjoyment ($p = 0.88$) or self-efficacy score between AHIIT-DWR and L-HIIT ($p = 0.57$). Meanwhile, exercise adherence was similar in both groups (>90% session completion rate, $p = 0.832$) (Table 6). There were no adverse events reported.

DISCUSSION

The purpose of this study was to examine the effects of 8-weeks of AHIIT-DWR and L-HIIT on cardio-metabolic parameters, cognitive tests and perceptions in inactive elderly women. The major findings found in this study following the 8-week intervention were the cardiorespiratory fitness improved in both the AHIIT-DWR and L-HIIT cohorts, but that there were greater increases in relative VO2, HR, VE and oxygen pulse in the AHIIT-DWR cohort.

There were decreases in metabolic blood markers (TC, LDL, triglycerides and blood glucose) without a significant group difference, while only triglycerides significantly decreased after AHIIT-DWR and L-HIIT. AHIIT-DWR and L-HIIT had no significant group differences in the cognitive tests in MMSE and MOCA, but a significant increase in the MOCA score was detected after both AHIIT-DWR and L-HIIT. In terms of psychological responses, no significant group difference for enjoyment and self-efficacy were found after either AHIIT-DWR and L-HIIT.

AHIIT-DWR can improve several cardio-metabolic health markers, particularly cardiorespiratory fitness in inactive older women. The current study extends the previous literature by using a more precise approach by adopting an incremental test performed on land and an aquatic medium to assess VO2 max and HR max in order to determine the HRR to monitor individuals' exercise intensity. HRR takes into account differences between individuals resting heart rate and has been recommended over percentage of maximal HR by ACSM (24). There were significant improvements in all the cardiorespiratory parameters after both interventions while AHIIT-DWR particularly demonstrated a significant group difference in relative VO2, VE, MET and O2 pulse versus L-HIIT. This finding agrees with the majority of previous studies comparing the efficacy of aquatic exercise and land-based exercise for cardiorespiratory improvement (28). Mechanistically, it has been suggested that the responses in water are caused by increased venous return to the heart with enhanced peripheral venous blood pressure due to the compression of the lower body by water pressure.

Low cardiovascular fitness, indicated by maximal oxygen uptake (VO2max), is in part a consequence of a physically inactive lifestyle and is a powerful predictor of premature cardiovascular mortality (29). Despite exercise using different mediums, our results revealed that AHIIT-DWR induced a similar absolute VO2max and HR max increase (approximately 5-6 $mL \cdot kg^{-1} \cdot min^{-1}$ in both groups) as L-HIIT. In our results, simple effect analyses showed that aerobic fitness in both the AHIIT-DWR and L-HIIT groups were significantly elevated. This suggests that aerobic fitness of inactive elderly individuals may be effectively improved by both interventions, regardless of the lack of significant main effects by group. As reported by most previous studies, HIIT is beneficial for improving aerobic fitness, either on land or in water (30, 31). In a recent AHA Scientific Statement, an increase in VO2 max of just 1 MET is valuable for increasing health outcomes and survival (32). Two other trials have reported an increase in VO2 max of ≥3,5mL/kg/ min using aquatic exercise (33, 34). AHIIT-DWR may be as beneficial as L-HIIT, which provides inactive elderly women another option for effective HIIT or potentially a more successful environment to start and continue with high-intensity training. In sum, the unique nature of physiological benefits of hydrostatic pressure and the enabling effect of buoyancy in water may facilitate such effectiveness. Traditional L-HIIT exercises may not be suitable for all inactive elderly women given that it requires a higher level of skill, impact, and physical function to perform.

Our results showed no group differences in any of the blood markers after either AHIITDWR nor L-HIIT. However, both AHIIT-DWR and L-HIIT markedly reduced triglycerides ($p < 0.05$), while both groups showed non-significant decreases in TC, HDL, and LDL after training ($p > 0.05$). The lack of differences between groups could be due to the timeframe of the study, as some previous evidence suggests that a minimal period of 8–12 weeks may be required for high intensity interval training to demonstrate a positive impact on physiological adaptions that improve metabolic health (4, 35). Another potential reason for our finding could be that most participants already presented with blood markers within the normal range at baseline, and hence the likelihood of observing notable differences was reduced. Further research in different clinical populations with longer timeframes will be required to determine if AHIIT-DWR is superior to L-HIIT in terms of metabolic blood markers.

There were no group differences in cognitive ability measured by MMSE and MOCA between AHIIT-DWR and L-HIIT. This suggests that both AHIIT-DWR and L-HIIT may be useful for improving cognitive ability in older women measured by MMSE and MOCA. The lack of difference between groups may be due to insufficient physiological changes from our 8-weeks intervention. A recent review reported the largest effects following interventions with longer sessions and intervention duration (36), suggests that a longer intervention may be required to produce a significant group-level change in global cognition. The within group difference in change in MMSE and MOCA, in favor of both AHIIT-DWR and L-HIIT, may be explained by increased cerebral blood flow and improvements in cardiovascular function (aerobic capacity, cardiac output, oxygen transport and metabolism) which can improve neurotransmitter function and brain health (37). As such, a higher cardiac output is associated with higher cerebral blood flow, which suggests that HIIT results in a better adaptation to the cardiovascular system and has a positive effect on cognitive ability (38). Another possible mechanism is related to the increased level of brain derived neurotrophic factor (BDNF) in the brain. Acute aerobic exercise increases BDNF levels, which is an important component of the brain's neuroplasticity (39). Physical exercises can improve the circulation level of BDNF, which has beneficial neurotrophic, neuroprotective and cognitive properties, consistent with a previous review (40).

In terms of psychological responses, no significant group differences for enjoyment or self-efficacy were found. While the cardio-metabolic benefits of AHIIT-DWR and L-HIIT have been demonstrated in this study and others (41), a typical public health concern is how the general population, particularly inactive and less fit elderly women, perceive AHIIT-DWR and L-HIIT and whether they can adhere to it in the long term (42). Our results agree with our previous study suggesting that a single bout of AHIIT or L-HIIT (matched with exercise intensity) elicited similar enjoyment, self-efficacy and exercise adherence (43). However, there were also conflicting findings with one study suggesting that AHIIT was perceived as being more effective and enjoyable than L-HIIT in men with obesity (44). Such mixed results are likely explained by the variability in water depth, subject characteristics, genders, exercise intensity, intervals, and depend on the modalities performed. Furthermore, both AHIIT-DWR and L-HIIT showed excellent exercise compliance rates (>90%), suggesting that both can be a practical exercise option for inactive elderly women in terms of enjoyment and self-efficacy.

Strengths and Limitations

This study has several strengths, including examining the effects of AHIIT-DWR and L-HIIT using a randomized research design. By adopting accurate and reliable measures of cardiorespiratory fitness in both aquatic and land environments, blood sampling, matching participant exercise intensity with HRR and achieving excellent adherence in both exercise groups are strengths of the study. These findings can provide valuable insights regarding the applications of AHIIT-DWR and L-HIIT. L-HIIT could be a time-efficient and efficacious approach for health professionals when designing individualized programs targeted to cardio-metabolic health benefits in women. However, because of the unique physical properties of water, AHIIT-DWR can be applied in appropriate individuals who need a lower weight bearing alternative to L-HIIT. Few randomized controlled trials have compared the impact of AHIIT-DWR and L-HIIT performed at matched intensities. Limitations of the present study included the fact that only elderly women were recruited and hence caution should be taken when generalizing to men, as well as young women. In addition, it is acknowledged that the relatively short intervention of 8-weeks may limit the ability to draw conclusions about the relative potency of AHIIT-DWR versus that of L-HIIT; this may explain why some secondary outcomes on metabolic blood markers were not statistically different between the AHIIT-DWR and L-HIIT programs. We believe, however, that our findings provide valuable insights regarding the potential applications of AHIIT-DWR versus L-HIIT and understand the effects on cardio-metabolic parameters and cognition perceptions were comparable to each other. From a practical point of view, our results may suggest that AHIIT-DWR could be an alternative to L-HIIT, and considered by healthcare professionals when designing programs that target cardio-metabolic health benefits, metabolic blood markers, cognitive function and perception in physically inactive elderlywomen. Future studies on a larger scale for longer duration may be warranted.

CONCLUSIONS

In summary, eight-week programs of AHIIT-DWR and L-HIIT showed no group differences in aerobic capacity and maximal HR, metabolic blood markers, cognitive function and psychological responses, but AHIIT-DWR improved oxygen capacity, metabolic equivalents, oxygen pulse and minute ventilation. This suggests that a practical model of AHIIT-DWR can offer cardio-metabolic health benefits, especially for cardiorespiratory fitness, comparable to traditional L-HIIT in inactive older women. AHIIT-DWR also showed a similar enjoyment level and self-efficacy to L-HIIT and had high adherence. Further research in different populations with longer duration is required to determine how AHIIT-DWR compares to L-HIIT in terms of overall cardio-metabolic health, cognitive and psychological benefits.

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fabrication, falsification, or inappropriate data manipulation. The results of the present study do not constitute endorsement by the American College of Sports Medicine.

Conflict of interest: The authors of this study declare that they have no conflicts of interest relevant to the content of this article.

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Ethical approval: This study was performed in accordance with the ethical standards of the Helsinki Declaration. Ethics approval was obtained from the ethics committee of the Hong Kong Polytechnic University (HSEARS20220926002).

Informed consent: Informed consent was obtained from all subjects involved in the study.

Consent for publication: Not applicable.

Availability of data and material: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions: M.M.Y.K. and J. M. participated in the design of the study, contributed to data collection and data analysis; B.C.L.S. and S.S.M.N. participated in the design of the study; M.M.Y.K and B.C.L.S. contributed to data collection; M.M.Y.K. S.S.M.N. J. M. and B.C.L.S. contributed to data analysis and the interpretation of results. All authors contributed to the manuscript writing. All authors have read and agreed to the published version of the manuscript.

FIGURE LEGENDS

Figure 1: Flow chart of proposed study

Figure 2: AHIIT-DWR form

Figure 1

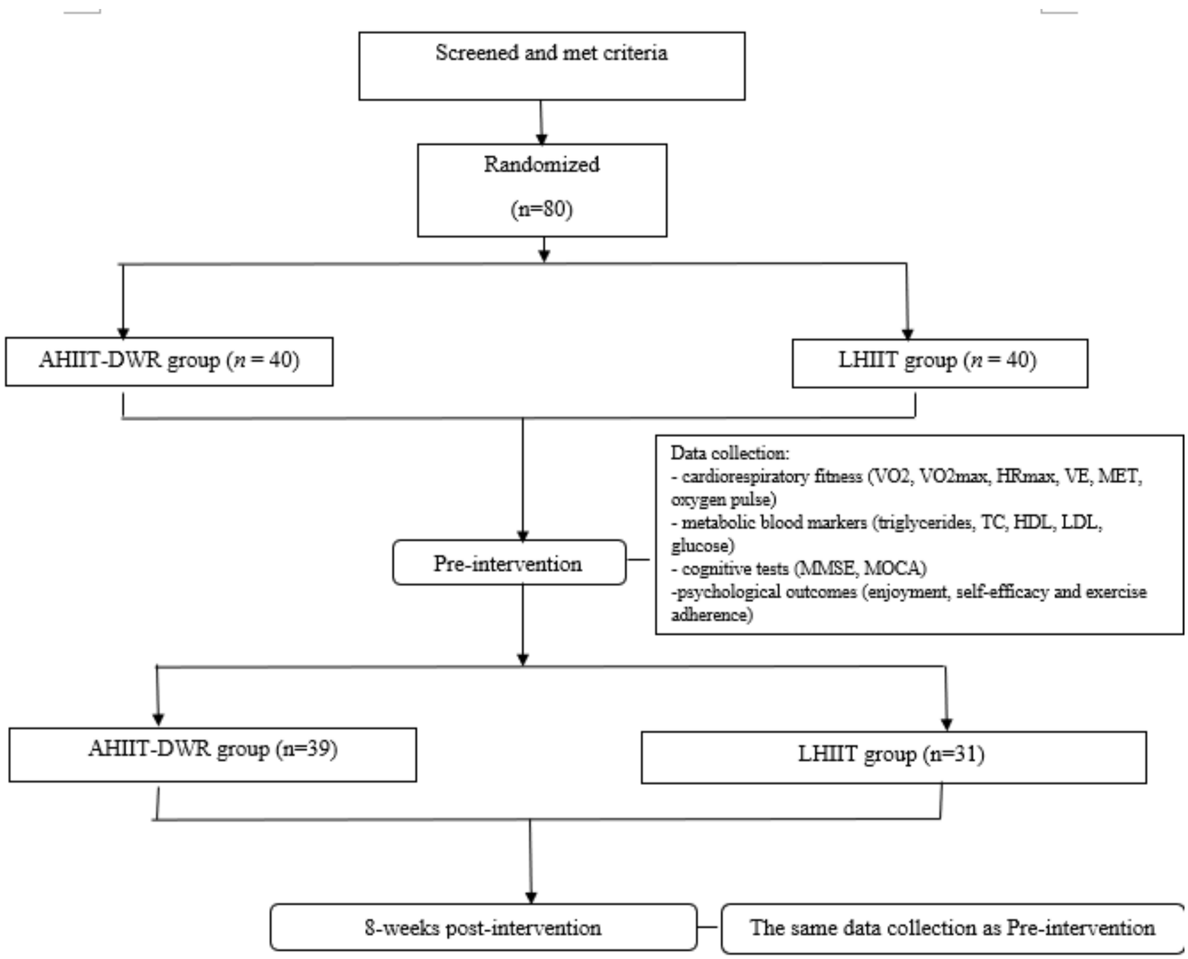


Figure 2

Weeks	Volume x Intensity	Total time
1-2	10 x (2min 80-85% HRR + 1min 50% HRR)	30 min
3-5	10 x (2min 85-90% HRR + 1min 50% HRR)	30 min
6-8	10 x (2min 90-95% HRR + 1min 50% HRR)	30 min

Table 1: AHIIT-DWR and L-HIIT training periodization

Parameters	AHIIT-DWR (n= 39)	L-HIIT (n= 31)
Age (yr)	66.33±4.99	65.68±6.19
Height (m)	156.31±6	154.65±5

	.12	.67
Weight (kg)	62.97±10	57.069±9
	.05	.05
BMI (kg/m^2)	25.73±3.	23.84±3.
	55	37

Table 2: Anthropometric parameters (mean ± SD)

	AHIIT-DWR (n= 39)		L-HIIT (n= 31)		Time effect		Group *time effect	
Parameters	Pre-training	Post-training	Pre-training	Post-training	P value	ES	P value	ES
HRmax	136.75±20.18	145.36±17.97*	138.88±21.06	142.88±19.56*	<0.01	0.15	0.21	0.02
VO2	1299.19±303.98	1738.22±468.10*#	1253.19±357.70	1529.29±448.14*	<0.01	0.55	0.04	0.06
RER	0.97±0.12	1.13±0.12*	1.01±0.11	1.11±0.15*	<0.01	0.44	0.07	0.05
VE	45.58±11.59	66.05±17.70*#	47.29±14.11	59.32±20.60*	<0.01	0.64	<0.01	0.11
MET	5.83±1.43	7.60±1.92*#	6.36±1.66	7.62±1.80*	<0.01	0.46	<0.05	0.06
O2 pulse	8.79±2.17	11.85±3.37*#	9.16±2.35	10.67±2.51*	<0.01	0.23	<0.05	0.07
VO2 max	20.76±4.14	26.62±6.73*	21.92±5.75	26.57±6.37*	<0.01	0.5	0.35	0.01

Table 3: Cardiovascular fitness in AHIIT-DWR and L-HIIT after the eight weeks intervention (mean ± SD)

#indicates significant group *time effect difference upon pairwise comparison (p<0.05).

Parameters	AHIIT-DWR (n= 39)		L-HIIT (n= 31)		Time effect		Group effect		Group* time effect	
	Pre-training	Post-training	Pre-training	Post-training	P-value	ES	P-value	ES	P-value	ES
TC (mmol/L)	5.53±0.95	5.50±1.09	5.36±1.06	5.55±0.91	0.06	0.06	0.40	0.01	0.83	0.00
HDL (mmol/L)	1.60±0.34	1.76±0.52	1.68±0.44	1.78±0.39	0.09	0.05	0.25	0.02	0.27	0.02
LDL (mmol/L)	3.26±0.86	3.09±1.0	3.48±0.92	3.30±0.81	0.05	0.06	0.36	0.02	0.93	0.00
Triglycerides(mmol/L)	1.47±0.71	1.32±0.56*	1.26±0.64	1.11±0.37*	0.04	0.07	0.16	0.03	0.10	0.00
Glucose(mmol/L)	5.38±0.44	5.39±.0.51	5.25±0.71	5.08±0.64	0.52	0.01	0.21	0.04	0.05	0.07

Table 4: Cardio-metabolic blood markers in AHIIT-DWR and L-HIIT after the eight weeks intervention (mean ± SD)

Parameters	AHIIT-DWR (n= 39)		L-HIIT (n= 31)		Time effect		Group effect		Group* time effect	
	Pre-training	Post-training	Pre-training	Post-training	P-value	ES	P-value	ES	P-value	ES
MMSE (Score of 30)	29.08±1.29	29.10±1.11	29.18±1.23	28.97±1.08	0.95	0.00	0.95	0.07	0.55	0.01
MOCA (Score of 30)	27.36±2.25	28.49±1.85	27.16±2.45	28.52±2.31	<0.01	0.22	0.73	0.35	0.69	0.00

Table 5: Cognitive batteries in AHIIT DWR and L-HIIT after 8-week intervention

	AHIIT-DWR (n=39)	L-HIIT (n=31)	P-value
Enjoyment (score out of 126)	110.5±8.5	104.6±13.5	0.88
Self-efficacy (score out of 100)	68.7±13.7	65.7±15.2	0.57
Exercise adherence (%)	93.26±6.69	92.29±11.04	0.81

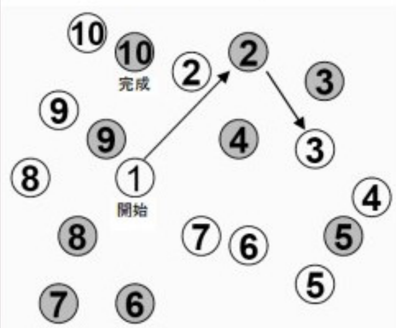
Table 6: Psychological outcomes in AHIIT-DWR and L-HIIT after the eight weeks intervention (mean ± SD)

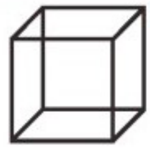
9.5.2 Montreal Cognitive Assessment Hong Kong version (HK-MOCA)

Montreal Cognitive Assessment Hong Kong version (HK-MoCA)
蒙特利爾認知評估香港版

姓名:
教育程度:
姓別/年齡: 日期:

		分數
視覺空間/執行性	複製圖形 / 畫時鐘 (十一點十分) (3 分)	
[ ]	[ ] / [ ] 輪廓 [ ] 數字 [ ] 時分針	___/5





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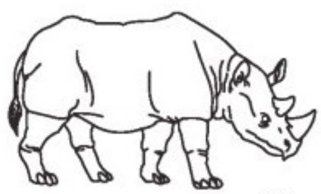
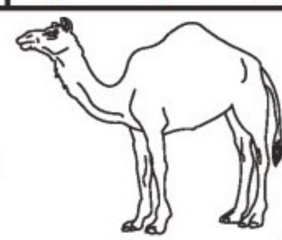




[ ] [ ] [ ] ___/3

記憶	讀出詞語再由病者重複 以上步驟做兩次 5分鐘後回憶		面孔	絲絨	教堂	雛菊	紅色	不用計分
		第一次嘗試						
		第二次嘗試						

專注	讀出數字 (每秒一個)	病者須把數字向前重複[ ] 2 1 8 5 4 病者須把數字向後重複[ ] 7 4 2	___/2
讀出數字: 當數字 '1' 出現時病者必須用手敲打桌面 (如≥2錯誤便不給予分數)		[ ] 5 2 1 3 7 4 1 1 8 0 6 2 1 5 1 7 4 5 1 1 1 4 1 7 0 5 1 1 2	___/1
由100開始連續 7減算		[ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65 4 或 5 個正確減算得 **3 分**, 2 或 3 個正確得 **2 分**, 1個正確得 **1 分**, 沒有正確得 **0 分**	___/3
語言	重複:	姨丈買魚腸 [ ] 西施四十四歲 [ ]	___/2
	流暢:	一分鐘內能說出的動物名稱的數目 [ ] (N ≥ 11 個名稱)	___/1
抽象	相似點: 例如: 香蕉 – 橙 =生果	[ ] 火車 – 單車 [ ] 手錶 – 間尺	___/2

延遲記憶	須回憶詞語 不可給提示	面孔 [ ]	絲絨 [ ]	教堂 [ ]	雛菊 [ ]	紅色 [ ]	分數只給予沒有提示的正確回憶	___/5
	類目提示 (見下表)							
	多項選擇 (見下表)							

定向	[ ] 日	[ ] 月	[ ] 年	[ ] 星期	[ ] 地點	[ ] 地區	___/6

延遲記憶備註表

	類目提示	多項選擇
面孔	身體的一部分	鼻子、面孔、手
絲絨	紡織品的一種	牛仔布、棉花、絲絨
教堂	建築物的一種	教堂、學校、醫院
雛菊	花的一種	玫瑰、雛菊、鬱金香
紅色	一種顏色	紅色、藍色、綠色

總分 如≤6年教育加1分 22分或以上為正常 ___/30

9.5.3 Mini Mental Status Examination (MMSE) Questionnaire

Form 2

MINI –MENTAL STATE EXAMINATION (MMSE)
簡短智能測驗

姓名:______________性別/年齡:__________ 評估日期: ________ 評估員:______________

最高分數	分數	
5	( )	**ORIENTATION** 依家係乜野日子? (年份)、(季節)、(月份)、(幾號)、(星期幾)
5	( )	我地依家係邊度? (九龍/新界/香港)、(九龍/新界/香港既邊區) 、(邊條街/邊個屋村)、(中心名字)、(邊層樓)
3	( )	**REGISTRATION** 依家我會講三樣野既名，講完之後，請你重複一次。 請記住佢地，因為幾分鐘後，我會叫你再講番俾我聽。 〔蘋果〕、〔報紙〕、〔火車〕。依家請你講番呢三樣野俾我聽。 （以第一次講的計分，一個一分；然後重複物件，直至全部三樣都記得住）
5	( )	**ATTENTION AND CALCULATION** 請你用一百減七，然後再減七，一路減落去，直至我叫你停為止。 (減五次後便停) ( )( )( )( )( ) 或:依家我讀幾個數目俾你聽，請你倒轉頭講番出黎。 〔42731〕 ( )
3	( )	**RECALL** 我頭先叫你記住既三樣野係乜野呀?
9	( )	**LANGUAGE** a. 呢樣係乜野？(鉛筆)(手錶) (2) b. 請你跟我講呢句話。(姨丈買魚腸) (1) c. 依家檯上面有一張紙，用你既右手拿起張紙，用兩隻手一齊將張紙摺成一半，然後放番張紙係檯上面。(3) d. 請讀出呢張紙上面既字，然後照住去做。(1) e. 請你講任何一句完整既句子俾我聽。 (1) 如:〔我係一個人〕、〔今日天氣好好〕 f. 呢處有幅圖，請你照住呢畫啦。 (1)

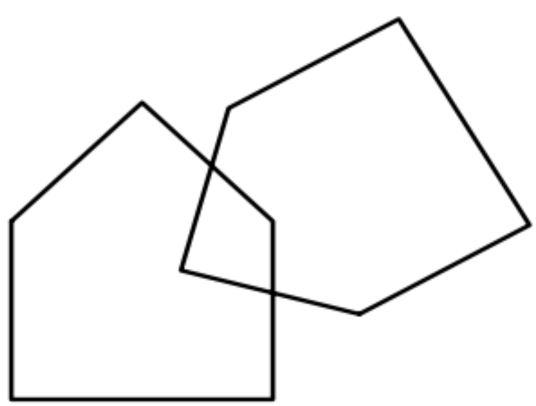
總分： ________/30

d. 請讀出哩張紙上面既字，然後照住去做。

拍手

e. 請你講任何一句完整既句子俾我聽。

f. 哩處有幅圖，請你照住呢畫啦。(畫在右邊空白位置)



< 問題完 >

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