

**Effects of Exercise Training Modalities
on Fat Oxidation
in Overweight and Obese Women**

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Abstract

Purpose: To compare the effects of aquatic-based and land-based exercise training on fat oxidation in overweight and obese women. **Methods:** Twenty healthy, overweight and obese women were randomly assigned to, and completed endurance training; deep water running (DWR) ($n = 11$, age 48 ± 7 years, BMI 30.0 ± 4.0 kg/m²), or endurance training combined with resistance training (DWR+RT) ($n = 9$, age 48 ± 8 years, BMI 29.7 ± 4.1 kg/m²) three times per week (70% mode specific HR_{peak}) for 12 weeks. Following the 12 week intervention there was an eight month washout period. At the end of the washout period, 17 of the 20 women originally enrolled in the first intervention participated in, and completed a second intervention, undertaking the same protocol as in the first study, in a land-based environment. Two additional participants recruited, were randomly assigned to, and completed respective land-based training. Nineteen participants in total completed endurance training; land based endurance (LBE) ($n = 9$, age 49 ± 7 years, BMI 30.0 ± 3.8 kg/m²), or endurance training combined with resistance training (LBE+RT) ($n = 10$, age 49 ± 7 years, BMI 29.4 ± 4.0 kg/m²) three times a week (70% mode specific HR_{peak}) for 12 weeks. Results from seventeen participants who completed both the aquatic and land-based interventions were pooled for analysis to compare aquatic-based and land-based exercise training modalities. For the aquatic and land-based interventions, pre and post intervention outcome measures included; resting and exercise fat oxidation and resting metabolic rate (RMR) measured by indirect calorimetry, resting and exercise plasma free fatty acid (FFA) and glycerol concentrations, cardiovascular (CV) fitness assessed during mode specific maximal oxygen consumption ($\dot{V}O_{2peak}$) tests, upper and lower body strength using a Biodex Isokinetic Dynamometer, body composition using dual-energy X-ray absorptiometry (DXA) and anthropometry, and plasma lipid profiles. Statistical analysis included between-group comparisons of outcome measures using analysis of covariance (ANCOVA). When there was no difference between groups, data was pooled and within modality comparisons were assessed by Student's paired t-test. Pearson correlation coefficients were used to investigate relationships between outcome measures. **Results:** Exercise fat oxidation rate did not change following DWR or DWR+RT, or aquatic

exercise training overall ($p > 0.05$). Following land-based exercise training, when training groups were combined, participants demonstrated significant increases in exercise fat oxidation rate (300 ± 92 to 359 ± 119 mg/min; $p < 0.01$), with no difference between LBE and LBE+RT ($p < 0.05$). When aquatic exercise training was compared directly with land-based exercise training using pooled analysis, there were significantly different responses pertaining to changes in exercise fat oxidation rate ($p = 0.03$). There was a significant increase in exercise fat oxidation rate following land-based exercise training (310 ± 91 to 373 ± 118 mg/min; $p < 0.01$); however, no change was observed following aquatic exercise training (265 ± 109 to 264 ± 73 mg/min; $p = 0.97$).

Conclusion: Overweight and obese healthy women demonstrate an increase in exercise fat oxidation rate following land-based exercise training, but not aquatic-based exercise training.

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List of Abbreviations

ANCOVA: analysis of covariance
ANOVA: analysis of variance
ATP: adenosine triphosphate
BMI: body mass index
CHO: carbohydrate
CO₂: carbon dioxide
CoA: coenzyme A
CPT: carnitine palmitoyl transferase
CV: cardiovascular
DWR: deep water running
DWR+RT: deep water running plus resistance training
DXA: dual energy x-ray absorptiometry
EE: energy expenditure
FA: fatty acid
FABP: fatty acid binding protein
FFA: free fatty acid
FM: fat mass
FFM: fat free mass
HDL: high density lipoprotein
HR: heart rate
HR_{peak}: heart rate peak
LBE: land based endurance
LBE+RT: land based endurance plus resistance training
LDL: low density lipoprotein
LPL: lipoprotein lipase
O₂: oxygen
RER: respiratory exchange ratio
RMR: resting metabolic rate
SD: standard deviation
TAG: triacylglycerol
TC: total cholesterol
TCA: tricarboxylic acid
TG: triglyceride
TMW: treadmill walk
 $\dot{V}O_2$: rate of oxygen consumption
 $\dot{V}CO_2$: rate of carbon dioxide production
 $\dot{V}O_{2max}$: maximal oxygen consumption
 $\dot{V}O_{2peak}$: peak oxygen consumption
WC: waist circumference
WHR: waist to hip ratio

Papers Published Relating To This Thesis

Phillips, V.K., Legge, M., & Jones, L. M. (2008). Maximal Physiological Responses between Aquatic and Land Exercise in Overweight Women. *Medicine and Science in Sports and Exercise*, 40, 959-964. (Appendix J).

Chapter 1: Introduction

Obesity research has become increasingly important as the incidence of obesity has risen internationally, leading the World Health Organisation (WHO) and the International Obesity Task Force to declare a 'global obesity epidemic' (Hill, Peters, & Wyatt, 2007; Li, Bowerman, & Heber, 2005; Taubes, 1998; WHO, 2000). Data from the 2002-03 New Zealand Health Survey indicated that more than half the adult population is currently either overweight or obese, with one in five New Zealand adults categorized as obese (Ministry of Health, 2004). If current trends continue, 29% of New Zealanders are likely to be obese by 2011 (Ministry of Health, 2004).

Obesity-related health problems increase beyond a body mass index (BMI) of 25 kg/m² (Panel, 1998). The Expert Panel on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults lists BMI for individuals of European descent of 25 to 29.9 kg/m² for overweight, and a BMI of greater than or equal to 30.0 kg/m² for obesity (ACSM, 2006; Panel, 1998). Co-morbidities, such as cardiovascular disease, hypertension and diabetes mellitus associated with obesity begin in those identified as 'overweight', worsening as an individual approaches an 'obese' classification (Golay & Ybarra, 2005; Li et al., 2005; Must et al., 1999). A U-shaped relationship between BMI and all cause mortality in men and women has been demonstrated, with higher rates of mortality as BMI increases from 25 kg/m² (Li et al., 2005). Consequently, evidence-based advice on the prevention and treatment of developing an overweight or obese state is needed to avert the onset of these co morbidities related to obesity and consequently mortality. Undertaking regular physical activity is one approach for obesity prevention and treatment (Goris & Westerterp, 2008), as participation in regular exercise reduces major health problems associated with excess adiposity (Burnham, 1998; Jeukendrup & Wallis, 2005; Vuori, 2001).

A contributing factor to the etiology of obesity may be an impaired ability to utilise fat as a fuel during exercise (Blaak & Saris, 2002; Jeukendrup & Wallis, 2005). Impairments occur in the ability to utilise plasma free fatty acids (FFA) during exercise, resulting in the development of a positive fat balance and increased fat storage, which is characteristic of obesity (Blaak & Saris, 2002). In normal weight individuals the ability

to utilise fat as a fuel during exercise, known as fat oxidation, is stimulated following endurance exercise training (Blaak & Saris, 2002; Brooks & Mercier, 1994; Phillips, Green et al., 1996; Turcotte, Richter, & Kiens, 1992). Therefore, optimization of fat oxidation through appropriate exercise training in overweight and obese individuals may compensate for impaired fat oxidation, and has the potential to reduce health risks associated with obesity (Jeukendrup & Wallis, 2005). However, contradictory results have been reported in overweight and obese individuals following endurance training with either increases (Dumortier et al., 2003; van Aggel-Leijssen, Saris, Wagenmakers, Senden, & van Baak, 2002; Venables & Jeukendrup, 2008), or no change in exercise fat oxidation (Kanaley, Weatherup-Dentes, Alvarado, & Whitehead, 2001; van Aggel-Leijssen et al., 2002; Venables & Jeukendrup, 2008).

Therefore, whether endurance exercise elicits an effect on fat oxidation is yet to be determined in overweight and obese individuals. There is also limited and inconclusive evidence on resistance training and fat oxidation. A lower rate of exercise fat oxidation occurs during acute resistance exercise compared to endurance exercise (Bloomer, 2005; Magkos et al., 2008). However, both increases and no change in fat oxidation have been reported following resistance training (Ballor, Harver-Berino, Ades, Cryan, & Calles-Escandon, 1996; Treuth, Hunter, Weinsier, & Kell, 1995). Investigation of exercise fat oxidation following resistance training, specifically in the overweight or obese population, is lacking.

When resistance exercises are performed intermittently with endurance activities, fat oxidative metabolism is similar to that during endurance exercise (Jurimae, Karelson, Smirnova, & Viru, 1990). This combined endurance and resistance training may be more beneficial than endurance training alone, as increases in fat oxidation attributed to endurance training may be combined with adaptations achieved through resistance training, including increases in fat free mass (FFM), resting metabolic rate (RMR) and strength (Dolezal & Potteiger, 1998; Maiorana, O'Driscoll, Goodman, Taylor, & Green, 2002; Pierson et al., 2001; Wallace, Mills, & Browning, 1997). Nevertheless, there are limited data on the effects of this combined type of training on substrate utilisation in overweight or obese individuals.

The most appropriate modality of exercise training to induce increases in fat oxidation has not been investigated in overweight or obese individuals. Exercise recommendations for individuals who are overweight or obese include non weight-bearing activities to minimise injury (ACSM, 2006; Wallace, 2003). Therefore, an aquatic, non weight-bearing environment may be an appropriate exercise setting for the overweight or obese population. Deep water running (DWR) is an alternate form of running in an aquatic environment. Both endurance and resistance exercise can be incorporated into a DWR programme; however the effects of this type of exercise on fat oxidation are unknown. Recent investigations demonstrate favorable changes in cardiovascular fitness, strength, body composition and lipid profile following an aquatic exercise intervention incorporating both endurance and resistance training (Meredith-Jones, Legge, & Jones, 2009; Nowak et al., 2008). These results indicate the potential for aquatic exercise training to demonstrate positive alterations in health indices, an area requiring further investigation.

The purpose of this thesis was to compare the effects of endurance, and a combination of endurance and resistance training, on fat oxidation in a group of middle aged overweight and obese women. Investigation into aquatic-based and land-based exercise training modalities will be undertaken independently and comparatively. From these investigations, the most appropriate exercise modality to enhance fat oxidation in a population of overweight and obese women may be determined.

It is worth noting that the purpose of this thesis was not to prescribe exercise for body fat loss, but rather investigate equivocal evidence in the literature regarding overweight and obese individuals and exercise fat oxidation following exercise training. In summary, low intensity exercise is recommended for individuals to oxidise fat as a fuel, which may be impaired in those who are overweight or obese. The aim of the thesis is not to stipulate that weight loss occurs at an exercise intensity maximising fat oxidation, but that oxidation of fat may improve the balance of FFA uptake and oxidation and limit excess circulating plasma FFA that may impose health problems. If an overweight or obese individual is able to increase fat oxidation during exercise following exercise training, weight loss will only occur if the individual is expending more energy than consuming.

There are three aims and hypotheses to this thesis:

1) To investigate whether there are differences between aquatic-based endurance DWR, and endurance DWR combined with aquatic resistance training, for changes in exercise fat oxidation. It is hypothesised that the combination of DWR and resistance training will result in a greater increase in exercise fat oxidation than DWR in overweight and obese women.

Responses following DWR and the combination of DWR and resistance training will also be compared for resting fat oxidation, and health related outcomes; cardiovascular (CV) fitness, upper and lower body strength, anthropometry and lipid profiles, in overweight and obese women.

As an additional aim, the graded DWR exercise test used to determine maximal responses for exercise prescription will be validated for a population of middle aged overweight and obese women. Comparison of maximal responses between aquatic and land maximal exercise tests will be undertaken.

2) To investigate whether there are differences between land-based endurance and land based endurance combined with resistance training for changes in exercise fat oxidation. It is hypothesised that a combination of LBE and resistance training will result in a greater increase in exercise fat oxidation than LBE in overweight and obese women.

Responses following LBE and the combination of LBE and resistance training will also be compared for resting fat oxidation, and health related outcomes; CV fitness, upper and lower body strength, anthropometry and lipid profiles, in overweight and obese women.

3) To compare responses between aquatic-based and land-based exercise training on exercise fat oxidation. It is hypothesised that both low intensity aquatic and land-based exercise training will result in an increase in exercise fat oxidation in overweight and obese women; with aquatic-based exercise training resulting in less of an increase in exercise fat oxidation than land-based exercise training.

Responses following aquatic-based and land-based exercise training interventions will also be compared for resting fat oxidation, and health related outcomes; CV fitness, upper and lower body strength, anthropometry and lipid profiles, in overweight and obese women.

Chapter 2: Review of Literature

2.1 Introduction

This literature review will begin with a discussion on substrate utilisation, with a primary focus on differences in fat metabolism between normal weight, and overweight and obese individuals. The effects of exercise training on land, including endurance training, resistance training, and a combination of endurance plus resistance training as a means to increase fat oxidation will be examined. An in depth discussion on the benefits of aquatic exercise for an overweight and obese population, and the adaptations that occur as a result of exercise in the water will conclude the review of literature.

2.2 Substrate Utilisation

During rest and physical activity, stored fuels are used to supply the body with energy (Jeukendrup, 2003). The two primary sources of energy are carbohydrate (CHO) and fat (Jeukendrup, Saris, & Wagenmakers, 1998a; Ranallo & Rhodes, 1998; Spriet, 2002). Fat provides energy through the process of oxidation. Carbohydrate provides energy through both oxidative and anaerobic pathways, referred to as CHO utilisation. The contribution of both fat and CHO toward energy provision is tightly regulated, and varies depending on a number of factors including; intensity, duration, and mode of physical activity, along with training status, body composition, and hormonal status of the individual (Jeukendrup, 2003).

2.2.1 Normal Substrate Utilisation Responses

Triacylglycerol (TAG) is the storage form of fat and comprises three fatty acids (FA) and a glycerol molecule. The majority (>95%) of the body's TAG are found in adipose tissue, with smaller amounts in skeletal muscle and plasma (Coppack, Jensen, & Miles, 1994; Horowitz & Klein, 2000a; Ranallo & Rhodes, 1998), and must be mobilised as an energy source through the process of lipolysis via its constituents, fatty acids and glycerol (Houston, 2001).

The most important regulators of lipolysis are insulin and catecholamines (Arner, 1995; Coppack et al., 1994). Insulin promotes fat storage by down regulating adipose triglyceride lipase (ATGL) levels, and inhibiting hormone sensitive lipase (HSL) levels (Kim, Tillison, Lee, Rearick, & Smas, 2006). Conversely, catecholamines promote lipolysis by activating HSL (Arner, 1995; Coppack et al., 1994). The rate limiting step for lipolysis of adipose tissue TAG was thought to be primarily hydrolysis by HSL (Bennard, Imbeault, & Doucet, 2005; Coppack et al., 1994; Jeukendrup et al., 1998a). However, recent evidence suggest that the combined action of ATGL, HSL and monoglyceride lipase (MGL) leads to the sequential hydrolysis of TAG, diacylglycerol and monoacylglycerol respectively (Zechner, Kiensberger, Haemmerle, Zimmermann, & Lass, 2009).

Once TAG is hydrolysed, FA may be released from adipose tissue, skeletal muscle, or plasma (Coppack et al., 1994). The glycerol released through TAG hydrolysis during lipolysis cannot be reused by adipose tissue cells, thus blood plasma measures of glycerol are often used to indicate whole body lipolysis (Bortz, Paul, Haff, & Holmes, 1972; Houston, 2001; Jeukendrup et al., 1998a). The glycerol is released from the adipocyte and is transported back to the liver where it is either phosphorylated to form TAG in the liver, or converted to dihydroxtacetone phosphate, which can then participate in either glycolysis or gluconeogenesis (Houston, 2001; Jeukendrup et al., 1998a). Fatty acids released during lipolysis have two major fates. Firstly, FA may form a new TAG molecule through re-esterification by triglyceride-fatty acid (TG-FA) cycling (Jeukendrup et al., 1998a; Wolfe, Klein, Carraro, & Weber, 1990). In this instance, re-esterification can occur within the adipocyte, or elsewhere, including the liver (Wolfe et al., 1990). Secondly, FA may exit the fat cell, attach to albumin and become a free fatty acid (FFA) in the blood stream to be used as an energy source (Corcoran, Lamon-Fava, & Fielding, 2007; Houston, 2001; Jeukendrup et al., 1998a). The release of FFA into the blood via lipolysis from its major storage source, adipose tissue, is important for increasing or maintaining the delivery of FFA to exercising muscle for oxidation (Hodgetts, Coppack, Frayn, & Hockaday, 1991; Spriet, 2002). Figure 1 depicts the pathways of FA metabolism.

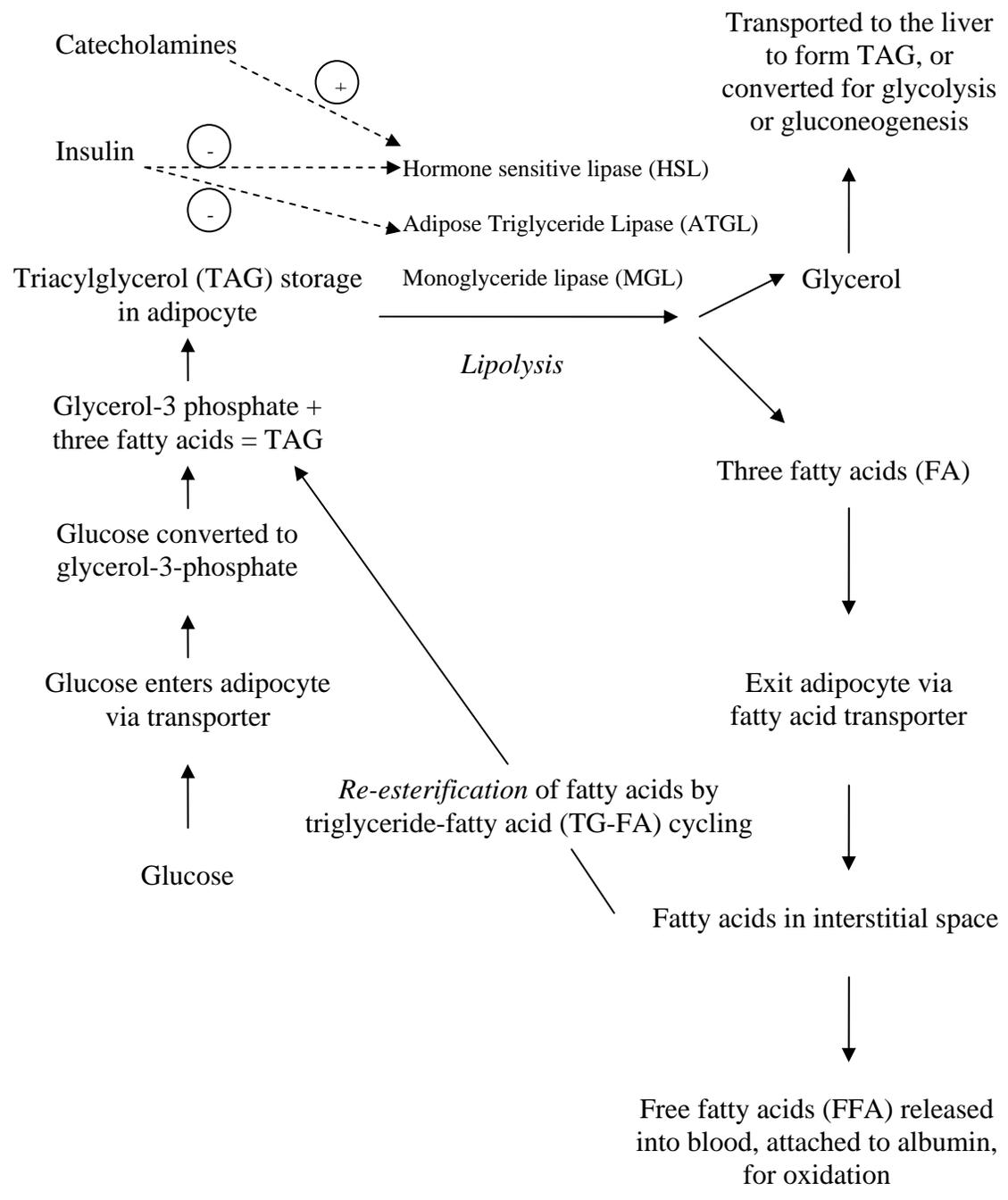


Figure 1. Schematic diagram showing simplified pathways of fatty acid metabolism.

Once FA are released via lipolysis, they are transported via the blood, across the muscle cell membrane and metabolized in a stepwise manner in the cytosol of the cell and mitochondria (Jeukendrup et al., 1998a; Spriet, 2002). The majority of FFA are transported or assisted across the muscle membrane by fatty acid binding proteins (FABP) (Jeukendrup et al., 1998a; Spriet, 2002). Fatty acids are then activated using adenosine triphosphate (ATP) and coenzyme A (CoA), allowing entry into the mitochondria for oxidation. The FA, now as fatty acyl CoA, cross the mitochondrial membrane with aid of the carnitine palmitoyl transferase (CPT) system (Jeukendrup et al., 1998a; Spriet, 2002). Once in the mitochondrion, carnitine is removed, and fatty acyl CoA molecules are metabolised (Jeukendrup et al., 1998a; Spriet, 2002). The metabolism of fatty acyl CoA involves the sequential removal of 2-carbon units during the process of β -oxidation, which is the initial process in fat oxidation (Jeukendrup et al., 1998a; Spriet, 2002). The β -oxidation pathway involves the production of acetyl-CoA and reducing equivalents, which are further metabolised in the tricarboxylic acid (TCA) cycle and used to drive the electron transport chain, synthesizing ATP for energy production (Houston, 2001; Jeukendrup, 2002; Jeukendrup et al., 1998a; Spriet, 2002).

Carbohydrate is stored in the body as glycogen in muscle and liver. There are limited CHO stores, whereas there is an abundance of stored TAG (Jeukendrup et al., 1998a; Maughan, Gleeson, & Greenhaff, 1997). Glycogen is catabolised through the process of glycogenolysis into glucose, which is then available for energy production through anaerobic glycolysis, or oxidation (Brooks & Mercier, 1994). Glycogenolysis is up regulated through increases in hormones such as adrenaline and glucagon, whereas insulin promotes CHO storage (Maughan et al., 1997). During high intensity muscular exercise, CHO is a more efficient fuel than FA as it generates acetyl CoA for the TCA cycle at a much higher rate (Houston, 2001). Therefore, CHO metabolism works in concert with fat metabolism depending on the demand for muscular work.

2.2.2 Substrate Utilisation during Exercise

At rest and during low to moderate intensity exercise, FFA are the major energy source (De Feo et al., 2003; Kanaley et al., 2001; Martin et al., 1993; Romijn et al., 1993;

Swan & Howley, 1993; Tarnopolsky, MacDougall, Atkinson, Tarnopolsky, & Sutton, 1990; van Loon, Greenhaff, Constantin-Teodosiu, Saris, & Wagenmakers, 2001). Oxidation of FFA is rate limited by two main factors; the availability of FFA which is determined by FFA mobilisation or lipolysis, and tissue capacity to oxidise FFA (Bulow, 1993; Hawley, Brouns, & Jeukendrup, 1998; Houston, 2001). The capacity of tissue to oxidise FFA is further influenced by blood FA concentration, capillary density, blood flow, transport efficiency, mitochondrial capacity, and neural and hormonal factors (van Baak, 1999).

Fatty acid availability normally exceeds fat oxidation at rest (Coppack et al, 1994); however the utilisation of fat during exercise can be limited by plasma FA availability, as demand may exceed supply. Increased blood flow to adipose tissue and muscle tissue during exercise decreases FA re-esterification and facilitates the delivery of FA to skeletal muscle for oxidation (Arner, 1995; Horowitz & Klein, 2000a; Jeukendrup, 2002; Wolfe et al., 1990). In absolute terms, CHO oxidation will increase proportionally with exercise intensity, whereas the rate of fat oxidation will initially increase but decreases at high exercise intensities (De Feo et al., 2003; Jeukendrup & Wallis, 2005). Fat oxidation increases from rest during low to moderate intensity exercise of 40-65% $\dot{V}O_{2\max}$ (Romijn et al., 1993; Spriet & Watt, 2003). However, fat oxidation is decreased during higher intensities of exercise, at power outputs above ~75% $\dot{V}O_{2\max}$ (Houston, 2001; Romijn et al., 1993). Thus, energy flux is determined by relative exercise intensity.

Although highly variable amongst individuals (Coggan, 1997), the release of FA into the bloodstream from adipose tissue increases in parallel with exercise intensity to approximately 50% of $\dot{V}O_{2\max}$, and then gradually declines (Brooks, 1997). The rate of FFA release declines progressively with increased exercise intensity to a point where plasma FFA concentration during high intensity exercise is markedly suppressed. It is possible that this reduced availability of plasma FFA may contribute to the decline in fat oxidation observed when the intensity of exercise is increased (Romijn et al., 1993). During high intensity exercise catecholamines continue to rise, stimulating lipolysis (Romijn et al., 1993). However, a reduction in adipose tissue blood flow during high intensity exercise may decrease FFA release from adipose tissue (Romijn et al., 1993). This decrease in FFA delivery to the working muscles during high intensity exercise,

corresponds with a large decrease in FA oxidation (Romijn et al., 1993). Furthermore, increased blood lactate accumulation during high intensity exercise also results in inhibition of lipolysis and subsequently fat oxidation (Maughan et al., 1997). Alternatively, release of glucose into the bloodstream from muscle and liver increases exponentially with exercise intensity, reflected by an increase in glycogen utilisation with increasing exercise intensity (Brooks, 1997). Brooks (1994) proposed a crossover concept which describes the balance between CHO and fat utilisation during exercise. Although considered controversial by some (Berger, 2004; Coggan, 1997), the crossover point describes the intensity at which increments in relative exercise effort result in increasingly greater dependence on CHO and less dependence on fat (Berger, 2004; Brooks & Mercier, 1994). The exercise intensity at which fat oxidation is maximal, defined as Fat_{max} , is dependent on sex, training status, maximal oxygen consumption ($\dot{V}O_{2max}$), and diet and therefore can be highly variable between individuals (Achten & Jeukendrup, 2003, 2004; Jeukendrup & Wallis, 2005; Meyer, Gabler, & Kindermann, 2007). In healthy, moderately trained men Fat_{max} is equivalent to $64 \pm 4\% \dot{V}O_{2max}$, and $74 \pm 3\% HR_{max}$ (Achten, Gleeson, & Jeukendrup, 2002). In the general population, the greatest absolute rates of fat oxidation occur between 47% and 52% $\dot{V}O_{2max}$ (Achten & Jeukendrup, 2004). However, one single exercise intensity does not appear to exist that uniformly leads to maximal fat oxidation in all individuals (Meyer et al., 2007).

The relative balance between FA released from adipose tissue through lipolysis and the re-esterification and FFA uptake by exercising muscles is indicative of plasma FFA concentration (Groop, Bonadonna, Shank, Petrides, & DeFronzo, 1991; Jeukendrup, 2003). Lipolysis increases as a function of power output at moderate intensity (25% to 65% $\dot{V}O_{2max}$) (Romijn et al., 1993). During the first 15 minutes of moderate-intensity exercise, plasma FFA concentrations usually decrease, as FA uptake by muscle exceeds lipolysis through utilisation of circulating FA (Jeukendrup, 2002). As exercise continues, plasma FFA concentration rises as lipolytic mobilisation of stored FA's exceeds the rate of plasma FFA uptake (Jeukendrup, 2003). The plasma concentration of FA can increase five to six fold during very prolonged exercise (Saltin & Astrand, 1993). As the availability of FA increases, via exercise induced catecholamine stimulated lipolysis, the extraction of FA from the blood by muscle increases. Therefore, FA uptake by muscle

and thus fat oxidation, is affected by the rate of lipolysis and plasma FFA concentration (Groop et al., 1991). Once exercise has stopped, fat oxidation is reduced, whereas lipolytic activity is maintained (Jeukendrup et al., 1998a). Peak FFA concentration is achieved 10-15 minutes after cessation of exercise, thereafter concentrations will decline to resting levels (Jeukendrup et al., 1998a).

Substrate utilisation may also be influenced by hormonal variations in the menstrual cycle. Studies using indirect calorimetry have reported a greater proportion of fat oxidised during low to moderate intensity exercise in the luteal phase of the menstrual cycle (Hackney, McCracken-Compton, & Ainsworth, 1994; Houston, 2001; Ruby & Robergs, 1994; Wenz, 1997; Zderic, Coggan, & Ruby, 2001). These researchers suggest that elevated levels of estrogen resulted in increased fat oxidation during this phase (Hackney et al., 1994; Houston, 2001; Ruby & Robergs, 1994; Wenz, 1997; Zderic et al., 2001). Schoeller and colleagues (1997) also suggest that resting metabolic rate (RMR) is significantly greater during the luteal phase than the follicular phase (Schoeller, Shay, & Kushner, 1997). Conversely, several researchers have demonstrated that fluctuations in ovarian hormones between the follicular phase and luteal phase of the menstrual cycle do not influence whole body fat oxidation at rest (Heiling & Jensen, 1992; Jacobs, Casazza, Suh, Horning, & Brooks, 2005; Suh, Casazza, Horning, Miller, & Brooks, 2002) or during exercise (Jacobs et al., 2005; Kanaley, Boileau, Bahr, Misner, & Nelson, 1992; Suh et al., 2002). The discrepancies between previous studies may be due to exercise intensity. Hackney et al (1994) observed differences in substrate utilisation between menstrual cycle phases at low and moderate intensity exercise (35% $\dot{V}O_{2\max}$ and 60% $\dot{V}O_{2\max}$); however, no differences in substrate utilisation were reported with exercise at higher intensity (75% $\dot{V}O_{2\max}$) (Hackney et al., 1994). The potential confounding influence of the menstrual cycle may be minimised by conducting metabolic studies during the follicular phase (3-9 days after onset of menses) in eumenorrhoeic women.

2.2.3 Measurement of Substrate Utilisation

Energy expenditure (EE) and substrate utilisation rates during rest and exercise are commonly determined using indirect calorimetry (Ferrannini, 1988; Simonson & DeFronzo, 1990; Westerterp, 2003). Ambient air of a known composition is inhaled and

change in the percentages of oxygen (O_2) and carbon dioxide (CO_2) in expired air provide an indirect indication of energy metabolism (Simonson & DeFronzo, 1990). These values are used to approximate EE using the formula developed by Weir (1949). Substrate utilisation rates can then be determined through the calculation of CHO and fat oxidation rates according to the non-protein respiratory quotient (RQ) technique (Peronnet & Massicotte, 1991).

Fat oxidation is most often measured by changes in the RQ, which is substrate turnover at the cellular level; or estimated by changes in the non-protein respiratory exchange ratio (RER), from concentrations of inspired and expired gases (Bennard et al., 2005; Ruby & Robergs, 1994). These equations are based on the assumption that protein breakdown contributes little to energy metabolism during exercise (Peronnet & Massicotte, 1991). The RER is calculated using the ratio of CO_2 produced to O_2 consumed. During steady-state exercise the RER can give a reasonable estimate of the proportions of CHO and lipid being oxidised (Jeukendrup et al., 1998a). An RER of 1.00 indicates that the primary substrate being oxidised is CHO, whereas an RER of between 0.69-0.73 indicates that the primary substrate being oxidised is fat (Jeukendrup et al., 1998a; Maughan et al., 1997). An RER of 0.82 is assumed to represent a mixed source of both CHO and fat (McArdle, Katch, & Katch, 2001).

The use of indirect calorimetry to measure substrate utilisation provides information on whole body processes (Ferrannini, 1988). Indirect calorimetry measures net fat oxidation, which includes oxidation of plasma FFA and oxidation of intracellular lipids (Frayn, 1983; Simonson & DeFronzo, 1990), therefore it is difficult to determine which pool of lipid is being oxidised with this technique (Coppack et al., 1994). To examine the exact source of fatty acid oxidation other techniques, such as infusions of labeled fatty acids and labeled isotopes, are used to provide information about oxidation of plasma and non-plasma fatty acid sources (Coppack et al., 1994; Horowitz, 2001).

Lipid mobilisation, or lipolysis, can be studied using a variety of techniques. An indication of lipolysis is obtained by measuring the changes in circulating glycerol and FFA, by-products of metabolism (Arner, 1995). Measurement of plasma FFA and glycerol concentrations is a systemic, in vivo method of studying lipolysis (Coppack et al., 1994). Changes observed in FFA and/or glycerol concentrations in blood may reflect

qualitative changes in adipose tissue lipolysis (Coppack et al., 1994). However, this should not be taken as a quantitative representation of lipolytic activity, due to a nonlinear relationship between FFA turnover and concentration (Miles, Ellman, McClean, & Jensen, 1987).

2.2.3 Overweight and Obese Individuals

Individuals of normal weight predominantly utilise stored fat as an energy source at rest and during low to moderate intensity exercise of long duration (Perez-Martin et al., 2001; van Loon et al., 2001). As exercise intensity increases there is an increased reliance on CHO (Brooks, 1997; Perez-Martin et al., 2001; van Loon et al., 2001). However, substrate utilisation in overweight and obese individuals is less clear.

Obesity is associated with metabolic disorders that may be related to disturbances in fat mobilisation and oxidation (Berggren, Boyle, Chapman, & Houmard, 2008; Golay & Ybarra, 2005; Horowitz, 2001; Houmard, 2008; Venables & Jeukendrup, 2008). Metabolic disturbances are not specific to obese individuals however, obesity-related health problems do increase beyond a body mass index (BMI) of 25 kg/m² (Panel, 1998). Westerterp and colleagues (2008) reported that fat oxidation is negatively related to percentage body fat, with obese individuals demonstrating lower rates of fat oxidation compared to normal weight individuals (Westerterp, Smeets, Lejeune, Wouters-Adriaens, & Westerterp-Plantenga, 2008). Similarly, Wade et al. (1990) observed that the RER during exercise was significantly correlated to body fatness (Wade et al., 1990), indicating lower exercise fat oxidation with increasing level of body fat. Conversely, one previous investigation also reported a lack of relationship between body fatness and RER (Geerling et al., 1994). Disorders in metabolism may therefore be accentuated with degrees of overweight and obesity, worsening with increasing levels of body fat. The lower rates of fat oxidation reported in obese individuals may be due to an impaired ability to oxidise fat during exercise, an etiological factor for obesity (Astrup, Raben, Buemann, & Toubro, 1997; Blaak & Saris, 2002; Colberg, Simoneau, Thaete, & Kelley, 1995; Guesbeck et al., 2001a; Kanaley et al., 2001; Kelley, Goodpaster, Wing, & Simoneau, 1999; Kim, Hickner, Cortright, Dohm, & Houmard, 2000; Perez-Martin et al.,

2001; Thyfault, Kraus et al., 2004; Tremblay, Simoneau, & Bouchard, 1994; van Baak, 1999; Wade et al., 1990; Zurlo et al., 1990). Significantly reduced fat oxidation during exercise has been reported in overweight and obese individuals compared to those of normal weight (Berggren et al., 2008; Chatzinkolaou et al., 2008; Colberg et al., 1995; Dumortier, Thoni, Brun, & Mercier, 2005; Perez-Martin et al., 2001). Perez-Martin and colleagues (2001) suggest that obese individuals experience a greater reliance on CHO at lower exercise intensities as reflected by an earlier point of crossover from fat to CHO sources for energy supply (Perez-Martin et al., 2001).

Studies have been undertaken, but are inconclusive, whether fat mobilisation is a limiting factor for fat oxidation in obese individuals during exercise, or if deficits are limited to uptake and oxidation in skeletal muscle (Astrup et al., 1997). The research to date indicates that basal lipolysis is elevated in obese individuals; however, there appears to be a blunted increase in lipolysis or reduced lipolytic response during exercise (Coppack et al., 1994; Jensen, Haymond, Rizza, Cryer, & Miles, 1989; Mittendorfer, Fields, & Klein, 2004; van Baak, 1999). These results indicate that fat mobilisation during exercise may be limited in obese individuals.

The decreased reliance on fat oxidation predisposes an individual toward weight gain and obesity (Berggren et al., 2008; Houmard, 2008; Thyfault, Kraus et al., 2004; Westerterp et al., 2008; Zurlo et al., 1990), as it leads to, or precedes the development and/or maintenance of increased fat stores (Blaak & Saris, 2002; Guesbeck et al., 2001b). Furthermore, circulating FFA concentrations are usually elevated in obese individuals (Corcoran et al., 2007; Lewis, Carpentier, Adeli, & Giacca, 2002). This, coupled with a reduced rate of fat oxidation, favors re-esterification of FFA, resulting in TAG deposition in adipose tissue, and non-adipose tissues including; muscle, liver and pancreas (Campbell, Carlson, & Nurjhan, 1994; Colberg et al., 1995; Coppack et al., 1994; Corcoran et al., 2007; Golay & Ybarra, 2005; Holloway, Bonen, & Spriet, 2009; Horowitz, 2001; Houmard, 2008; Kelley et al., 1999; Lewis et al., 2002; Mensink, Blaak, Wagenmakers, & Saris, 2005; Thyfault, Richmond, Carper, Potteiger, & Hulver, 2004). In addition, limitations in lipolysis (Coppack et al., 1994; Jensen et al., 1989; van Baak, 1999), may lead to a higher concentration of TAG and larger TAG stores in adipose tissue. Consequently, obese persons may have increased TAG stores in adipose tissue

and non-adipose tissue, contributing to the metabolic abnormalities associated with obesity (Coppack et al., 1994; Horowitz, 2001; Phillips, Caddy et al., 1996).

2.2.4 Health Implications

The ability to oxidise fat during exercise has implications for weight control and consequently health outcomes (ACSM, 2001; Houmard, 2008; Pasanisi, Contaldo, de Simone, & Mancini, 2001). Elevated FFA levels in many obese individuals, combined with lower daily fat oxidation, results in the imbalance between uptake and oxidation of plasma FFA. It is this reduced lipid turnover that is thought to underpin adverse health outcomes related to obesity (Corcoran et al., 2007). In overweight and obese individuals, the reduction in lipid turnover and subsequent accumulation of lipids is strongly associated with insulin resistance (Coppack et al., 1994; Corcoran et al., 2007; Despres, 1994; Golay & Ybarra, 2005; Holloway et al., 2009; Houmard, 2008; Pan et al., 1997), hyperlipidemia (Coppack et al., 1994; Horowitz, 2001), coronary heart disease (CHD) (Coppack et al., 1994; Horowitz, 2001), hypertension, and Type 2 diabetes (ACSM, 2001; Golay & Ybarra, 2005; Pasanisi et al., 2001; Vuori, 2001). Furthermore, with increased body fat, low levels of cardio protective high density lipoprotein (HDL) are reported (Mansfield, McPherson, & Koski, 1999; Sardinha, Teixeira, Guedes, Going, & Lohman, 2000), in conjunction with elevated low density lipoprotein (LDL) levels (Despres, 1994; Lemieux et al., 2000; Mansfield et al., 1999). Low levels of HDL and high levels of LDL are frequently associated with increased levels of triglyceride (TG) (Lemieux et al., 2000), which is regarded as a stimulus for an atherogenic lipid profile (Coppack et al., 1994; Herd, Kiens, Boobis, & Hardman, 2001).

It is important to elucidate effective treatments that reverse and/or compensate for the obesity induced impairment in fat oxidation (Berggren et al., 2008). Physical activity facilitates the equilibrium between energy intake and expenditure, and most importantly between fat intake and fat oxidation (Tremblay & Therrien, 2006). Exercise training with the purpose of increasing exercise fat oxidation, has been recommended to reduce the complications of obesity; including the risk of developing Type 2 diabetes, cardiovascular disease (Dumortier et al., 2005; Horowitz, 2001; Pasanisi et al., 2001),

hypertension (Achten & Jeukendrup, 2004; Pasanisi et al., 2001) and improve the blood lipid profile (Achten & Jeukendrup, 2004; Horowitz, 2001; Pasanisi et al., 2001).

2.3 Exercise Training Modalities

Endurance training and resistance training are two common exercise training modalities. Endurance training is typically associated with increased CV fitness and increases in fat oxidation (Friedlander, Casazza, Horning, Buddinger, & Brooks, 1998; Horowitz, Leone, Feng, Kelly, & Klein, 2000), while resistance training is typically associated with improved muscular strength and increased fat free mass (FFM) in normal weight individuals (Williams et al., 2007). However, there is contradictory and limited evidence which suggests that either endurance or resistance training elicits fat oxidation adaptations in overweight or obese individuals (Amati, Dube, Shay, & Goodpaster, 2008; Ballor et al., 1996; Kanaley et al., 2001; Treuth et al., 1995). Alternatively, a combination of endurance and resistance training may result in adaptations specific to both modes of exercise. It is unknown whether addition of resistance exercise to an endurance based exercise programme further improves the ability to increase exercise fat oxidation, specifically in overweight and obese individuals. The majority of studies investigating endurance, resistance and a combination of endurance and resistance training on fat oxidation and related adaptations have been conducted using land-based weight-bearing exercise; these will be addressed in section 2.3.1 and section 2.3.2. However, weight-bearing exercise may not be optimal in overweight or obese individuals; therefore investigation into the effects of exercise training undertaken in an aquatic non weight-bearing environment will be addressed in section 2.3.3.

2.3.1 Endurance and Resistance Training Adaptations

2.3.1.1 Substrate Utilisation

Endurance training results in specific adaptations that allow an individual to exercise at a given power output for a longer duration (Coggan, Raguso, Gastaldelli,

Sidossis, & Yeckel, 2000; Spriet, 2002). These adaptations involve modifications in substrate utilisation patterns by increasing an individual's capacity to utilise fat as an energy source during exercise (Bergman et al., 1999; Blaak & Saris, 2002; Brooks & Mercier, 1994; Maughan et al., 1997; Phillips, Green et al., 1996; Turcotte et al., 1992). Rates of fat oxidation are significantly greater in endurance trained men during rest (Bouchard, 1993; Calles-Escandon, Goran, O'Connell, Nair, & Danforth, 1996) and exercise (Klein, Coyle, & Wolfe, 1994; Tremblay, Coveney, Despres, Nadeau, & Prud'homme, 1992). Furthermore, data from cross-sectional (Bergman & Brooks, 1999; Coggan et al., 2000; Klein et al., 1994; Sidossis, Wolfe, & Coggan, 1998; Turcotte et al., 1992) and longitudinal studies (Bergman et al., 1999; Friedlander et al., 2007; Girandola & Katch, 1976; Horowitz et al., 2000; Hurley et al., 1986; Kiens, Essen-Gustavsson, Christensen, & Saltin, 1993; Martin et al., 1993; Phillips, Green et al., 1996; Pruchnic et al., 2004; Sial, Coggan, Hickner, & Klein, 1998) in normal weight individuals, have demonstrated that endurance training increases the oxidation of fat and reduces the reliance on CHO as an energy source at a given absolute exercise intensity. Observations of increased exercise fat oxidation following endurance training have also been made in women (Friedlander et al., 1998; Horowitz et al., 2000), and elderly persons (Sial et al., 1998).

According to the crossover concept (Brooks & Mercier, 1994), during acute submaximal exercise the relative oxidation of lipid will increase, and CHO will decrease, after chronic exercise training (Brooks & Mercier, 1994). Whole body fat oxidation may increase 25% to 41% following endurance exercise training in normal weight individuals (Horowitz et al., 2000; Martin et al., 1993). Early work undertaken by Christensen and Hansen (1939) demonstrated a reduction in the respiratory exchange ratio (RER), during moderate-intensity exercise after endurance training. This reduction in the RER indicates a decrease in the proportion of CHO oxidised, and a corresponding increase in the proportion of fat oxidised following endurance training (Christensen & Hansen, 1939).

A number of adaptations occur in skeletal muscle following endurance training, which result in its increased capacity to oxidise fatty acids (Hurley et al., 1986). Skeletal muscle plays a major role in the balance of fat and CHO oxidation at rest and during exercise as it is one of the most metabolically active body tissues, due in part, to its mass

(Houmard, 2008; Thyfault, Richmond et al., 2004). Endurance training increases blood flow and oxygen delivery to muscle, which are key elements in fat oxidation (McMurray & Hackney, 2005) thereby promoting an increased ability to oxidize FFA (Holloszy & Coyle, 1984), resulting in glycogen sparing and an increase in exercise capacity. This results in an increase in fatty acid transporter synthesis within the muscle fiber and increased fatty acid transporters within the sarcolemma (Saltin & Astrand, 1993). Proliferation of capillaries within skeletal muscle also enhances fatty acid delivery to muscle (Hawley et al., 1998; Jeukendrup, 2002; McMurray & Hackney, 2005; Saltin & Astrand, 1993), while increases in oxidative and mitochondrial enzyme activity, and mitochondria (size and number) in trained muscles enhances the capacity for fat oxidation (Holloszy & Coyle, 1984; Horowitz et al., 2000; Hurley et al., 1986; Jeukendrup, 2002; Jeukendrup, Saris, & Wagenmakers, 1998b; McMurray & Hackney, 2005; Saltin & Astrand, 1993; Spina et al., 1996). Thus, as a result of endurance training, muscle is able to oxidise a greater amount of fat due to adaptations that occur within the muscle itself.

Resistance training, on the other hand, is usually undertaken to develop muscular strength (Reilly, 1983; Williams et al., 2007). Resistance exercise involves activities that use muscular movement to work against a resistive load and/or gravity (Williams et al., 2007). Strength increases occur when substantial force is placed on the muscle itself, causing muscle overload (Williams et al., 2007). Resistance and endurance exercise can differ considerably in the type of muscular contraction, motor unit recruitment, muscle cell metabolism, and thus whole body substrate use (Knuttgen, 2007). Low intensity endurance training relies on aerobic metabolism, which can result in greater fat oxidation through adaptations in skeletal muscle (Knuttgen, 2007; Thyfault, Richmond et al., 2004). However, resistance training, consisting of repeated muscular contractions at high mechanical power output results in greater anaerobic metabolism (Knuttgen, 2007; McArdle et al., 2001; Thyfault, Richmond et al., 2004; Williams et al., 2007). Anaerobic metabolism utilises the glycolytic energy systems, as anaerobic glycolysis of glucose accounts for a large portion of the energy necessary for power production (Knuttgen, 2007; Thyfault, Richmond et al., 2004). A greater RER, indicative of higher CHO oxidation, has been demonstrated during an acute resistance exercise bout compared to

endurance exercise matched for total time and relative intensity (Bloomer, 2005; Magkos et al., 2008). Consequently, resistance training may result in CHO being the preferred substrate during this exercise mode (Thyfault, Richmond et al., 2004), as there is less reliance on aerobic metabolism and thus lower fat oxidation.

The effects of resistance training on substrate utilisation have been investigated in response to a single bout of resistance exercise (Chatzinkolaou et al., 2008; Gillette, Bullough, & Melby, 1994; Magkos et al., 2008; Melanson et al., 2002; Melby, Scholl, Edwards, & Bullough, 1993; Ormsbee et al., 2007; Osterberg & Melby, 2000), and following resistance training interventions (Ballor et al., 1996; MacDougall et al., 1979; Treuth et al., 1995). While endurance exercise training studies largely measure fat oxidation rates during a single exercise bout, resistance training studies primarily measure and investigate fat oxidation in the hours immediately after exercise, or over 24 hours following exercise. The discrepancy between substrate measurement times makes it difficult to compare the effects of resistance training and endurance training on fat metabolism during exercise.

In contrast to the limited work investigating substrate use during resistance exercise (Bloomer, 2005; Magkos et al., 2008), studies investigating the acute effects of a single bout of resistance exercise have reported a significant decrease in the RER immediately following exercise, indicative of increased fat oxidation (Gillette et al., 1994; Melby et al., 1993; Ormsbee et al., 2007; Schuenke, Mikat, & McBride, 2002), and prolonged (15 to 40 hours) decreases in the RER following the exercise bout (Gillette et al., 1994; Magkos et al., 2008; Melby et al., 1993; Osterberg & Melby, 2000; Schuenke et al., 2002). Resistance exercise therefore, elicits increased post exercise fat oxidation; although the effects of chronic resistance training on fat oxidation during an exercise bout are limited (Ballor et al., 1996).

Results of studies investigating the long-term effects of resistance training on substrate utilisation are scarce and findings equivocal. While increases in 24 hour and resting fat oxidation following 16 weeks of resistance training have been demonstrated in healthy older women (Treuth et al., 1995), no difference in substrate utilisation has also been reported (Ballor et al., 1996). Other investigators consider it unlikely that brief maximal contractions associated with resistance training would provide an adequate

training stimulus for the oxidative energy delivery system and thus would not have an effect on fat oxidation ability (MacDougall et al., 1979).

Following endurance training an enhanced rate of lipolysis may be expected due to the increased oxidative potential of endurance trained skeletal muscle. However, lower (Hurley et al., 1986; Martin et al., 1993), or unaltered (Klein et al., 1994) plasma FA concentrations after training have been demonstrated in those of normal weight, which may imply greater levels of fat oxidation. Endurance training therefore, does not appear to have an effect on lipolysis (Horowitz et al., 2000; Martin et al., 1993; Phillips, Green et al., 1996; Sial et al., 1998), as chronic training demonstrates increases in total exercise fat oxidation without any significant change in lipolysis; assessed as the difference in glycerol and FFA concentrations (Saltin & Astrand, 1993; Sial et al., 1998).

Little is known about the effects of resistance training on lipolysis. The lipolytic response to resistance exercise may be similar to the response to endurance exercise (Chatzinkolaou et al., 2008). Following acute resistance exercise, it also appears that exercise induced hormonal secretions may lead to enhanced lipolysis during recovery (Goto, Ishii, Sugihara, Yoshioka, & Takamatsu, 2007; Ormsbee et al., 2007). However, it is unclear what effect chronic resistance training, and potential post exercise increase in lipolysis, would have on lipolysis during exercise following resistance training.

Overweight and Obese Individuals

In overweight and obese individuals the effects of both endurance and resistance training on substrate utilisation are controversial. Following endurance training, a group of obese women demonstrated an increased reliance on CHO oxidation with no increase in fat oxidation during exercise (Kanaley et al., 2001). In contrast, endurance training has resulted in an increase in exercise fat oxidation in overweight and obese adults (Amati et al., 2008). As little as 10 consecutive days of endurance training can increase fatty acid oxidation in skeletal muscle in obese individuals, correcting the defect in the ability to oxidise lipid (Berggren et al., 2008).

Variations in the intensity of endurance training utilised across the literature may account for the differences in fat oxidation observed in the overweight and obese population. Obese men have exhibited an increase in total exercise fat oxidation

following endurance training at low intensity (40% $\dot{V}O_{2\max}$), but not high intensity (70% $\dot{V}O_{2\max}$) (van Aggel-Leijssen et al., 2002). Consequently, low intensity endurance training has been recommended to increase exercise fat oxidation in overweight and obese individuals (Thompson, Townsend, Boughey, Patterson, & Bassett Jr, 1998; van Baak, 1999). Low intensity endurance training between 47% and 52% $\dot{V}O_{2\max}$ for the general population (Achten & Jeukendrup, 2004), and at 42% to 46% $\dot{V}O_{2\max}$ in obese males (Deriaz et al., 2001; Venables & Jeukendrup, 2008) has been demonstrated to result in greatest rates of fat oxidation. Accordingly, improved exercise fat oxidation can be achieved in obese individuals if exercise intensity is individually prescribed at maximal fat oxidation (Dumortier et al., 2003; Dumortier, Perez-Martin, Pierrisnard, Mercier, & Brun, 2002; Venables & Jeukendrup, 2008). Venables and Jeukendrup (2008) alternated exercise intensity 20% above and below the intensity of maximal fat oxidation at five minute intervals in obese men, and demonstrated that a continuous endurance training protocol prescribed at the intensity of maximal fat oxidation is more effective in enhancing exercise fat oxidation compared to an interval training program (Venables & Jeukendrup, 2008).

The effect of endurance training on resting fat oxidation is also equivocal in overweight and obese individuals (Horowitz, 2001). Neither low nor high intensity endurance training is found to have an effect on resting fat oxidation in the otherwise healthy overweight or obese population (van Aggel-Leijssen et al., 2002).

Due to the limited number of studies investigating the effects of resistance exercise training on substrate utilisation in overweight and obese individuals, conclusions cannot be made. Obese individuals demonstrate a higher RER during acute resistance exercise compared to lean individuals, suggesting lower fat oxidation (Chatzinkolaou et al., 2008). Therefore, there appears to be less stimulus to increase exercise fat oxidation following resistance training in obese individuals.

Comparable increases in fat oxidation have been demonstrated following a single bout of either resistive or endurance exercise, of similar relative intensity and duration, compared to pre exercise values in young healthy males (Jamurtas et al., 2004). Thus, both endurance and resistance exercise training have the potential to result in increases in fat oxidation. In overweight and obese individuals however, fat oxidation is higher

during endurance exercise training compared with resistance training (Ballor, Katch, Becque, & Marks, 1988; Ballor, McCarthy, & Wilterdink, 1990). Therefore, endurance training, which stimulates the mobilisation and oxidation of fatty acids, may be the best stimulus to utilise excessive fat stores, having a positive influence on metabolic risk (Horowitz & Klein, 2000b; Mittendorfer et al., 2004). Nevertheless, the impact of the addition of resistive exercises to an endurance based exercise training programme on fat oxidation in overweight or obese individuals is yet to be determined.

2.3.1.2 Anthropometry and Muscular Strength

Body composition may be positively influenced by endurance training, possibly as a consequence, or preceding, favorable alterations in fat oxidation. Positive changes in body composition can occur following endurance training in men and women of normal weight, including reductions in waist and hip circumference measures, waist to hip ratio (WHR), body mass index (BMI), total body mass, fat mass, percentage body fat, abdominal fat and increases in fat free mass (FFM), as demonstrated in the HERITAGE Family Study (Wilmore et al., 1999). However, it appears that a higher intensity of training is more effective in improving body composition. Several researchers reported improvements in body composition following 10-24 weeks of endurance training at intensity of 45-80% $\dot{V}O_{2max}$ in overweight and obese individuals (Asikainen et al., 2002; Murphy & Hardman, 1998; Solomon et al., 2008). Conversely, van Aggel-Leijssen and colleagues (2001) did not report changes in body composition following 12 weeks of endurance training at 40% $\dot{V}O_{2max}$ in obese women, despite an increase in exercise fat oxidation.

As documented in normal weight individuals, fat oxidation is proportional to FFM in those who are overweight (Dumortier et al., 2003), thus increases in FFM through exercise training may favour increases in fat oxidation. In overweight, older women, resistance training resulted in similar increases in FFM and reductions in percent body fat compared to normal weight individuals (Nichols, Omizo, Peterson, & Nelson, 1993; Schmitz, Jensen, Kugler, Jeffery, & Leon, 2003). Resistance training enhances FFM and muscular strength to a greater extent than endurance training (Williams et al., 2007). Coinciding with significant increases in FFM are significant improvements in upper and

lower body strength in obese women following resistance training (Ryan, Pratley, Elahi, & Goldberg, 1995; Schmitz et al., 2003). Strength measurements, although indirect, may be indicative of increased FFM. Therefore, exercise training that elicits increases in FFM, and consequently strength, may improve fat metabolism, both at rest and during exercise in overweight and obese women.

2.3.1.3 Resting Metabolic Rate

Resting metabolic rate (RMR) is the energy expended by an individual that is necessary to maintain physiological processes during rest, accounting for approximately 60-75% of daily EE (Poehlman, 1989). As RMR is the largest component of daily EE, any increase in RMR will contribute to enhanced daily total EE. Thus, RMR is important in managing energy balance and weight control, having long term benefits for the prevention or treatment of obesity (Byrne & Wilmore, 2001; Speakman & Selman, 2003).

Resting metabolic rate can be influenced by endurance training; however, the literature is inconclusive as to the direction and magnitude of the effect (Ravussin & Bogardus, 1989). Endurance trained individuals generally demonstrate a greater RMR when compared to matched sedentary individuals (Tremblay et al., 1992). However, no change in RMR has also been demonstrated following endurance training of 20 weeks at progressive duration and intensity in normal weight men and women (Wilmore et al., 1998). A decrease in RMR has also been reported following an endurance training intervention, consisting of running for 44 weeks, in men and women of normal weight (Westerterp, Meijer, Schoffelen, & Janssen, 1994). Similarly, in obese individuals, RMR has been demonstrated to both increase (Tremblay, 1986) or display no change (van Aggel-Leijssen, Saris, Wagenmakers, Hul, & van Baak, 2001) following endurance training of 12 weeks.

Differing results from endurance training investigations may be due to methodological variations in the timing of the RMR measurement relative to the last exercise bout, intensity and duration of training interventions, mode of exercise, and changes in body composition. A review by Poehlman (1989) suggested that a threshold of exercise intensity may be necessary to increase RMR. Therefore, exercise intensity effects both exercise fat oxidation and RMR adaptations.

Fat free mass also appears to represent a key determinant in the level of RMR (Dolezal & Potteiger, 1998; Nielsen et al., 2000; Stiegler & Cunliffe, 2006); therefore chronic exercise training resulting in an increase in FFM may result in an increase in RMR. Further, Byrne and Wilmore (2001) demonstrated that an increase in FFM after 20 weeks of resistance training increased RMR in obese women (Byrne & Wilmore, 2001). Accordingly, exercise prescription which takes into account the appropriate mode, duration, intensity, frequency and volume of training that will affect FFM, may be essential in influencing RMR (Sharp, Reed, Sun, Abumrad, & Hill, 1992; Speakman & Selman, 2003).

Despite the association between increases in FFM following resistance training, and the link between increases in FFM and RMR, there is inconclusive evidence regarding the magnitude of the effects of resistance training on RMR. Researchers have reported increases in RMR in obese women following 16 weeks and 20 weeks of resistance training respectively (Byrne & Wilmore, 2001; Ryan et al., 1995). However, other researchers fail to demonstrate any change in RMR following resistance training of 12 weeks (Treuth et al., 1995), despite an increase in FFM in male participants (Van Etten, Westerterp, & Verstappen, 1995). Although resistance exercise training has the ability to increase FFM, an increase in RMR may not always occur, therefore further investigation is required to determine the effect of resistance training on RMR in overweight and obese women (Byrne & Wilmore, 2001; Stiegler & Cunliffe, 2006).

2.3.1.4 Lipid Profile

A blood lipid profile consists of various measures of cholesterol, frequently reported in a clinical setting as; total cholesterol (TC), triacylglycerol (TAG), low density lipoprotein (LDL) and high density lipoprotein (HDL) (NCEP, 2001). Elevated TC, TG (Yarnell et al., 2001), and LDL concentrations have been identified as risk factors in coronary heart disease (CHD) (Sharrett et al., 2001; Wallace, Moffatt, Haymes, & Green, 1991). Whereas, high levels of HDL are regarded as cardio protective (NCEP, 2001; Sharrett et al., 2001; Wallace et al., 1991). Regular physical activity has shown to favorably improve the blood lipid profile (Durstine et al., 2001; Houston, 2001). Moderate exercise, therefore diminishes the atherogenic stimulus, as higher fat oxidation

following endurance training is likely to reflect enhanced use of FA derived from TG lipoproteins (Herd et al., 2001). This indicates the importance of regular exercise in the prevention of disease through increased fat oxidation.

Endurance exercise training induces small but favorable modifications to the blood lipid profile in previously sedentary adults (Durstine et al., 2001; Halbert, Silagy, Finucane, Withers, & Hamdorf, 1999; Kokkinos & Fernhall, 1999). A popular form of endurance training in overweight and obese individuals is walking, as it is low impact and can be undertaken in a safe and cost-effective manner (Morris & Hardman, 1997). An exercise training programme incorporating walking has been shown to improve lipid profiles in healthy middle-aged adults (Kukkonen-Harjula et al., 1998), and overweight and obese women (Despres et al., 1991; Lamarche et al., 1992). Conversely, Hinkleman and Nieman (1993) found that a moderate intensity walking programme, without diet modification, in overweight women was not a sufficient stimulus to alter lipid profiles. Favorable alterations in blood lipids are demonstrated in obese women when combining walking training with weight loss or a reduced caloric intake (Nieman, Brock, Butterworth, Utter, & Nieman, 2002). Therefore, it is uncertain whether endurance walking will result in positive alterations in blood lipid profiles in overweight and obese women when it is not combined with caloric restriction.

A meta-analysis of walking interventions found significant reductions in LDL, with a non significant trend toward beneficial changes in TC, HDL and TG (Kelley, Kelley, & Vu Tran, 2004). However, TC does not appear to be influenced by an endurance walking training programme in women, despite increases in pace or volume (Duncan, Gordon, & Scott, 1991; Hardman, Hudson, Jones, & Norgan, 1989; Ready et al., 1996).

Exercise training utilising endurance-based walking has the ability to increase levels of HDL in women (Duncan et al., 1991; Hardman et al., 1989; Morris & Hardman, 1997), however others have reported no change (Ready et al., 1996; Santiago, Leon, & Serfass, 1995). There may be a dose-dependent increase in HDL, or a level in training intensity, duration and frequency that must be attained before significant and favorable changes in HDL occur (Kokkinos & Fernhall, 1999).

Walking may also result in the characteristic decrease in TG observed with endurance exercise, as demonstrated in more active individuals (Morris & Hardman, 1997). This is believed to be associated with improved circulation to active muscle, with faster degradation of TAG rich lipoproteins at the capillary luminal surface, resulting in enhanced HDL (Morris & Hardman, 1997).

Research on the effects of resistance training on changes in blood lipid profiles have also reported mixed results (Halbert et al., 1999; Williams et al., 2007). Lipoprotein levels are known to vary depending on the timing of measurement and preceding diet, and many resistance training interventions have not adequately controlled for these influences (Williams et al., 2007). However, Honkola, Forsen and Eriksson (1997), demonstrated improvements in TC and LDL following five months of resistance training in obese men (Honkola, Forsen, & Eriksson, 1997), signifying the positive effects of resistance training on blood lipid profiles. Furthermore, Magkos et al (2008) have demonstrated resistance training to be considered as an alternative to, or in addition to endurance exercise for effective control of plasma TG concentration. An acute bout of resistive exercise training resulted in lower fasting plasma TG, and increased plasma TG clearance compared to endurance exercise, for the same total energy expenditure in untrained men of normal weight (Magkos et al., 2008).

A threshold of exercise training may need to be reached in order for substantial changes in blood lipids to occur (Halbert et al., 1999), however a successful training programme that will produce the greatest improvement in the blood lipid profile is yet to be determined (Halbert et al., 1999). As both endurance and resistance exercise training demonstrate the ability to positively improve the blood lipid profile in those of normal weight, further investigation is required in overweight and obese individuals to ascertain the optimal exercise training regime to improve the blood lipid profile.

2.3.1.5 Cardiovascular Fitness

Trained individuals with high levels of cardiovascular (CV) fitness demonstrate greater rates of fat oxidation than untrained individuals (Achten & Jeukendrup, 2003; Klein et al., 1994; Tremblay et al., 1992). Thus increases in CV fitness, and hence fat

oxidation, with exercise training may be an important mechanism for altering the imbalance between available FFA and oxidation in overweight and obese individuals.

Both endurance and resistance training can elicit increases in CV fitness; however the magnitude of improvement in CV fitness varies between endurance and resistance training (ACSM, 1998; Williams et al., 2007). An increase in CV fitness is evident following endurance training in normal weight individuals (Duncan et al., 1991; Friedlander et al., 1998; Friedlander, Casazza, Horning, Huie, & Brooks, 1997; Hanson & Nedde, 1974; Horowitz et al., 2000; Hurley et al., 1986; Poehlman, Gardner, Arciero, Goran, & Calles-Escandon, 1994), and overweight and obese individuals (Amati et al., 2008; Asikainen et al., 2002; Despres et al., 1991; Murphy & Hardman, 1998; Solomon et al., 2008). Increases in maximal oxygen consumption ($\dot{V}O_{2\max}$) ranging from 15% to 28% have been demonstrated after 12 weeks of endurance training in males of normal weight (Horowitz et al., 2000; Hurley et al., 1986; Martin et al., 1993) and females of normal weight (Friedlander et al., 1998; Martin et al., 1993; Morris & Hardman, 1997). Endurance training is known to induce greater improvements in CV fitness than resistance training (Williams et al., 2007), as early studies investigating resistance training reported less of an increase in CV fitness (Gettman & Pollock, 1981). Accordingly, a combination of endurance training with resistance exercise may be more beneficial for increasing CV fitness and eliciting aerobic adaptations necessary for increases in fat metabolism.

As endurance and resistance training demonstrate varying magnitudes of improvement in CV fitness, differing CV responses may be apparent between the two training modes. During endurance exercise, HR may be maintained at a constant intensity during this steady state exercise. However, as resistance exercise training involves sub-maximal efforts of intensity, HR may fluctuate from high intensity during the resistive movement, to low intensity during recovery. In particular, upper body exercise demonstrates a greater increase in HR at a given $\dot{V}O_2$ compared to lower body exercise (Astrand & Rodahl, 1970; Tulppo, Makikallio, Laukkanen, & Huikuri, 1999). Although resistance training, particularly upper body resistance training may elevate HR because of sympathetic activity and catecholamine responses, the HR is disproportionate to oxygen consumption (ACSM, 2006; Beckham & Earnest, 2000). Several mechanisms

have been proposed to explain this difference in the $\dot{V}O_2$ /HR relationship observed during resistance exercise including; a pressor response (exaggerated blood pressure response relative to the amount of work performed); and lower stroke volumes, requiring an increase in HR to maintain cardiac output (Beckham & Earnest, 2000). Consequently, to increase CV fitness during resistance training, active recovery periods consisting of endurance exercise between resistive exercises may be recommended. This may maintain HR, and $\dot{V}O_2$, at a consistently higher level. Therefore, exercise prescription, involving HR is easily maintained and provides an accurate intensity for $\dot{V}O_2$ during constant load endurance exercise. During resistance training however, HR may be limited in its ability to accurately measure exercise intensity (ACSM, 2006; Beckham & Earnest, 2000).

In summary, both endurance and resistance training may result in specific adaptations in substrate utilisation. In normal weight individuals there is ample evidence to suggest that endurance training results in an enhanced ability to utilise fat during exercise; however, in overweight and obese individuals the evidence is inconclusive. The limited data that exists on the effects of a resistance training programme on exercise or resting fat oxidation in normal weight individuals indicates a disparity between increases in both fat oxidation and CHO utilisation. Furthermore, little evidence exists on adaptations in substrate utilisation following resistance training in overweight and obese individuals. Potential increases in FFM in overweight and obese individuals following resistance training may be coupled with enhanced fat utilisation following endurance training. Therefore, a combination of endurance and resistance training may provide the greatest stimulus to improve fat oxidation in these individuals.

2.3.2 Combined Endurance and Resistance Training Adaptations

Both endurance and resistance exercise have beneficial effects on substrate utilisation and training adaptations (Maiorana et al., 2002), and are outlined in chapter 2.3.1. Thus, combining endurance and resistance training may demonstrate further benefit due to a combination of training adaptations from each type of exercise.

Significant increases in $\dot{V}O_{2\max}$ are demonstrated when combining both endurance and resistance training (Byrne & Wilmore, 2001; Maiorana et al., 2002; McCarthy, Agre, Graf, Pozniak, & Vailas, 1995; Pierson et al., 2001). Although combined training elicits only modest increases in CV fitness compared with purely endurance training, greater increases in CV fitness are demonstrated following combined training than purely resistance training alone (Byrne & Wilmore, 2001; Gettman & Pollock, 1981). As increased rates of fat oxidation occur in endurance trained individuals with high CV fitness (Klein et al., 1994; Tremblay et al., 1992), combined endurance and resistance training that results in a significant improvement in CV fitness and aerobic adaptation, may be superior in enhancing fat oxidation than resistance training alone.

When resistance exercises are performed intermittently with endurance exercises, metabolic responses are similar to those during endurance training (Jurimae et al., 1990). Combined endurance and resistance training may be more beneficial than endurance training alone, as increases in fat oxidation attributed to endurance training may be combined with adaptations achieved through resistance training (Dolezal & Potteiger, 1998). The rationale behind this combined method of training is that endurance exercise alone increases CV fitness and skeletal muscle capillary density and reduces body fat, while resistance exercise increases FFM, RMR and strength (Dolezal & Potteiger, 1998; Maiorana et al., 2002; Pierson et al., 2001; Wallace et al., 1997). Therefore a combination of endurance and resistance training may provide all these benefits. Furthermore, in an investigation by McCarthy and colleagues (1995), when resistance and endurance training were combined, increases in strength and CV fitness were of the same magnitude as when resistance and endurance training were performed alone (McCarthy et al., 1995). Improvements in CV fitness, favorable changes in blood lipid profile, preserved FFM and increases in RMR have also been reported in overweight women by Svendsen and colleagues (1993) following combined endurance and resistance training.

Greater improvements in body composition and muscular strength are demonstrated following combined endurance and resistance training, compared to endurance training alone (Beckers et al., 2008; McCarthy et al., 1995; Pierson et al., 2001; Wallace et al., 1997). However, whether combined endurance and resistance

training can elicit favorable increases in exercise fat oxidation is unknown. Therefore, further research is required to investigate the effects of this combined modality on fat oxidation in overweight and obese individuals, with the goal to determine the optimal exercise prescription to improve fat oxidation in this population.

2.3.3 Aquatic Exercise

Optimal exercise prescription for overweight and obese individuals should facilitate EE; yet minimise the potential for injury (Wallace, 2003). An increased risk of orthopedic injury may be associated with overweight and obese individuals due to excess body weight, and exacerbate existing joint conditions during weight bearing exercise (Gappmaier, Lake, Nelson, & Fisher, 2006; Wallace, 2003). The buoyancy effect of water makes aquatic training an optimal exercise environment for overweight and obese individuals, as impact and stress on joints is reduced (Gappmaier et al., 2006). One aquatic exercise technique uses simulated running movements in water deep enough to prevent contact with the bottom of the pool, often with participants wearing a flotation belt. This deep water running (DWR) technique is an effective form of CV conditioning for both injured athletes and individuals who require a low-impact aerobic workout (Butts, Tucker, & Greening, 1991; Dowzer, Reilly, Cable, & Nevill, 1999; Frangolias & Rhodes, 1996; Frangolias, Rhodes, Taunton, Belcastro, & Coutts, 2000; Reilly, Dowzer, & Cable, 2003; Town & Bradley, 1991; Wilder & Brennan, 1993). However, there is limited information on the effectiveness of DWR training on increasing fat oxidation in any population. As an aquatic environment can be manipulated to provide either an endurance, or combined endurance and resistance training regimen, comparisons may be made between these training types to determine effects on fat metabolism.

Water has several properties that make it an ideal environment for exercise. The buoyancy of water supports the submerged body from the downward pull of gravity, providing up to a 90% reduction in body weight (Darby & Yaekle, 2000; di Prampero, 1986; Wilder & Brennan, 2004). Benefits of this buoyant effect include less stress and pressure on bone, muscle and connective tissue, while the viscosity and drag force of water provides a resistance proportional to the exerted effort (Wilder & Brennan, 2004). When the velocity of movement doubles, the drag force produced by water quadruples,

providing a resistance training stimulus (Tsourlou, Benik, Dipla, Zafeiridis, & Kellis, 2006). As the density of water is approximately 800 times that of air (di Prampero, 1986), this resistance contributes to the CV challenge of aquatic exercise with no impact stress on joints and soft tissue. The resistance placed upon a moving body by water may be greater in overweight and obese individuals, who have an increased frontal surface area, however may also be offset by increased buoyancy. According to Evans and colleagues (1978), the dual effects of buoyancy and resistance make possible high levels of energy expenditure with relatively little movement or strain on lower-joint extremities. Additionally, enhanced temperature regulation during water exercise makes this an ideal environment for obese individuals who have an increased risk of heat intolerance (Wallace, 2003).

2.3.3.1 Comparison between Aquatic-Based and Land-Based Exercise

The mechanics of DWR appear to be qualitatively similar to treadmill running. However during DWR different proportions of upper to lower body muscle mass are activated, compared to land based running (Michaud, Rodriguez-Zayas, Andres, Flynn, & Lambert, 1995). Greater fatigue is reported in the arms and shoulders during DWR, possibly due to greater use of the upper body and less use of the lower body (Michaud, Rodriguez-Zayas et al., 1995). The propulsion mechanics of the muscles in the legs when running or walking on land are different than in water, where the body is suspended and not working against gravity (Michaud, Rodriguez-Zayas et al., 1995; Reilly, Dowzer et al., 2003), but rather in an opposing role as it works against the buoyancy effect of water (Moening, Scheidt, Shepardson, & Davies, 1993). As DWR is not weight bearing, there is no push off phase as there is in land based running. Furthermore, leg range of motion is different between DWR and land-based running at the hip and knee joints, and differences in leg coordination are evident at similar stride frequencies (Kilding, Scott, & Mullineaux, 2007; Moening et al., 1993). The lack of a stretch-shortening cycle during DWR is likely to contribute to these differences, affecting muscle recruitment patterns (Kilding et al., 2007). In addition, the absence of ground contact during DWR, and therefore non-weight bearing environment, results in movement of the lower extremities in an open kinetic chain, which differ to that of a closed kinetic chain as during running

on land (Moening et al., 1993). Consequently, DWR demonstrates differing biomechanical and physiological variables when compared to similar land-based exercise.

There are some inherent differences between exercise responses observed on land and in water. At a given rate of oxygen consumption ($\dot{V}O_2$), heart rate (HR) is decreased by approximately 7-13 beats per minute in water compared to on land (Darby & Yaekle, 2000). The hydrostatic pressure exerted by water on the submerged body is thought to aid cardiovascular function by promoting venous return, resulting in a greater stroke volume and thus lower HR (Chu, Rhodes, Taunton, & Martin, 2002; Darby & Yaekle, 2000; Dowzer et al., 1999; Svedenhag & Seger, 1992; Wilder & Brennan, 2004). Lower maximal values for HR and $\dot{V}O_{2max}$, expressed in relative (ml/kg/min) and absolute (L/min) units, have been reported during DWR when compared to running on a treadmill (Butts et al., 1991; Dowzer et al., 1999; Frangolias & Rhodes, 1995, 1996; Frangolias et al., 2000; Mercer & Jensen, 1997; Michaud, Rodriguez-Zayas et al., 1995; Nakanishi, Kimura, & Yokoo, 1999; Svedenhag & Seger, 1992; Town & Bradley, 1991), and performing aquatic exercises immersed to the axillary region compared with land (Barbosa, Garrido, & Bragada, 2007). Maximal HR during DWR ranges from 86% to 95%, and $\dot{V}O_{2max}$ values of 73% to 92% of those obtained during land-based running (Wilder & Brennan, 2004). The pressure of water also leads to a sequence of events which reduces the partial pressure of oxygen contributing to the increased work of breathing in water by 60% (Becker, 2004), further challenging the CV system. The physiological responses to DWR do not differ across age or gender, as older individuals (Chu et al., 2002), and both men and women (Glass, Wilson, Blessing, & Miller, 1995; Mercer & Jensen, 1997), have similarly reduced HR_{max} and $\dot{V}O_{2max}$ in water compared to land based activities.

Literature regarding substrate utilisation during DWR in comparison to land-based running is limited and contradictory. A significantly higher RER (DeMaere & Ruby, 1997; Svedenhag & Seger, 1992), higher CHO oxidation (DeMaere & Ruby, 1997), and lower fat oxidation (DeMaere & Ruby, 1997) have been demonstrated during DWR compared to treadmill running at sub-maximal intensities in trained male athletes (DeMaere & Ruby, 1997; Michaud, Rodriguez-Zayas et al., 1995; Svedenhag & Seger,

1992). Conversely, lower RER (Bishop, Frazier, Smith, & Jacobs, 1989) and similar RER (Butts et al., 1991) during DWR have also been reported. Nevertheless, higher anaerobic metabolism is found during submaximal DWR, when compared to the same absolute workload on land (Svedenhag & Seger, 1992), indicating potential for less aerobic fat oxidation. It has been suggested that there is greater involvement of the anaerobic energy system during water exercise because of the additional recruitment of the smaller muscle groups of the upper body (Michaud, Rodriguez-Zayas et al., 1995).

Due to the differences in physiological responses between exercise on land and water, it is important to prescribe aquatic and land-based exercise using a percentage of maximum derived from mode specific exercise testing. A graded maximal DWR test utilising addition of weight to increase resistance has been determined as reliable and valid, when compared to a graded maximal treadmill walk (TMW) test in normal weight individuals (Mercer & Jensen, 1997). The DWR test may be useful in determining maximal physiological responses for specific prescription of DWR training in overweight and obese women. However, in an overweight or obese population, the validity of the DWR test is unknown. Therefore, the maximal responses between DWR and treadmill walking need to be determined and validated in an overweight and obese population.

2.3.3.2 Aquatic Training Adaptations

Like exercise training undertaken on land, training adaptations also result from exercise undertaken in an aquatic, non weight-bearing environment. Of particular interest is whether adaptations in substrate utilisation occur after a non weight-bearing aquatic exercise training programme similar to that on land. However, to date, literature regarding substrate utilisation following aquatic-based exercise training in either a normal weight or overweight and obese population is lacking. Due to disparity in substrate utilisation responses during a single acute aquatic exercise session compared to a land-based exercise session, it is difficult to predict the training adaptations in substrate utilisation that may result from aquatic exercise training, specifically fat oxidation.

Although there is little research on the effects of aquatic exercise training on substrate utilisation, the effects of aquatic exercise on CV adaptations has been extensively studied. Aquatic exercise is commonly used in fitness training and

rehabilitation (Broman, Quintana, Lindberg, Jansson, & Kaijser, 2006; Takeshima et al., 2002; Taunton et al., 1996). A programme of DWR is successful in increasing CV fitness in relatively unfit, young individuals (Davidson & McNaughton, 2000; Michaud, Brennan, Wilder, & Sherman, 1995) and in maintaining CV fitness in trained athletes (Bushman et al., 1997; Wilber, Moffatt, Scott, Lee, & Cucuzzo, 1996). Furthermore, trained runners are able to elicit equivalent increases in CV fitness following both DWR and land-based running (Wilber et al., 1996), indicating the potential for DWR to be as effective as land-based training for CV conditioning. High intensity DWR also elicits improved $\dot{V}O_{2\max}$ in elderly women (Broman, Quintana, Lindberg et al., 2006). During DWR, the hydrostatic pressure may impart a pressure overload, and hence stimulate capillary proliferation and oxidative enzyme activities, which are mechanisms for adaptations resulting in increases in CV fitness (Broman, Quintana, Lindberg et al., 2006). Furthermore, raised cardiac blood volume, stroke volume, cardiac output, and hence volume overload during water immersion, may also lead to an increased stimulus for the myocardium of the heart to adapt to training (Sheldahl, 1987). These training studies suggest that when performed with proper technique and intensity, DWR may be as effective as land-based training as a form of CV conditioning, and may potentially result in similar endurance training adaptations.

In contrast to the extensive research on the effects of aquatic training and CV responses, studies investigating the effects of DWR training on body composition are scarce. Wilber and colleagues (1996) did not report any significant change in body fat percentage in normal weight men after six weeks of DWR. However, it is considered that a minimum of eight weeks are necessary for training effects to occur in physiological variables, while body fat decreases require longer duration interventions (Takeshima et al., 2002). A trend for body fat decreases have been observed in DWR training programs lasting eight weeks or longer (Michaud, Brennan et al., 1995). Recently, Barbosa and colleagues (2007) compared a 13 week aquatic endurance exercise programme to a land-based programme of equivalent intensity, duration and frequency and demonstrated comparable decreases in body fat percentage in a population of obese women. Thus aquatic exercise may elicit similar decreases in body fat and changes in body composition

as land-based training when prescribed at appropriate intensity, duration and frequency. However, further research is needed to clarify these findings.

Due to continuous movement of limbs against the resistance of water, aquatic based exercise training provides a stimulus for muscular development (Tsourlou et al., 2006). However, Taunton and colleagues (1996) did not report a significant improvement in muscular strength after 12 weeks of aquatic training, despite demonstrating an increase in $\dot{V}O_{2\text{peak}}$ (Taunton et al., 1996). The aquatic programme employed by Taunton and colleagues (1996) did not include specific resistance training exercises and thus may not have elicited enough of a training response. Poyhonen and colleagues (2002) reported significant increases in knee extensor and flexor strength following a 10 week resistance training program in water that employed specific resistance exercises (Poyhonen et al., 2002). Specific resistance exercises targeted to increase muscular strength may therefore be recommended, in addition to an endurance-based aquatic exercise programme.

The majority of aquatic resistance training interventions have been undertaken in a shallow water environment, allowing foot contact with the bottom of the pool. While this differs from DWR, where there is no foot contact with the bottom of the pool, shallow water aquatic resistance training interventions provide valuable information on the effects of aquatic resistance training. In contrast to the lack of evidence for improved muscular strength following deep water exercise, increases in upper and lower body strength have been reported following a shallow water training programme combining resistance exercises (Tsourlou et al., 2006). The increase in muscular strength following shallow water training was accompanied by a significant increase in FFM (Tsourlou et al., 2006), potentially explaining the increase in muscle strength. The magnitude of muscular strength increases following aquatic training is partly in accordance with studies conducted on land in elderly women (Tsourlou et al., 2006). Therefore, the resistive properties of water may facilitate the development of muscular strength when resistance exercises are combined with endurance based exercise; however more research in this area is needed, specifically in DWR.

There is little research on the effects of combined endurance and resistance training in an aquatic-based environment. Only recently, investigations have been

undertaken in overweight and obese women performing combined endurance and resistance aquatic-based exercise (Meredith-Jones et al., 2009; Nowak et al., 2008). A 12 week combined aquatic-based exercise training programme significantly increased CV fitness (Meredith-Jones et al., 2009), upper and lower body strength (Meredith-Jones et al., 2009), decreasing WC and WHR (Meredith-Jones et al., 2009; Nowak et al., 2008) and improving the blood lipid profile in overweight and obese women (Nowak et al., 2008). These studies substantiate the benefits of a combined endurance and resistance aquatic-based exercise training programme.

The adaptations that occur following combined endurance and resistance exercise in an aquatic environment are similar to that undertaken on land. This is demonstrated by similar positive adaptations in CV fitness, body composition, lipid profile and muscular strength after training in both aquatic-based and land-based modalities (Taunton et al., 1996; Volaklis, Spassis, & Tokmakidis, 2007). Accordingly, the American College of Sports Medicine (ACSM) guidelines recommend non weight-bearing exercise and resistance training as suitable modes of exercise for obese individuals (ACSM, 2006; Wallace, 2003). Consequently, a combination of both endurance and resistance DWR training may provide greater physiological benefits and training adaptations for fat oxidation in overweight and obese women compared to a purely endurance based training programme.

2.4 Summary

Due to the rise in the incidence of obesity and associated health problems, exercise interventions aimed at preventing and reducing the impact of excess body fat are essential (Golay & Ybarra, 2005; Goris & Westerterp, 2008; Li et al., 2005; Vuori, 2001). An increase in fat oxidation in overweight or obese people may be possible following exercise training, as seen in normal weight individuals (Friedlander et al., 1998; Friedlander et al., 2007; Horowitz et al., 2000; Pruchnic et al., 2004). Therefore, investigation into the optimal modality of exercise training to maximise fat oxidative potential in overweight and obese individuals may assist exercise prescription. An aquatic environment is suitable for exercise in those who are overweight or obese;

however there is a paucity of data investigating substrate utilisation following this modality of exercise. Alternatively, weight-bearing exercise such as walking is frequently prescribed for exercise training but the effects of this training mode on substrate utilisation in the overweight or obese population are unclear.

The addition of resistance exercise to endurance training in overweight or obese individuals has the potential to enhance fat oxidation. This is seen with traditional endurance training programmes in normal weight individuals, and the combined training regime can be implemented in an aquatic or land-based environment. Further investigation is required to determine effects of aquatic and land-based exercise training on fat oxidation and associated adaptations. Whether addition of a resistance training component to endurance training will alter changes in fat oxidation and associated adaptations in overweight and obese women requires further study. Furthermore, comparison of aquatic exercise training and land-based exercise training in overweight and obese women will provide relevant information for exercise prescription, with particular reference to changes in fat oxidation. This will aid in determining whether there is an optimal modality of exercise training to elicit increases in fat oxidation in overweight and obese women.

Chapter 3: Aquatic-Based Exercise Training

3.1 Methods

3.1.1 Participants

A total of 23, Caucasian women, classified as overweight or obese ($\text{BMI} \geq 25 \text{ kg/m}^2$) (WHO, 2000) were recruited by advertisement (Appendix 1) and accepted into this study. Only Caucasian participants were selected, as BMI values defining 'overweight and obese' in Maori and Pacific Islanders differ from those used to classify Caucasians (Swinburn, Ley, Carmichael, & Plank, 1999). After delivery of information sheets for participants (Appendix 2), written consent was obtained from each individual. This study was approved by the University of Otago Human Ethics Committee (Ethics Number 05/108). A screening questionnaire (Appendix 3), obtaining medical and exercise history was completed by each participant. The screening questionnaire was based on the Physical Activity Readiness Questionnaire (PAR-Q), which is a standardised questionnaire to determine suitability for participation in an exercise programme (ACSM, 2006). Exclusion criteria included; $\text{BMI} < 25 \text{ kg/m}^2$, a diagnosis of heart disease, pulmonary disease, Type 1 and Type 2 diabetes mellitus, orthopaedic limitations and any other health problem that may interfere with exercise. Participants who reported significant weight change in excess of 4.5kg in the two months before enrollment were also excluded (Horner et al., 2001). Individuals were considered for eligibility if they were prescribed medication or substances that could impact metabolism; however, dosage and frequency of the medication must have been stable for a minimum of the past six months. These medication/substances included low dose hormone replacement therapy, antidepressant medication and tobacco smoking. Participants were instructed to do the following - "continue with your original exercise, dietary and medication routines during the course of the intervention." In addition, none of the subjects had participated in a supervised systematic exercise program for at least six months before the study (Volaklis et al., 2007). However, all participants disclosed in their pre-screening questionnaire that they undertook sporadic, recreational activity.

3.1.2 Sample Size

To obtain a power of 0.80 at $\alpha < 0.05$, the paired t-test for pre to post exercise intervention measures in exercise fat oxidation required a total overall sample of nine individuals (Bausell & Li, 2002). Analysis of covariance (ANCOVA) testing to compare changes pre to post intervention in fat oxidation between training groups required a sample size of 12 per group at a 0.80 power and 5% significance (Bausell & Li, 2002). Both sample size estimates are based on detecting a significant difference in exercise fat oxidation of 459 $\mu\text{mol}/\text{min}$ (177 mg/min) with a standard deviation of 408 $\mu\text{mol}/\text{min}$ (158 mg/min). These estimates are based on the significant difference identified in exercise fat oxidation following training by van Aggel-Leijssen et al. (2002) in obese individuals. As no study to date has investigated changes in exercise fat oxidation following aquatic exercise training, these reference values were established from this land-based exercise intervention (van Aggel-Leijssen et al., 2002).

3.1.3 Procedure

Participants completed a 12 week aquatic training intervention, to which they were randomly assigned to endurance-based deep water running (DWR), or deep water running combined with aquatic resistance training (DWR+RT). Randomisation was carried out using a random number generator (graphpad, 2005). Baseline and post intervention testing included measures of; CV fitness, exercise and resting metabolism, muscular strength, anthropometry and lipid profiles, as detailed in section 3.1.5. Baseline testing was undertaken during the four weeks leading up to the intervention, with post testing undertaken within one week of completion of the intervention. In pre-menopausal participants, baseline and post testing for oxidation rates and RMR was completed during the follicular phase of the menstrual cycle. All participants continued training until post testing was completed.

In addition, the validity of the DWR maximal exercise test was assessed through comparison of maximal responses to that undertaken on a treadmill. This is described in section 3.1.5.

3.1.4 Exercise Intervention

Following pre intervention testing, each participant was randomly assigned to one of two aquatic exercise training groups:

- 1) Deep water running (DWR)
- 2) Deep water running combined with resistance training (DWR+RT)

DWR: Twelve women were allocated to the supervised DWR training programme. Participants attended a 60 minute DWR class, three days per week for 12 weeks. Each class began with a five minute warm up in the pool where participants performed DWR. This was followed by 50 minutes of continuous DWR combined with variations of DWR motion performed at random (Appendix 6). The class concluded with a five minute cool down phase where participants performed DWR.

DWR+RT: Eleven women were allocated to the supervised DWR+RT programme. Participants attended a 60 minute, combined aquatic endurance and resistance training class, three days per week for 12 weeks. Each class consisted of a five minute warm up in the pool where participants performed DWR. This was followed by 50 minutes of DWR+RT where DWR was interspersed with resistance exercises incorporating dumbbell shaped flotation devices (Swimjoy Ltd, NZ). The class concluded with a five minute cool down phase where participants performed DWR. The DWR+RT programme consisted of a ratio of 1:2 for RT and DWR respectively. The RT periods utilised resistive exercises against water for all major muscle groups for 90 seconds. These included exercises which targeted; chest, back, shoulders, triceps, biceps, hip adductors, hip abductors, hip flexors, gluteal muscles, hamstrings, quadriceps, calf muscles, rectus abdominals and oblique abdominals (Appendix 7). The exercise load associated with the resistance training component was rationalised and quantified through the Biodex strength testing component. The load associated with resistance training was originally determined through mimicking the speed of contraction during Biodex strength testing during the resistance training component of the exercise intervention. Exercise intensity and load for the resistance training portion of the DWR+RT program was determined in the first training session each participant attended. Participants were asked to perform the DWR RT exercises for 90 seconds at the same speed undertaken during

Biodex strength testing. Depending on the body part moved, this equated to approximately 45 repetitions per work interval. As the resistance of water provides the training load, participants were required to increase the surface area moved through water to maintain their prescribed target HR as the program progressed. Once the increase in surface area reached maximum, the velocity of the movement had to be increased in order to increase muscular load and hence maintain target HR. In some instances the typical number of repetitions ranged from 45 to 90 per 90 seconds. Therefore, due to the nature of the aquatic exercise modality, contraction speed had to be increased in some circumstances to maintain target HR during aquatic RT. The endurance period consisted of DWR for three minutes.

Exercise sessions were undertaken at Moana Pool, Dunedin. Sessions took place in a dive pool with a depth of 3.8 m, and a temperature of 28.5°C. Participants wore standard DWR belts (Water Jogger, Swimjoy Ltd, NZ) during all sessions, and were immersed to mid-chest depth, ie between the xiphoid process and axillary region. Individuals unfamiliar with DWR were given advice on techniques to assist with correct DWR motion and resistance exercises in the water. Participants were instructed to undertake their respective supervised exercise programme at an intensity of 60% heart rate maximum (HR_{max}), which was calculated from the DWR pre test. Participants wore HR monitors in the pool (Polar A3, Kempe, Finland) to ensure correct intensity during the exercise sessions. As well as exercise prescription based off HR, participants were also advised to select an exercise intensity corresponding to between 'very light' (RPE 9) to 'fairly light' (RPE 11) on the 6-20 point Borg RPE scale (Borg, 1982). This prescription of exercise intensity is in the range demonstrated to result in the greatest rates of fat oxidation (Achten & Jeukendrup, 2004; Deriaz et al., 2001; Venables & Jeukendrup, 2008). Training heart rates were recorded at each session and an average calculated for each individual over the 12 week period. A training log was given to each participant detailing their training HR with information on the days and times of sessions for the 12 week training duration. Details of respective exercise programs were also given to each participant to ensure they had sufficient information to correctly perform each session. Sessions were available one to two times each day, and participants were instructed to have three sessions signed off each week after attendance by the instructor.

Three sessions per week were required to meet the ACSM recommended quantity of exercise for developing and maintaining CV fitness and muscular fitness in healthy adults (ACSM, 1998).

Energy expenditure during the aquatic intervention was determined retrospectively using values obtained during the graded maximal DWR test. Using the Weir (1949) formula, EE was calculated from corresponding values for $\dot{V}O_2$ and $\dot{V}CO_2$ and the relationship with HR plotted. From the linear trend line, EE was calculated from the average training HR for each individual.

3.1.5 Pre and Post Intervention Outcome Measures

3.1.5.1 Cardiovascular Fitness

To determine aquatic-based CV fitness, a graded DWR test was conducted in the swimming flume at the School of Physical Education, University of Otago, following the protocol of Mercer and Jensen (1997) (Figure 2). Water temperature was held constant at 29°C, with water depth at 1.65 m; preventing participant foot contact with the bottom of the pool. Participants were submerged, with the water level between the xiphoid process and the axillary region, and wore a standard DWR belt (Water Jogger, Swimjoy Ltd, NZ) to which a tether was attached and run through a series of pulleys. A bucket was attached to the other end of the tether and suspended in front of the participant 0.75 m above a wooden plank that rested on either side of the pool deck. The DWR protocol was continuous and consisted of one minute stages. To provide a graded response, a weight of 0.57 kg was placed in the bucket at the beginning of each stage. Continuous respiratory gas analysis using indirect calorimetry (Sensormedics, California, USA) was undertaken throughout the test. Participants wore a custom made head piece to which the mouthpiece was attached so that it would not interfere with the DWR movement. Heart rate was monitored throughout the test with a waterproof HR monitor (Polar A3, Kemple, Finland) and recorded every 20 seconds. Strong standardised verbal encouragement was provided throughout the entire session; however, if the participant was unable to meet the intensity at any given stage, they were pulled back and the bucket dropped onto the wooden plank, indicating the endpoint of the test (Figure 2). The highest oxygen uptake

($\dot{V}O_2$) and corresponding HR were taken as peak oxygen uptake ($\dot{V}O_{2peak}$) and peak heart rate (HR_{peak}), respectively. The corresponding respiratory exchange ratio (RER) to $\dot{V}O_{2peak}$ was determined. Ratings of perceived exertion (RPE) were taken on completion of the test using the 6-20 Borg scale (Borg, 1982).

Participants were instructed on proper DWR technique prior to the test and encouraged to maintain correct form during the graded DWR test. Involuntarily hyperventilation, measured by indirect calorimetry, was avoided by allowing a familiarisation period with the breathing apparatus before testing began (Simonson & DeFronzo, 1990).

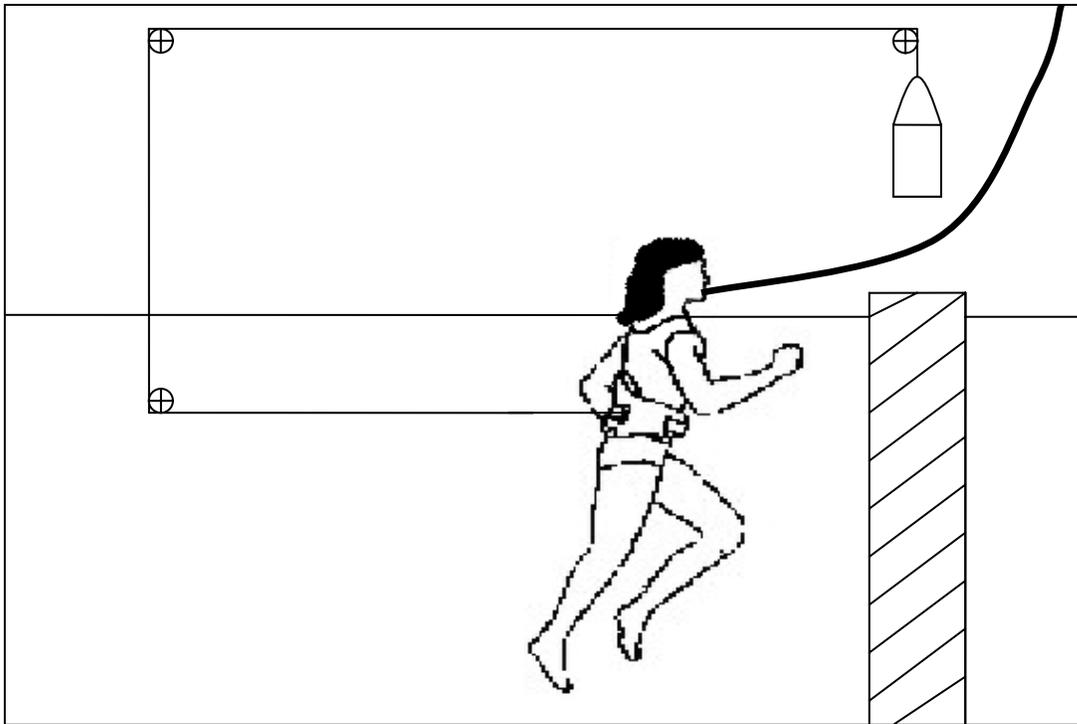


Figure 2. Illustration of the graded maximal deep water run (DWR) test set up. The test end point was when the bucket touched the plank, indicating that the participant could no longer meet the exercise demand. Figure adapted with permission from Mercer and Jensen (1997).

The DWR protocol used in the current investigation has been found to be valid and reliable in assessing peak oxygen consumption ($\dot{V}O_{2\text{peak}}$) in young men and women (mean age 21-24 years) of normal weight (Mercer & Jensen, 1997). To ensure the DWR test was valid in the current population of overweight to obese women, comparison of maximal exercise responses was made between DWR and treadmill walking (TMW). Participants completed a TMW graded exercise test a minimum of 24 hours following the DWR test. The graded TMW test was conducted in an exercise physiology laboratory at the School of Physical Education, University of Otago on a motorized treadmill (Quinton Instrument Company, Series 90 Q65, WA, USA) following the Harbor Protocol (Wasserman, Hansen, Sue, Stringer, & Whipp, 2005). The TMW protocol was also continuous and consisted of one minute stages. Participants completed three minutes of warm up at a zero grade walking at a comfortable speed, which was determined during familiarisation with the mouthpiece and head gear. The grade was increased at a constant pre selected amount of 1-1.5% each minute until volitional exhaustion. Continuous respiratory gas analysis using a Sensormedics 2900 metabolic cart (Sensormedics, California, USA) and HR (Polar A3, Kempe, Finland) was undertaken throughout the test. Participants received standardised strong verbal encouragement throughout the entire test; however, the test was terminated when the participant could no longer continue and signaled to stop. Again, the highest $\dot{V}O_2$ and corresponding HR were taken as $\dot{V}O_{2\text{peak}}$ and HR_{peak} , respectively. The corresponding respiratory exchange ratio (RER) to $\dot{V}O_{2\text{peak}}$ was also determined, with a rating of perceived exertion (RPE) taken on completion of the test (Borg, 1982).

3.1.5.2 Metabolism and Substrate Utilisation

Exercise Metabolism

To determine exercise metabolism participants completed 30 minutes of steady state DWR at 60% HR_{peak} in the swimming flume at the School of Physical Education, University of Otago. Participants were submerged to a water level between the xiphoid process and axillary region and wore a standard DWR belt (Water Jogger, Swimjoy Ltd, NZ), which was tethered to the side of the pool. The swimming flume was held at a

constant temperature of 29°C with water depth of 1.65 m. Intensity was calculated from HR_{peak} obtained during the DWR graded maximal exercise test. Participants exercised at the same absolute intensity during pre and post testing. Indirect calorimetry was performed for one minute at five minute intervals while DWR, using a Sormedics 2900 metabolic cart (Sormedics, California, USA). The first 20 second segment was discarded for each measurement. Gas analyzers were calibrated prior to each test with a known concentration of oxygen and carbon dioxide. Volume was also calibrated prior to each test using a standard 3 L syringe. As the mouthpiece system is not recommended to be used continuously for greater than 15-30 minutes, measurements at spaced intervals may prevent this limitation (Simonson & DeFronzo, 1990). Once DWR was commenced, every five minutes the participant breathed through the mouthpiece, which was suspended in front of them while maintaining DWR technique. A mean of the five sampling periods, excluding the first 20 seconds of measurement, for $\dot{V}CO_2$ and $\dot{V}O_2$ (ml/min) was used to calculate fat and carbohydrate oxidation rates according to the non-protein respiratory quotient technique (Peronnet & Massicotte, 1991):

$$\text{CHO oxidation rate (mg/min)} = 4.585 (\dot{V}CO_2) - 3.2255 (\dot{V}O_2)$$

$$\text{Lipid oxidation rate (mg/min)} = -1.7012 (\dot{V}CO_2) + 1.6946 (\dot{V}O_2)$$

Energy expenditure during the 30 minute steady state exercise session was calculated using the equation of Weir, (1949):

$$EE \text{ (kJ)} = 16.318 (\dot{V}O_2) \text{ (L)} + 4.602 (\dot{V}CO_2) \text{ (L)}$$

Energy expenditure was expressed as both kJ and kcal. Nitrogen excretion as a result of protein oxidation was assumed to be constant accounting for 15% of total EE (Weir, 1949).

The approximate contribution of substrates to energy metabolism during exercise was determined from the RER, defined as $\dot{V}CO_2/\dot{V}O_2$. The measurement of RER is

based on the assumption that the exchange of O₂ and CO₂ measured at the lungs reflect the actual gas exchange at the cellular level. During steady state exercise this assumption is valid (Kanaley, Mottram, Scanlon, & Jensen, 1995).

The contribution of fat and CHO toward steady state EE during the 30 minute DWR session was determined by multiplying the oxidation rate of fat by nine and the oxidation rate of CHO by four, using the Atwater general conversion factor (Atwater, 1909).

Heart rate was monitored continuously using a HR monitor (Polar A3, Kempe, Finland). Participants were asked to keep the DWR intensity at the prescribed HR in order to ensure steady state exercise. In addition, participants were instructed to exercise within a RPE of 9 to 11 (Borg, 1982). This was monitored by the investigator during the session and HR was recorded during breath analysis, every five minutes.

Blood was collected by venepuncture from a superficial vein in the antecubital fossa at rest prior to, and on completion of the 30 minute DWR session. A total of 10 ml of blood was collected into EDTA tubes. Following centrifugation, plasma was pipetted into Eppendorf tubes and stored at -80°C until analysis. Plasma samples were analysed for total free fatty acids and glycerol in the Department of Biochemistry, University of Otago and are described below.

Free fatty acids (μmol/L) were measured in all samples according to the manufacturer's instructions (Roche Diagnostics GmbH, Nonnenwald 2, Penzberg, Germany). The concentration of FFA on completion of the 30 minute exercise session was subtracted from the FFA concentration at the beginning of exercise to determine the exercise FFA concentration (ΔFFA), pre and post aquatic training.

Glycerol (μmol/l) was measured in plasma samples using colorimetric method, according to the manufacturer's specifications (RANDOX laboratories Ltd., Ardmore, Crumlin). The concentration of glycerol on completion of the 30 minute exercise session was subtracted from the glycerol concentration at the beginning of exercise to determine the exercise-induced glycerol concentration (Δglycerol), pre and post aquatic training.

Resting Metabolism

Resting metabolic rate is typically measured using indirect calorimetry, deriving expenditure estimates from O₂ consumption and CO₂ production from expired gases (Horner et al., 2001). A single 10 minute measurement using indirect calorimetry, excluding the first five minutes of data, produces reliable results with minimal subject burden (Horner et al., 2001). Ideally, achievement of steady state is considered an accurate and reliable RMR test. Steady state is often defined as five consecutive minutes during which O₂ consumption and CO₂ production vary by less than 10% (Reeves, Davies, Bauer, & Battistutta, 2004). Reducing the time period of steady state to four minutes also produces measurements of RMR that are within clinically acceptable, predetermined limits and normal individual variation in EE is in the order of 3-5% for RMR (Reeves et al., 2004). Prediction methods of RMR, including equations, produce considerable error compared with measured EE when predicting RMR of healthy individuals (Reeves et al., 2004), therefore measurement of RMR with indirect calorimetry is advantageous despite its limitations.

Resting metabolic rate was measured using indirect calorimetry based on best practice methods (Compher, Frankenfield, Keim, & Roth-Yousey, 2006). Measurement was taken after an overnight fast, with no alcohol or nicotine consumption for at least 10 hours prior to the test. Participants were asked not to undertake any strenuous activity (including exercise intervention sessions) in the 24 hours prior to this measurement. Participants rested for 15 minutes in a semi-recumbent position, in an exercise physiology laboratory at the School of Physical Education, University of Otago, and were instructed not to fall asleep. The laboratory was maintained at a thermoneutral temperature of between 21-26°C on land (Wilmore & Costill, 2004). Following the rest period, participants breathed through a mouthpiece with their nose clipped, for a 15 minute period. Expired gas was collected using a SensorMedics 2900 metabolic cart (SensorMedics, California, USA). Gas analyzers were calibrated prior to each test with a known concentration of oxygen and carbon dioxide. Volume was also calibrated prior to each test using a standard 3 L syringe. The first five minutes of data collection were discarded and the remaining 10 minutes used to determine a four minute period having a coefficient of variation (CV) for $\dot{V}O_2$ (L/min) and $\dot{V}CO_2$ (L/min) of $\leq 10\%$ for analysis. When participants did not display a four minute period having a CV for $\dot{V}O_2$ (L/min) and

$\dot{V}CO_2$ (L/min) of $\leq 10\%$, values for the lowest CV were used in analysis. The abbreviated Weir equation (Weir, 1949) was used to determine RMR from mean $\dot{V}O_2$ (L/min) and $\dot{V}CO_2$ (L/min), as used previously (Reeves et al., 2004):

$$\text{RMR (kcal/day)} = 3.941 (\dot{V}O_2) + 1.106 (\dot{V}CO_2) \times 1440$$

The four minute period for RMR measurement was used for further analysis of substrate metabolism under resting conditions. Approximate contribution of substrate to resting metabolism was determined from the mean RER. Resting fat and CHO oxidation rates were calculated according to the non-protein respiratory quotient technique (Peronnet & Massicotte, 1991) using mean values for $\dot{V}O_2$ (ml/min) and $\dot{V}CO_2$ (ml/min) during the four minute RMR measurement, and contribution of fat and CHO to RMR were determined using the Atwater general conversion factors (Atwater, 1909).

3.1.5.3 Muscular Strength

Upper and lower body strength was assessed using isokinetic testing on a Biodex Isokinetic Dynamometer (Biodex Corporation, New York, USA). Muscular strength was defined as the amount of torque exerted volitionally at various speeds. For upper body strength, maximal voluntary concentric isokinetic torque was assessed in Newton meters at an angular velocity of 30° per second during a seated chest press. Wrists were fixed in a neutral position throughout the full range of motion, and the test began with the elbows at approximately 90° , moving through to full extension without 'locking' the joint. For lower body strength, knee extension and flexion were measured using maximal voluntary concentric isokinetic torque, assessed in Newton meters (Nm) at angular velocities of 90° per second. The hip joint was at approximately 90° during testing. The axis of rotation of the lever arm was aligned with the rotation axis of the knee joint. Range of motion around the knee joint was from 90° at flexion to 170° at extension. For standardisation purposes, the right leg was used for all tests. The rationale for selecting the certain velocities to assess the strength of upper and lower body muscles using the Biodex was to ensure the majority of women could produce peak torque, and replicated the speeds at

which they would perform chest press, knee flexion and knee extension during the training intervention. Furthermore, upper and lower body movement speeds were selected based on velocities previously used in older women (Symons, Vandervoort, Rice, Overend, & Marsh, 2005). Each participant was instructed to move their limb in the required manner 'as fast as they possibly could'. Support straps around the chest, pelvis and thigh were used to stabilize the trunk, hip joint and thigh. After three familiarisation trials, which also served as the warm up, each participant was given thirty seconds of rest before proceeding with the recorded tests. All subjects received verbal encouragement to exert the maximal force possible. A total of three trials were undertaken with ten seconds rest between each trial. Data were collected using Chart 4 for Windows (ADInstruments Pty Ltd, Bella Vista, NSW, Australia). The maximum value recorded during the three trials for chest press, knee extension and knee flexion was recorded as the final peak torque value and used in the analysis.

3.1.3.4 Anthropometry

Height, weight, and waist and hip circumferences were recorded at the initial visit, pre and post intervention. Height and weight were measured without shoes and in a swimsuit using a stadiometer (School of Physical Education, Otago University, Dunedin, NZ) and Digi electronic scales (D1-10, Teraoka Seiko Ltd, Tokyo, Japan), respectively. Height was measured to the nearest 0.5cm and weight to the nearest 0.1kg. Waist and hip circumferences were taken as close to the skin as possible using a fibre-glass standard anthropometric tape measure. Waist circumference (WC) was measured at the narrowest part in the trunk between the last rib and the anterior superior iliac spine, and hip circumference was measured at the widest part between the anterior superior iliac spine and the greater trochanter, both to the nearest 0.5cm. Measurements were taken in duplicate, with a pre selected maximum variation of 0.5 kg for the weight measurement, and 0.5 cm for height and circumference measurements. A third measurement was taken if the difference of the first two measures was greater than the pre selected limit. An average of the two closest measurements was used in the analysis. Waist to hip ratio

(WHR) was calculated for each participant. Body mass index (BMI) was calculated as weight (kg) / height squared (m²).

Body composition was determined using dual-energy x-ray absorptiometry (DXA) (LUNAR DPX-L, Lunar Corporation, Madison, Wisconsin, USA) pre and post intervention at Dunedin Hospital. Participants disrobed and wore a hospital gown to minimise artifacts. The scanning procedure involved passing a beam of dual energy radiation through the participant, taking a transverse scan of the whole body while in a supine position. Fat percentage (%), fat mass (FM) (kg) and fat free mass (FFM) (kg) were obtained for total body, and the trunk regions. Fat mass comprises both subcutaneous and visceral adipose tissue, while FFM consists of the non-bone mineral protein and water body fractions (Kohrt, 1995). The procedure is safe with a low radiation exposure, taking approximately between 10-20 minutes (Mazess, Barden, Bisek, & Hanson, 1990b). Although precision errors (CV) of 5-6% have been found from repeated measurements for FM and FFM (Mazess, Barden, Bisek, & Hanson, 1990a), other results confirm that DXA is a precise and reproducible method for measuring body composition (DeVita & Stall, 1999; Thomsen, Jensen, & Henriksen, 1998). Furthermore, a DXA scan of the truncal region may provide valid and reliable measures of abdominal obesity (Glickman, Marn, Supiano, & Dengel, 2004).

3.1.5.5 Lipid Profile

Blood was collected by venepuncture from a superficial vein in the antecubital fossa of the forearm following an overnight fast of at least 10 hours, both pre and post intervention. A total of 10 ml of blood was collected by syringe, placed into an EDTA vacutainer and centrifuged at 3000 rpm for 10 minutes. Following centrifugation the plasma fraction was pipetted into Eppendorf tubes and stored at -80°C until analysis. These samples were analysed using a Cobas Mira Plus Analyser (Roche, New Jersey, USA) at the Department of Human Nutrition, Lipid and Mineral testing laboratory, University of Otago. Values for triacylglycerol (TAG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL) were determined using manufacturers instructions (Roche Diagnostics, Indianapolis, USA). Low density lipoprotein cholesterol (LDL) was calculated using the Friedwald equation (Friedwald, Levy, & Fredrickson, 1972).

Triacylglycerol

Triacylglycerol was measured using a standard enzymatic colorimetric test, according to the manufacturer's instructions (Roche Diagnostics, Indianapolis, USA). Absorbance was read at 500 nm. The inter-assay CV was 2.5%.

Cholesterol

Cholesterol was measured using a standard enzymatic colorimetric test based on manufacturer's specifications (Roche Diagnostics, Indianapolis, USA). The absorbance wavelength was measured at 500nm. Inter-assay CV was 1.0%.

High-Density Lipoprotein Cholesterol

Determination of HDL was measured according to the precipitation method (Assman, Schriewer, Schmitz, & Hagele, 1983). Precisely 250 μ l of plasma was pipetted into a micro centrifuge tube and 500 μ l of HDL precipitating reagent added. The HDL precipitating reagent consisted of 2.54g of magnesium chloride and 0.80g of phosphotungstic acid in 500ml of deionised water. After mixing, the solution was left undisturbed for 10 minutes at room temperature. Following this, the sample was centrifuged at 3000 G for 10 minutes, at room temperature. Approximately 250 μ l of the yellow precipitate was transferred into a Cobas sample cup and analysed according to the reactions for the total cholesterol method. Results were multiplied by a factor of 3 to correct for dilution. Absorbance wavelength was read at 500nm. The inter-assay CV for this test was 2.5%.

Low-Density Lipoprotein Cholesterol

Low-density lipoprotein cholesterol was calculated using the Friedwald equation (Friedwald et al., 1972). This equation is the most common indirect method for estimating LDL (Warnick et al, 1990). The Friedwald formula is regarded as an accurate estimation of LDL when TAG is below 2.3mmol/l in fasting samples (Warnick et al,

1990). Concentrations are represented in millimoles per litre. The CV of the calculated LDL concentrations was 2.4%.

$$[\text{LDL cholesterol}] = [\text{TC}] - [\text{HDL}] - [\text{TG}]/2.22$$

3.1.6 Standardisation of Testing Procedures

Each participant undertook the 30 minute DWR pre and post test at the same time of the day to control for any diurnal effects on substrate utilisation (Galliven et al, 1997). All metabolic testing for exercise and resting substrate oxidation, and RMR took place during the follicular phase (days 3-9) of the menstrual cycle in eumenorrhoeic participants. This was done to avoid the possibility of elevated estrogen levels promoting lipolytic activity during the luteal phase of menstruation (Ruby & Robergs, 1994).

The relative contribution of substrate being oxidised is influenced by the composition of the last meal eaten (Christensen & Hansen, 1939). To minimise the effects of diet on substrate utilisation, participants were instructed to record their diet in the 24 hour period prior to the 30 minute DWR pre test (Appendix 5). They were required to follow this same diet before undertaking the 30 minute DWR post test. Analysis of the 24 hour diet was undertaken with Diet Cruncher for Windows (version 1.6.0, Way Down South Software, Dunedin, New Zealand) to ensure equality in nutrient composition between pre and post intervention testing.

As bioavailability of substrates in postprandial periods varies with the time since eating (Dumortier et al., 2005), a standardised time period is advisable between eating and measurement to minimise the effects of diet on substrate utilisation measured by IC. At three hours following a meal insulin and blood glucose return to values close to baseline (Dumortier et al., 2005), therefore at least three hours of no eating is required to prevent alterations in substrate utilisation due to diet. Each participant was asked to refrain from eating for at least four hours before each 30 minute DWR pre and post test as substrate utilisation is altered by prior nutrient consumption. Consumption of caffeine and smoking were also eliminated four hours prior to the 30 minute DWR pre and post test.

3.1.7 Statistical Analysis

Means and standard deviations (SD) were used to describe group data. Results were analysed using Statistics Package for Social Sciences (SPSS version 14.0, Chicago, Illinois). Independent t-tests were undertaken to investigate baseline participant characteristics. Analysis of Covariance (ANCOVA) was conducted to compare the effectiveness of DWR and DWR+RT training on metabolic and physiological parameters. The independent variable was the type of intervention (DWR, DWR+RT) and the primary dependent variable was the post-intervention measure of exercise fat oxidation. The pre-intervention measure of exercise fat oxidation was used as the covariate in the analysis. Secondary dependent variables and covariates included other post-intervention measures and pre-intervention measures respectively. These consisted of; exercise CHO oxidation and RER; resting CHO oxidation, RER and RMR; basal and exercise plasma FFA and glycerol concentrations; $\dot{V}O_{2peak}$; chest, quadriceps and hamstring strength; weight, BMI, hip and waist circumference; total body and trunk fat mass, fat free mass and percent body fat; and total cholesterol, HDL, LDL and TAG. Preliminary checks were conducted to ensure that there was no violation of the assumptions of normality, linearity and homogeneity of variances. Residuals were examined for normality using histograms. If a large studentised residual was identified, this value was removed from the original data set and analyses re-run. If the re-run analysis reached the same statistical conclusion, the original data set was used for analysis. Linearity and the association between pre and post test scores were visually assessed using scatter plots. Levene's test of equality of error variances was used to check homogeneity of variances (Pallant, 2007). If ANCOVA detected evidence of a difference in post test scores, controlling for pre test scores, between DWR and DWR+RT training, one-sample t-tests were performed on the change score for each group. However, when no evidence of a difference between groups existed, DWR and DWR+RT groups were combined and paired t-tests undertaken to investigate the overall effects of aquatic exercise training on changes in metabolic and physiological parameters. Linear regression analysis was used to examine the relationships between change scores in exercise and resting fat metabolism, with change scores in anthropometry, CV fitness,

lipid profile and blood measures of lipolysis (change in FFA and glycerol and basal FFA and glycerol). Associations were also examined between change scores in total body and trunk DXA data with anthropometric data, lipid profile and blood measures of lipolysis. The strength of a relationship between two variables was measured using Pearson correlation coefficient and partial correlation where appropriate. Maximal physiological responses between DWR and TMW exercise tests were examined using paired t-tests. Validity of the DWR protocol was tested by calculating the Pearson's correlation coefficient for $\dot{V}O_{2\max}$ and HR_{\max} between DWR and TMW for each individual. Statistical significance was set at $p < 0.05$.

3.2 Results

3.2.1 *Pre Exercise Training Data*

3.2.1.1 Participant Characteristics

A total of 23 participants were recruited and began participation in the aquatic-based exercise intervention. Of these 23 participants, 20 completed the aquatic-based exercise intervention; 11 completed DWR, and nine completed DWR+RT (Figure 3). Three participants were unable to complete 12 weeks of aquatic-based exercise training due to work commitments (n = 2), and injury which occurred outside of the training programme (n = 1). The aquatic-based exercise intervention was well tolerated with no adverse events or injury as a result of training. Amongst participants that completed the intervention, attendance was 100% to all 36 sessions for DWR and DWR+RT groups.

Individuals on low dose HRT, antidepressants or who smoked tobacco did not change their habits over the course of the study. To check for potential confounding from medications and other substances that could impact metabolism, participants were removed from the data set (n = 4) and statistical analyses re-run. No confounding or effect modification was present, therefore results were pooled.

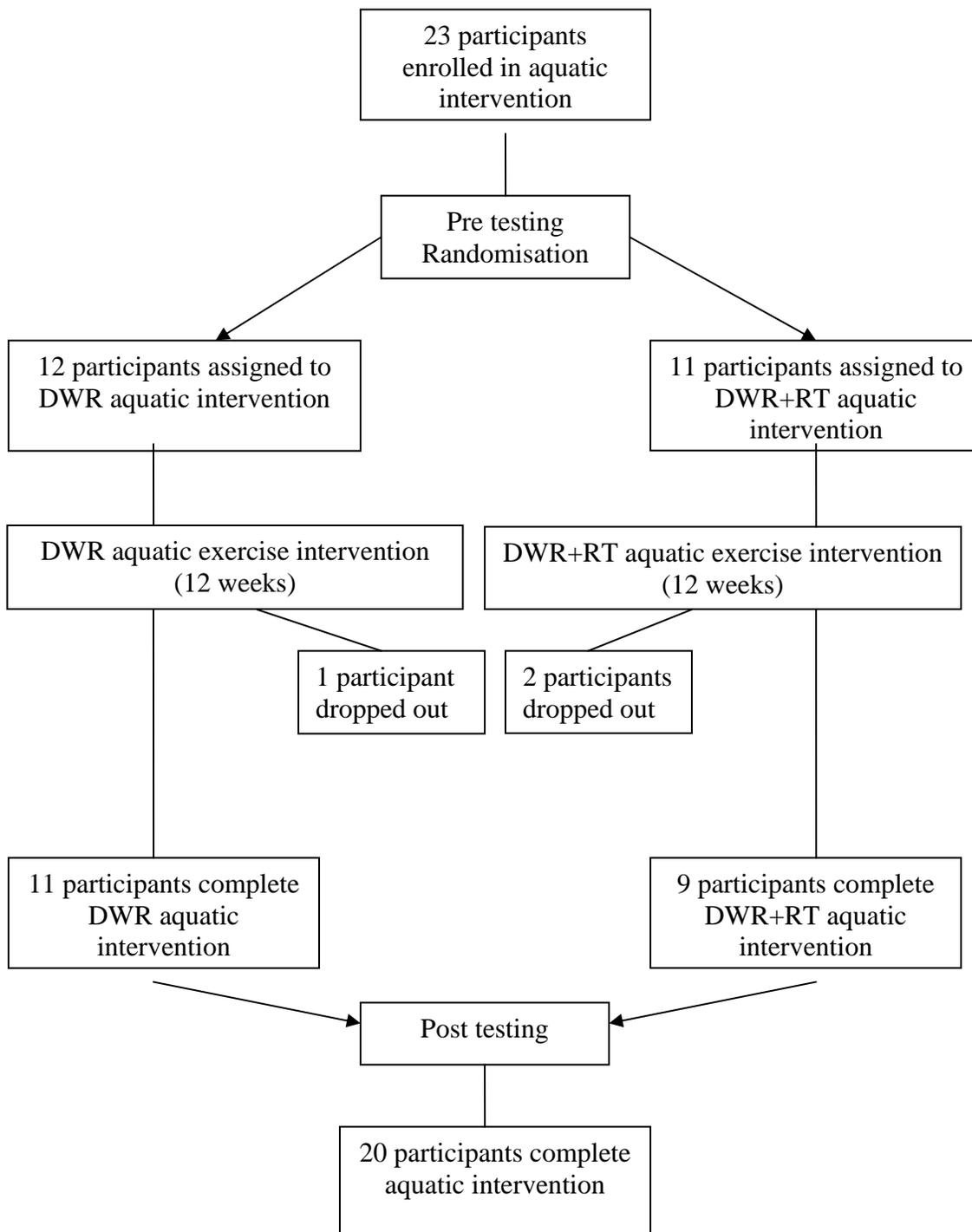


Figure 3. Flow diagram demonstrating the number of participants completing each stage of the aquatic-based exercise intervention.

Baseline characteristics of participants who completed the aquatic-based exercise intervention are displayed in Table 1 (n = 20). These characteristics did not differ between participants in DWR and DWR+RT groups ($p > 0.05$) (Table 1). Of the 11 women who completed the DWR aquatic training intervention, five women were pre-menopausal and six women were post-menopausal. Of the nine women who completed the DWR+RT aquatic training intervention, five women were pre-menopausal and four women were post-menopausal. Post-menopausal status was defined as having at least 12 months since last menses (Horner et al., 2001).

Both the DWR and DWR+RT groups had a waist circumference ≥ 88 cm and WHR of > 0.80 (Table 1), which places them at 'high' risk for Type 2 diabetes, hypertension and cardiovascular disease (ACSM, 2006; McArdle et al., 2001).

Both the DWR and DWR+RT aquatic training groups were below the 10th percentile for fitness (Table 1) using normative values for $\dot{V}O_{2max}$ when expressed as ml/kg/min, with specific reference to age and sex (ACSM, 2006). This percentile corresponds to a 'well below average' description of fitness in this group (ACSM, 2006). However, these normative values were determined from treadmill testing, which is not specific to aquatic exercise.

Pre intervention screening indicated that the majority of participants (18 out of 20) had previous experience with DWR. However, none of the participants were undertaking regular DWR at commencement of the aquatic intervention.

Table 1. Baseline characteristics of participants who completed the deep water running (DWR) and deep water running combined with resistance training (DWR+RT) interventions

	DWR (n = 11)	DWR+RT (n = 9)	p value
Age (years)	48 ± 7	48 ± 8	0.90
Weight (kg)	79.0 ± 13.5	78.4 ± 10.6	0.91
Body mass index (kg/m ²)	30.0 ± 4.0	29.7 ± 4.1	0.86
Waist circumference (cm)	93.7 ± 11.8	90.1 ± 7.6	0.43
Hip circumference (cm)	111.6 ± 8.6	108.1 ± 9.7	0.40
Waist to hip ratio	0.84 ± 0.05	0.83 ± 0.05	0.91
DWR $\dot{V}O_{2peak}$ (L/min)	1.71 ± 0.45	1.74 ± 0.36	0.85

Data represent mean ± SD

Note. $\dot{V}O_{2peak}$ = peak oxygen consumption

3.2.2 Exercise Training Data

3.2.2.1 Exercise Training Intensity and Energy Expenditure

Participants were initially instructed to undertake their respective supervised exercise programmes at moderate intensity (60% HR_{peak}), which was calculated from their DWR maximal oxygen consumption pre-test. However, to prevent a mild hypothermic response, participants worked at a higher intensity to maintain body temperature in the pool. When DWR and DWR+RT groups were combined for analyses, participants exercised at 71% HR_{peak} . The relationship between HR and $\dot{V}O_2$ during the DWR pre-test was plotted and the corresponding training percentage of $\dot{V}O_{2peak}$ estimated retrospectively. Using the linear trend line, it was determined that participants were training at 50% of $\dot{V}O_{2peak}$. Training intensity, expressed as % HR_{peak} and % $\dot{V}O_{2peak}$, was not significantly different between DWR and DWR+RT groups ($p > 0.05$) (Table 2). When using RPE as an alternate gauge of exercise intensity, the DWR group reported an average RPE of 11 and the DWR+RT group an average RPE of 11.

Using the Weir (1949) formula, EE was calculated retrospectively from corresponding values for $\dot{V}O_2$ and $\dot{V}CO_2$, and the relationship with HR plotted during the DWR pre test. From the linear trend line, EE was estimated using the average training HR for each individual. Amount of estimated weekly EE as a result of the intervention was not significantly different between DWR and DWR+RT groups ($p > 0.05$) (Table 2).

Table 2. Training intensity and total energy expenditure for deep water running (DWR) and deep water running combined with resistance training (DWR+RT) groups

	DWR (n = 11)	DWR+RT (n = 9)	p value
%HR _{peak}	72 ± 11	71 ± 5	0.79
% $\dot{V}O_{2peak}$	52 ± 19	48 ± 12	0.59
Energy expenditure (kcal.week ⁻¹)	707 ± 213	713 ± 219	0.95
Energy expenditure (kJ.week ⁻¹)	2959 ± 890	2985 ± 918	0.95

Data represent mean ± SD

Note: %HR_{peak} = percentage of heart rate peak, % $\dot{V}O_{2peak}$ = percentage of peak oxygen consumption

3.2.2.2 Pre and Post Intervention Outcome Measures

3.2.2.3 Cardiovascular Fitness

There was no difference between DWR and DWR+RT for changes in CV fitness, expressed as $\dot{V}O_{2\text{peak}}$ (L/min), controlling for baseline fitness (DWR 1.71 ± 0.46 to 1.76 ± 0.42 L/min; DWR+RT 1.74 ± 0.36 to 1.77 ± 0.36 L/min; $p = 0.71$). Additionally, when DWR and DWR+RT groups were combined to investigate the overall effects of aquatic training, there was no significant change in CV fitness (1.72 ± 0.40 to 1.76 ± 0.39 L/min, $p = 0.34$).

3.2.2.4 Metabolism and Substrate Utilisation

A 30 minute DWR exercise session was undertaken to assess fat and CHO oxidation rates, with measurement of RER, and determination of the percentage contribution of fat and CHO to EE. Participants were instructed to undertake the exercise session at 60% of their pre-intervention HR_{peak} . Paired t testing within DWR and DWR+RT exercise groups confirmed that exercise was undertaken at the same percent of $\%HR_{\text{peak}}$, $\% \dot{V}O_{2\text{peak}}$, HR, $\dot{V}O_2$ and total EE at pre and post testing for both training groups ($p > 0.05$) (Table 3). When training groups were combined, retrospective analysis of HR demonstrated that participants were exercising at 64% HR_{peak} during pre and post testing, which corresponded to 49% $\dot{V}O_{2\text{peak}}$ for both pre and post testing.

Table 3. Exercise intensity and energy expenditure during 30 minutes of steady state deep water running pre and post aquatic-based exercise intervention for deep water running (DWR) and deep water running combined with resistance training (DWR+RT) groups

	DWR (n = 11)		p value	DWR+RT (n = 9)		p value
	Pre	Post		Pre	Post	
HR beats/min	98 ± 9	98 ± 9	0.22	104 ± 8	105 ± 8	0.49
%HR _{peak}	64 ± 5	63 ± 5	0.25	64 ± 3	64 ± 3	0.53
$\dot{V}O_2$ (ml/kg/min)	9.9 ± 2.9	10.0 ± 2.6	0.91	11.2 ± 2.0	11.4 ± 2.2	0.41
$\dot{V}O_2$ (L/min)	0.79 ± 0.27	0.77 ± 0.17	0.79	0.88 ± 0.20	0.89 ± 0.25	0.62
% $\dot{V}O_{2peak}$	46 ± 9	46 ± 8	0.85	51 ± 13	52 ± 13	0.76
Total EE (kJ)	474 ± 167	463 ± 103	0.72	528 ± 120	542 ± 155	0.52

Data represent mean ± SD

Note. %HR_{peak} = percentage of heart rate peak, % $\dot{V}O_{2peak}$ = percentage of peak maximal oxygen consumption, $\dot{V}O_2$ = rate of oxygen consumption, HR = heart rate, EE = energy expenditure

Following training, although DWR and DWR+RT groups displayed fat oxidation rates in opposite directions, statistically there was no difference in follow up values for exercise fat oxidation rate when controlling for baseline rates ($p = 0.13$) (Figure 4). When aquatic training groups were combined, there was also no significant change in the rate of fat oxidation during exercise (269 ± 105 v 271 ± 70 mg/min, $p = 0.89$).

There was evidence of a difference between DWR and DWR+RT for the post intervention rate of CHO oxidation during exercise ($p < 0.01$). Participants from the DWR group demonstrated a decrease in the rate of exercise CHO oxidation following training (-100 ± 215 mg/min, $p = 0.16$), whereas the DWR+RT group demonstrated an increase in the rate of exercise CHO oxidation following training (111 ± 146 mg/min, $p = 0.053$) approaching significance (Figure 4).

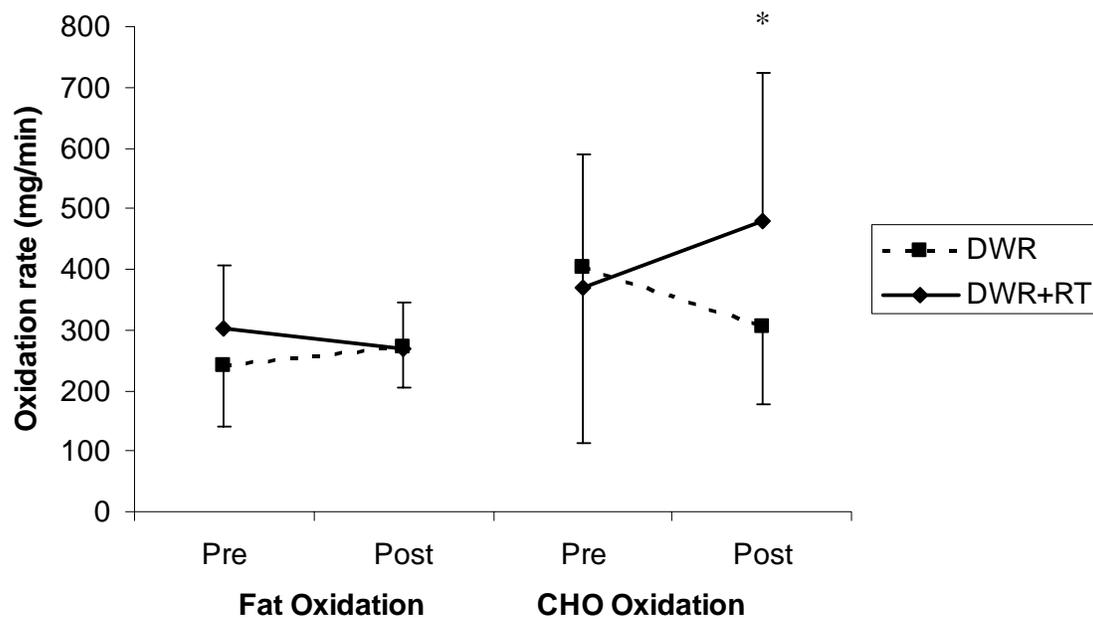


Figure 4. Fat and carbohydrate (CHO) oxidation rates during 30 minutes of steady state deep water running (DWR), pre and post aquatic exercise training in DWR (n = 11) and deep water running combined with resistance training (DWR +RT; n = 9) groups.

* $p < 0.01$ significant difference between DWR and DWR+RT groups for CHO oxidation

Analysis of covariance also indicated evidence of a difference in exercise RER post intervention, between DWR and DWR+RT groups after training ($p = 0.03$). The DWR group demonstrated a decrease in RER ($- 0.02 \pm 0.06$, $p = 0.17$), corresponding to an increase in fat and decrease in CHO oxidation. Whereas the DWR+RT group demonstrated an increase in RER ($+ 0.02 \pm 0.04$, $p = 0.15$), corresponding to an increase in CHO and decrease in fat oxidation (Figure 5, a).

Results for the exercise RER were also confirmed by the contribution of fat and CHO to total EE during the exercise bout. There was a significant difference between DWR and DWR+RT for the percent contribution of fat and CHO to total EE during the 30 minute DWR exercise session pre to post training ($p = 0.04$). The DWR group demonstrated an increase in the percent of fat oxidation and corresponding decrease in CHO oxidation of 8% ($p = 0.24$). Whereas, the DWR+RT groups demonstrated a decrease in the percent of fat oxidation and corresponding increase in CHO oxidation of 8% ($p = 0.12$) (Figure 5, b).

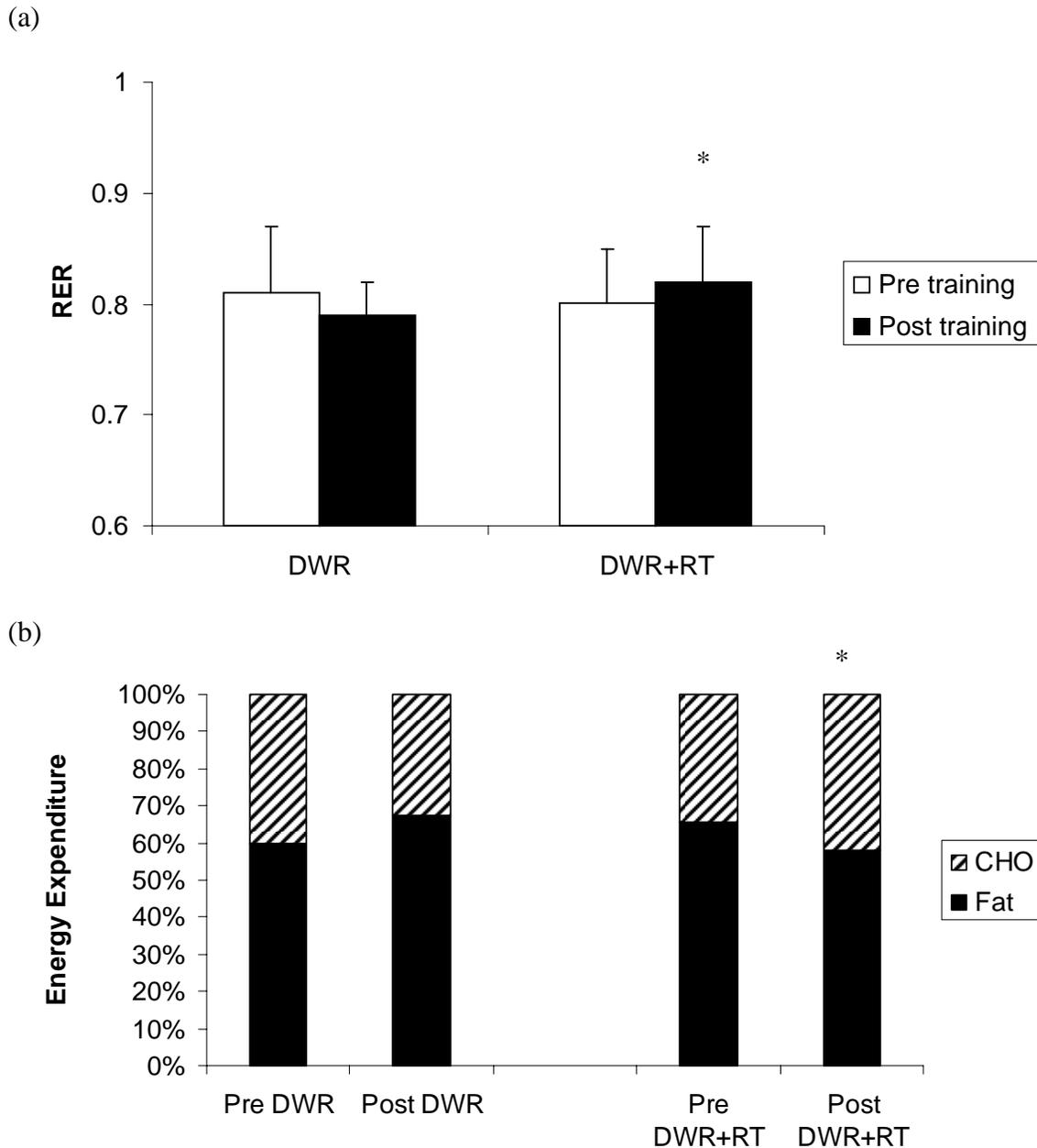


Figure 5. (a) Respiratory exchange ratio (RER), (b) and contribution of fat and CHO to total EE during 30 minutes of steady state deep water running (DWR), pre and post DWR training (n = 11) and deep water running combined with resistance training (DWR+RT, n = 9).

* $p < 0.05$ significant difference between training groups

Data from 16 participants, from whom complete blood samples were obtained, were used for analysis of plasma FFA and glycerol. Four participants were unable to provide complete samples when blood could not be obtained from the superficial forearm vein on at least one occasion. Analysis of covariance (ANCOVA) was undertaken to compare DWR to DWR+RT groups. However, due to the small number of participants in the DWR+RT group ($n = 6$), non-parametric statistical analysis was also undertaken using Mann-Whitney U Test for FFA and glycerol measures. Both parametric and non-parametric analyses resulted in the same statistical conclusion for measures of plasma FFA and glycerol, therefore parametric analyses are reported. When controlling for baseline release of plasma FFA and plasma glycerol, ANCOVA indicated no significant difference between DWR and DWR+RT groups for post intervention release of plasma FFA or plasma glycerol during acute DWR exercise ($p > 0.05$) (Table 4). Furthermore, ANCOVA demonstrated no significant difference between DWR and DWR+RT groups for basal concentrations of plasma FFA or plasma glycerol after respective aquatic interventions ($p > 0.05$) (Table 4). Therefore, training groups were combined to determine the overall effects of aquatic training on plasma FFA and glycerol concentrations.

Both plasma FFA and plasma glycerol concentrations were significantly higher on completion of the 30 minute DWR exercise session with no difference in FFA or glycerol release after training when DWR and DWR+RT groups were combined ($p > 0.05$) (Table 4). Paired t-testing indicated that aquatic training overall, did not significantly influence the release of plasma FFA or plasma glycerol during a 30 minute DWR exercise session pre to post training respectively (Δ FFA; 151 and 92 $\mu\text{mol/L}$, $p = 0.24$; Δ Glycerol; 46 and 46 $\mu\text{mol/L}$, $p = 0.98$). The concentration of FFA increased significantly after the 30 minute DWR exercise session during both pre intervention (510 ± 252 to 661 ± 209 $\mu\text{mol/L}$, $p = 0.02$) and post intervention (436 ± 169 to 527 ± 219 $\mu\text{mol/L}$, $p = 0.04$). Similarly, the concentration of plasma glycerol increased significantly after the 30 minute DWR exercise session during both pre (123 ± 54 to 169 ± 50 $\mu\text{mol/L}$, $p < 0.001$) and post intervention (112 ± 44 to 158 ± 53 $\mu\text{mol/L}$, $p < 0.001$).

When training groups were combined, basal plasma FFA concentrations (510 ± 252 v 436 ± 169 $\mu\text{mol/L}$, $p = 0.15$) and basal plasma glycerol concentrations (123 ± 54 v

$112 \pm 44 \mu\text{mol/L}$, $p = 0.12$) were not different before and after the exercise interventions, respectively.

Table 4. Basal FFA and glycerol concentrations, and difference in free fatty acid and glycerol concentrations during 30 minutes of steady state deep water running (DWR), for deep water running (DWR) and deep water running combined with resistance training (DWR+RT) groups

	DWR (n = 10)		DWR+RT (n = 6)		p value
	Pre	Post	Pre	Post	
Basal FFA ($\mu\text{mol/L}$)	544 \pm 296	443 \pm 183	453 \pm 163	423 \pm 157	0.80
Basal glycerol ($\mu\text{mol/L}$)	134 \pm 57	121 \pm 49	105 \pm 48	97 \pm 33	0.81
ΔFFA ($\mu\text{mol/L}$)	73	67	281	133	0.85
$\Delta\text{Glycerol}$ ($\mu\text{mol/L}$)	32	45	69	46	0.75

p value column indicates difference between training groups

Note. ΔFFA = change in free fatty acid concentration from pre to post 30 minute DWR exercise testing, $\Delta\text{Glycerol}$ = change in glycerol concentration from pre to post 30 minute DWR exercise testing

There was no evidence of a difference between DWR and DWR+RT groups for follow up scores, controlling for baseline scores for RMR (kcal/day) ($p > 0.05$) (Table 5). Furthermore, when groups were combined for analysis there was no significant change in RMR pre to post training (1452 ± 332 to 1382 ± 276 kcal/day, $p = 0.24$).

There was also no difference between DWR and DWR+RT groups for changes in resting RER, resting fat oxidation rate or resting CHO oxidation rate, or contribution of fat and CHO during the RMR measurement ($p > 0.05$) (Table 5). Following the combination of DWR and DWR+RT to assess effects of aquatic training over time there were also no significant changes in resting RER (0.81 ± 0.04 to 0.81 ± 0.05 , $p = 0.92$), resting fat oxidation rate (66.74 ± 18.86 to 63.28 ± 21.49 mg/min, $p = 0.53$), resting CHO oxidation rate (99.88 ± 40.81 to 95.82 ± 49.68 mg/min, $p = 0.79$), or the contribution of fat (61 ± 13 to 60 ± 19 %, $p = 0.98$) or CHO (39 ± 13 to 40 ± 19 %, $p = 0.98$) during the RMR measurement.

Table 5. Resting metabolism measures pre and post aquatic based exercise intervention for deep water running (DWR) and deep water running combined with resistance training (DWR+RT) groups

	DWR (n = 11)			DWR+RT (n = 9)			p value
	Pre	Post	Chg (%)	Pre	Post	Chg (%)	
RMR (kcal/day)	1466 ± 371	1429 ± 320	-2.5	1434 ± 298	1326 ± 215	-7.5	0.39
Resting RER	0.80 ± 0.04	0.82 ± 0.05	2.5	0.82 ± 0.03	0.80 ± 0.06	-2.4	0.65
Resting fat ox (mg/min)	69.27 ± 19.93	63.96 ± 24.26	-7.7	63.63 ± 18.12	62.45 ± 18.97	-1.9	0.97
Resting CHO ox (mg/min)	96.19 ± 46.56	102.68 ± 42.21	6.7	104.39 ± 34.69	87.44 ± 59.08	-16.2	0.54
% resting fat oxidation	63 ± 14	58 ± 17	-5.0	58 ± 12	64 ± 23	6.0	0.65
% resting CHO oxidation	37 ± 14	42 ± 17	5.0	42 ± 12	36 ± 23	-6.0	0.65

Data are presented as mean ± SD

p value column indicates difference between training groups

Note. Chg = change, RMR= resting metabolic rate, RER = respiratory exchange ratio, fat ox = fat oxidation, CHO ox = carbohydrate oxidation, % percentage

3.2.2.5 Muscular Strength

There was no difference between DWR and DWR+RT for changes in chest strength or quadriceps strength (Nm) ($p > 0.05$) pre to post training (Table 6). On further analysis, when groups were combined to assess the overall effects of aquatic training, significant increases were evident in chest strength ($p = 0.02$) (Figure 6) and quadriceps strength ($p = 0.01$) (Figure 7).

Analysis of covariance provided evidence of a significant and greater improvement in hamstring strength for the DWR group (16 ± 12 Nm, $p < 0.01$) compared to the DWR+RT group (1 ± 7 Nm, $p = 0.62$) following training ($p < 0.01$) (Table 6).

Table 6. Strength data pre and post aquatic based exercise intervention for deep water running (DWR) and deep water running combined with resistance training (DWR+RT) groups

	DWR (n = 11)			DWR+RT (n = 9)			p value
	Pre	Post	Chg (%)	Pre	Post	Chg (%)	
Chest strength (Nm)	205 ± 57	214 ± 43	4.4	187 ± 59	219 ± 46	17.1	0.14
Quadriceps strength (Nm)	97 ± 26	108 ± 26	11.3	110 ± 33	114 ± 31	3.6	0.42
Hamstring strength (Nm)	61 ± 18	77 ± 14	26.2	71 ± 19	72 ± 17	1.4	< 0.01**

Data are presented as mean ± SD

** p < 0.01 significant difference between training groups

Note. Chg = change

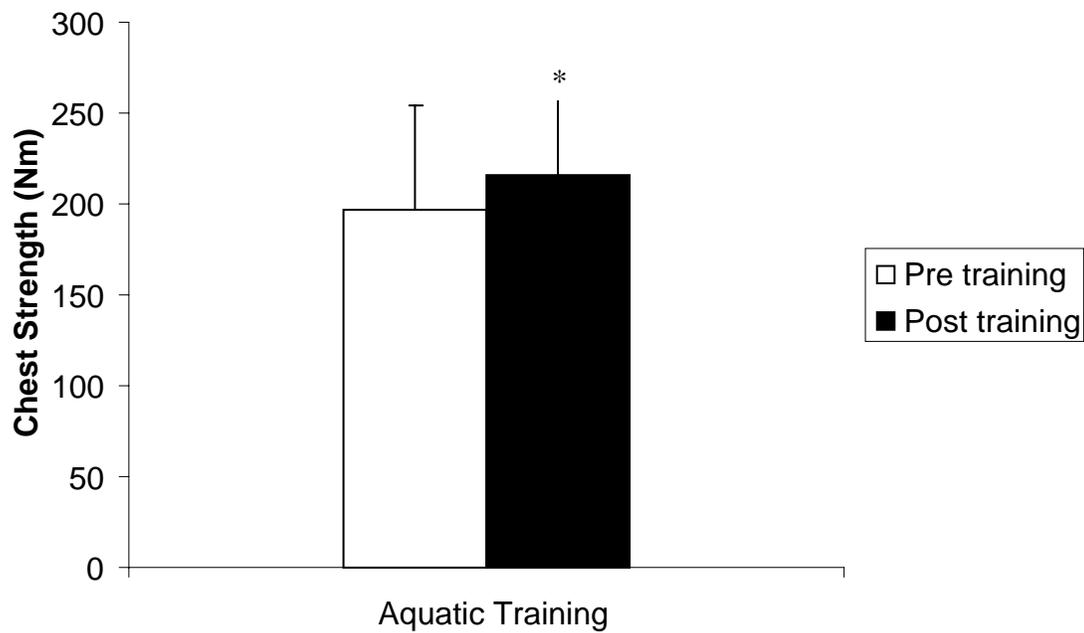


Figure 6. Chest strength pre and post aquatic-based exercise training (combined DWR and DWR+RT) in overweight and obese women (n = 20).

* $p < 0.05$ significant difference pre to post training

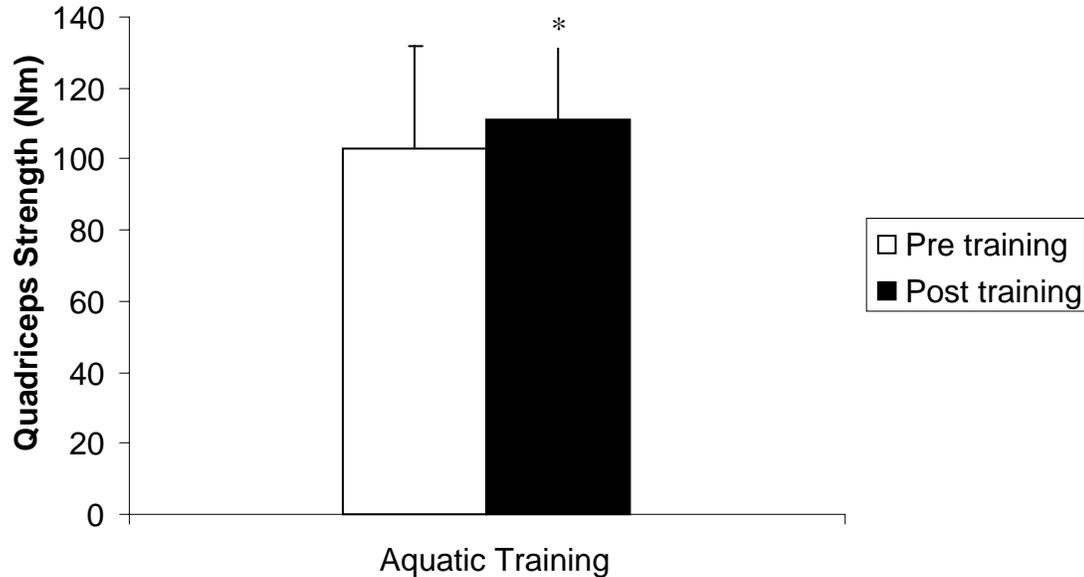


Figure 7. Quadriceps strength pre and post aquatic-based exercise training (combined DWR and DWR+RT) in overweight and obese women (n = 20).

* $p < 0.05$ significant difference pre to post training

3.2.2.5 Anthropometry

Weight or BMI did not change significantly between DWR and DWR+RT groups ($p > 0.05$) (Table 7). When groups were combined, there was no significant change in either weight or BMI following aquatic training (78.7 ± 12.0 to 78.0 ± 12.2 kg, $p = 0.13$; 29.9 ± 4.0 to 29.6 ± 4.0 kg/m², $p = 0.13$), respectively.

There was evidence of a difference between DWR and DWR+RT groups for hip circumference ($p = 0.04$) when controlling for baseline scores (Table 7). Within group analysis indicated that participants in the DWR+RT group demonstrated a greater reduction in hip circumference following training ($- 2.3 \pm 3.5$ cm, $p = 0.09$), compared to the DWR group ($- 0.8 \pm 1.5$ cm, $p = 0.95$). However, there was no difference between DWR and DWR+RT groups for changes in WC or WHR following training ($p > 0.05$) (Table 7). The aquatic intervention overall had a positive effect on abdominal adiposity as reflected by a significant reduction in WC ($p < 0.001$) (Figure 8) and WHR ($p = 0.02$) (Figure 9) when training groups were combined.

DWR and DWR+RT groups did not differ for DXA measures of total body or trunk fat percentage, FM or FFM following training when baseline scores were controlled ($p > 0.05$) (Table 8). Furthermore, when DWR and DWR+RT groups were combined to determine the overall effect of aquatic training on body composition, there were no significant changes in total body or trunk fat percentage (44 ± 5 to $43 \pm 5\%$, $p = 0.34$; 44 ± 5 to $43 \pm 5\%$, $p = 0.23$), total body or trunk FM (34.5 ± 7.8 to 34.0 ± 7.7 kg, $p = 0.34$; 17.0 ± 4.5 to 16.7 ± 4.8 kg, $p = 0.30$), or total body or trunk FFM (40.9 ± 5.9 to 41.1 ± 6.0 kg, $p = 0.46$; 20.4 ± 3.4 to 20.6 ± 3.4 kg, $p = 0.40$), respectively.

Table 7. Anthropometric data pre and post aquatic based exercise intervention for deep water running (DWR) and deep water running combined with resistance training (DWR+RT) groups

	DWR (n = 11)			DWR+RT (n = 9)			p value
	Pre	Post	Chg (%)	Pre	Post	Chg (%)	
Weight (kg)	79.0 ± 13.5	78.9 ± 14.1	-0.1	78.4 ± 10.6	76.9 ± 10.0	-1.9	0.16
Body mass index (kg/m ²)	30.0 ± 4.0	30.0 ± 4.3	0.0	29.7 ± 4.1	29.1 ± 3.8	-2.0	0.13
Waist circumference (cm)	93.7 ± 11.8	91.1 ± 12.4	-2.8	90.1 ± 7.6	87.4 ± 7.2	-3.0	0.99
Hip circumference (cm)	111.6 ± 8.6	110.8 ± 8.2	-0.7	108.1 ± 9.7	105.8 ± 7.0	-2.1	0.04*
Waist to hip ratio	0.84 ± 0.05	0.82 ± 0.06	-2.4	0.84 ± 0.05	0.83 ± 0.04	-1.2	0.40

Data are presented as mean ± SD

* p value column indicates difference between training groups (p < 0.05)

Note. Chg = change

Table 8. Dual-energy x-ray absorptiometry data pre and post aquatic-based exercise intervention for deep water running (DWR) and deep water running combined with resistance training (DWR+RT) groups

	DWR (n = 11)			DWR+RT (n = 9)			p value
	Pre	Post	Chg (%)	Pre	Post	Chg (%)	
Total body fat (%)	44 ± 3	44 ± 4	0.0	43 ± 6	42 ± 5	-1.0	0.20
Total fat mass (kg)	34.8 ± 7.2	35.0 ± 7.9	0.6	34.1 ± 8.9	32.7 ± 7.7	-4.1	0.14
Total fat free mass (kg)	40.9 ± 7.2	41.0 ± 7.4	0.2	40.9 ± 4.2	41.1 ± 4.0	0.5	0.82
Trunk fat (%)	45 ± 4	45 ± 5	0.0	43 ± 6	42 ± 5	-1.0	0.38
Trunk fat mass (kg)	17.8 ± 4.8	17.6 ± 5.5	-1.1	16.1 ± 4.2	15.6 ± 3.8	-3.1	0.60
Trunk fat free mass (kg)	20.7 ± 4.1	20.6 ± 4.3	-0.5	20.1 ± 2.4	20.6 ± 2.2	2.5	0.29

Data are presented as mean ± SD
p value column indicates difference between training groups

Note. Chg = change

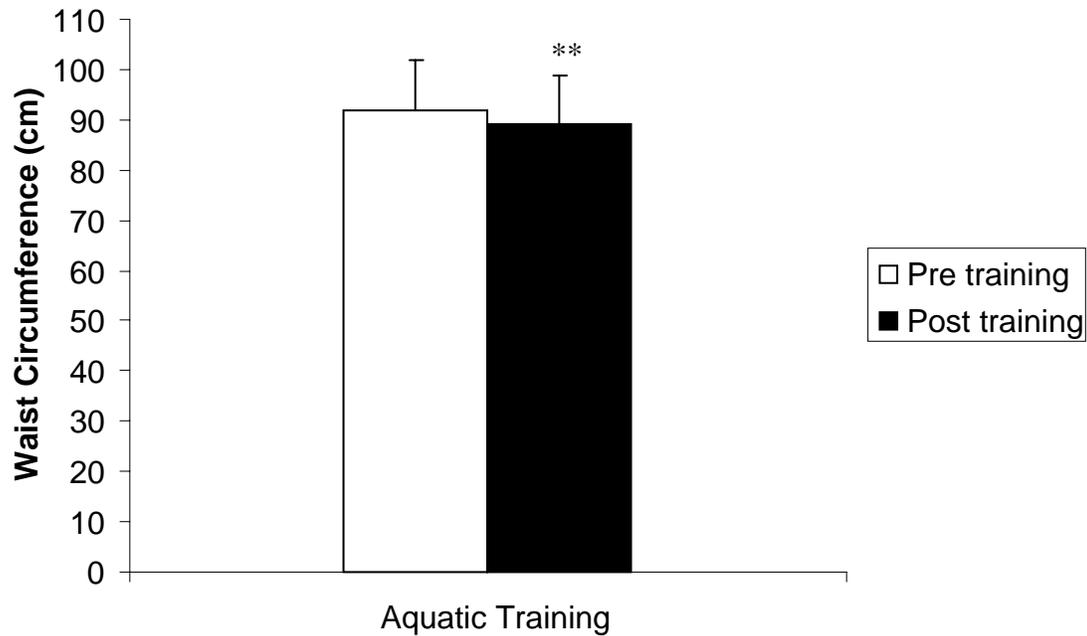


Figure 8. Waist circumference pre and post aquatic-based exercise training (combined DWR and DWR+RT) in overweight and obese women (n = 20).

** p < 0.01 significant difference pre to post training

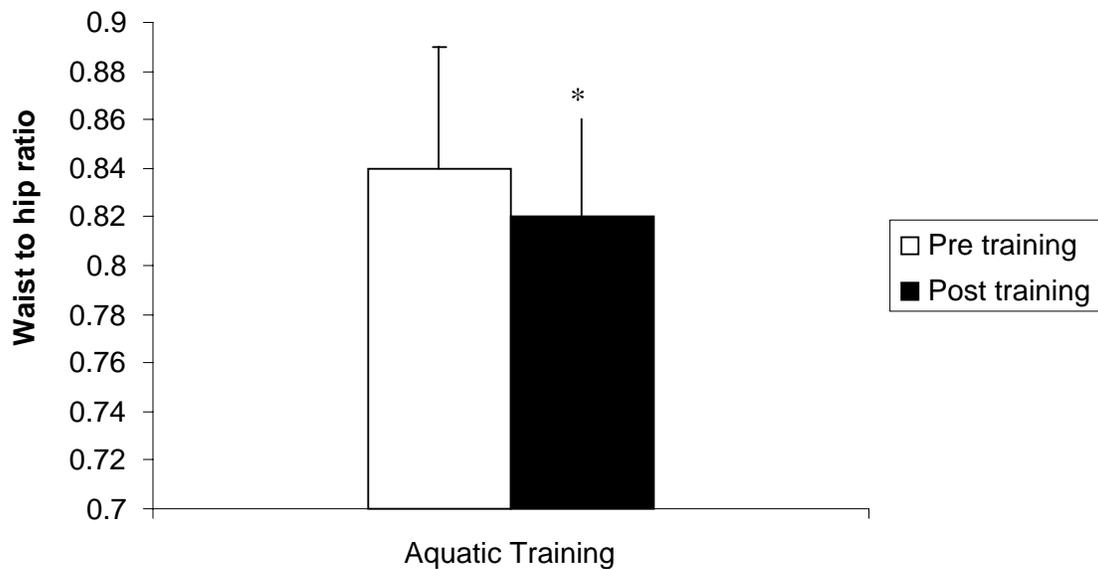


Figure 9. Waist to hip ratio (WHR) pre and post aquatic-based exercise training (combined DWR and DWR+RT) in overweight and obese women (n = 20).

* p < 0.05 significant difference pre to post training

3.2.2.6 Lipid Profile

There was no evidence of a difference in post intervention lipid profile, when controlling for baseline values, between DWR+RT and DWR groups ($p > 0.05$) (Table 9). Furthermore, when DWR and DWR+RT groups were combined to determine the overall effect of aquatic training across time, there were no significant changes in HDL (1.32 ± 0.24 to 1.30 ± 0.26 mmol/L, $p = 0.39$), LDL (3.18 ± 0.80 to 3.09 ± 0.89 mmol/L, $p = 0.19$), TAG (1.04 ± 0.67 to 1.01 ± 0.43 mmol/L, $p = 0.75$), or total cholesterol (4.98 ± 0.82 to 4.84 ± 0.92 mmol/L, $p = 0.07$).

Table 9. Lipid profile pre and post aquatic-based exercise intervention for deep water running (DWR) and deep water running combined with resistance training (DWR+RT) groups

	DWR (n = 11)			DWR+RT (n = 9)			p value
	Pre	Post	Chg (%)	Pre	Post	Chg (%)	
HDL (mmol/L)	1.31 ± 0.30	1.30 ± 0.33	-0.8	1.34 ± 0.17	1.29 ± 0.13	-3.7	0.65
LDL (mmol/L)	2.93 ± 0.74	2.77 ± 0.87	-5.5	3.48 ± 0.81	3.47 ± 0.81	-0.3	0.34
TAG (mmol/L)	1.12 ± 0.88	1.06 ± 0.51	-5.4	0.94 ± 0.25	0.94 ± 0.34	0.0	0.80
TC (mmol/L)	4.76 ± 0.79	4.56 ± 0.91	-4.2	5.25 ± 0.82	5.19 ± 0.86	-1.1	0.40

Data are presented as mean ± SD
p value column indicates difference between groups

Note. Chg = change, HDL = high density lipoprotein, LDL = low density lipoprotein, TAG = triacylglycerol, TC = total cholesterol

3.2.2.7 Correlation Analyses

Data for DWR and DWR+RT groups were combined to investigate relationships between fat oxidation measures and outcome variables following aquatic exercise training overall. There were significant positive correlations between the change in exercise fat oxidation rate, and changes in both BMI ($r = 0.49$, $p = 0.03$) and total body fat percentage ($r = 0.45$, $p = 0.046$) (Table 10). A significant negative correlation also existed between the change in exercise fat oxidation rate and LDL cholesterol ($r = -0.53$, $p = 0.02$) (Table 10). In the combined group of 16 participants, for whom complete blood samples were obtained, the change in glycerol concentration during acute aquatic exercise pre to post training was significantly positively correlated with changes in exercise fat oxidation following aquatic training ($r = 0.63$, $p < 0.01$) (Table 10). Partial correlation was used to explore the relationship between the change in exercise fat oxidation rate with change in glycerol concentration, while controlling for total body FM, trunk FM and BMI. The significant positive relationship remained between changes in exercise fat oxidation rate and glycerol concentration, controlling for total body FM ($r = 0.59$, $p = 0.02$), trunk FM ($r = 0.61$, $p = 0.02$) and BMI ($r = 0.62$, $p = 0.01$). This suggests that controlling for body composition measures had very little effect on the strength of the relationship between these two variables. No significant relationships existed between changes in resting fat oxidation and any outcome variable analysed ($p > 0.05$) (Table 10).

A significant positive correlation was evident between changes in total body FFM and RMR ($r = 0.58$, $p < 0.01$), and between trunk FFM and RMR ($r = 0.57$, $p < 0.01$) following aquatic training overall, when DWR and DWR+RT groups were combined. In addition, the change in RMR was negatively correlated with changes in body fat percentage ($r = -0.49$, $p < 0.05$) (Table 10).

Relationships between DXA data with anthropometric and blood variables were also investigated following aquatic training using combined group data on change scores. Significant positive associations were apparent between change in BMI and changes in total body fat percentage ($r = 0.74$, $p < 0.01$), trunk fat percentage ($r = 0.73$, $p < 0.01$),

total body FM ($r = 0.88, p < 0.01$) and trunk FM ($r = 0.86, p < 0.01$) (Table 11). Changes in hip circumference were positively related to changes in total body fat percentage ($r = 0.49, p = 0.03$) and total body FM ($r = 0.63, p < 0.01$) (Table 11). The change in trunk FM following aquatic training demonstrated significant positive relationships with both changes in waist circumference ($r = 0.51, p = 0.02$) and hip circumference ($r = 0.45, p = 0.04$) (Table 11).

Table 10. Pearson correlation coefficients (r) and significance for change scores in exercise and resting fat oxidation rate and resting metabolic rate, with change scores in cardiovascular fitness, body composition, lipid profile, and FFA and glycerol concentrations, following aquatic exercise training (DWR plus DWR+RT).

	Exercise Fat Oxidation Rate (mg/min)	Resting Fat Oxidation Rate (mg/min)	Resting Metabolic Rate (kcal/day)
	Pearson r	Pearson r	Pearson r
n = 20			
DWR VO _{2peak} (L/min)	0.02	0.08	N/A
Body Mass Index (kg/m ²)	0.49*	-0.10	N/A
Waist (cm)	0.19	0.04	N/A
Total fat (%)	0.45*	-0.25	-0.49*
Total FM (kg)	0.43	-0.15	-0.36
Total FFM (kg)	-0.19	0.25	0.58**
Trunk fat (%)	0.38	-0.33	-0.40
Trunk FM (kg)	0.32	-0.10	-0.14
Trunk FFM (kg)	-0.23	0.34	0.57**
HDL (mmol/L)	-0.21	-0.05	N/A
LDL (mmol/L)	-0.53*	-0.22	N/A
TAG (mmol/L)	0.43	-0.02	N/A
TC (mmol/L)	-0.33	-0.25	N/A
n = 16			
ΔFFA (μmol/L)	0.36	-0.13	N/A
ΔGlycerol (μmol/L)	0.63**	-0.13	N/A
Basal FFA (μmol/L)	-0.12	-0.24	N/A
Basal Glycerol (μmol/L)	-0.24	-0.15	N/A

* p < 0.05, ** p < 0.01; *Note.* FM = fat mass, FFM = fat free mass, HDL = high density lipoprotein, LDL = low density lipoprotein, TAG = triacylglycerol, TC = total cholesterol, FFA = free fatty acid, N/A = not applicable for that measure

Table 11. Pearson correlation coefficients (r) and significance for change scores in total body and trunk DXA data with anthropometric data, lipid profile, and FFA and glycerol data, following aquatic-based exercise training (DWR plus DWR+RT)

	Total fat (%)	Total FM (kg)	Total FFM (kg)	Trunk fat (%)	Trunk FM (kg)	Trunk FFM (kg)
	Pearson r	Pearson r	Pearson r	Pearson r	Pearson r	Pearson r
n = 20						
BMI (kg/m ²)	0.74**	0.88**	-0.16	0.73*	0.86**	-0.27
Waist (cm)	0.27	0.42	0.22	0.26	0.51*	0.22
Hip (cm)	0.49*	0.63**	-0.20	0.39	0.45*	-0.28
WHR	-0.14	-0.12	0.33	-0.07	0.09	0.41
HDL (mmol/L)	0.02	0.09	0.08	0.06	0.14	0.11
LDL (mmol/L)	-0.08	-0.01	0.23	-0.07	-0.00	0.21
TAG (mmol/L)	-0.17	-0.19	0.20	-0.16	-0.10	0.24
TC (mmol/L)	-0.17	-0.09	0.38	-0.14	-0.00	0.40
n = 16						
ΔFFA (μmol/L)	0.10	0.05	-0.05	0.15	0.12	-0.02
ΔGlycerol (μmol/L)	0.34	0.27	-0.24	0.27	0.22	-0.14
Basal FFA (μmol/L)	0.03	0.00	-0.09	-0.04	-0.15	-0.15
Basal Glycerol (μmol/L)	0.10	0.07	-0.15	0.06	-0.03	-0.17

* p < 0.05, ** p < 0.01

Note. BMI = body mass index, WHR = waist to hip ratio, FFM = fat free mass, HDL = high density lipoprotein, LDL = low density lipoprotein, TAG = triacylglycerol, FFA = free fatty acid

3.2.3 Standardisation of Testing Procedures

Of the 20 participants who completed the aquatic-based exercise intervention, nine women were still menstruating. These women were tested during the follicular phase of their menstrual cycle for both the 30 minute DWR and RMR test, on average at day six.

All 20 participants completed the 24 hour diet record and refrained from eating at least four hours prior to the 30 minute DWR session. Nutrient composition was maintained during the 24 hours leading up to the 30 minute DWR session between pre and post testing for fat (14 ± 6 v 14 ± 6 %), CHO (61 ± 7 v 60 ± 7 %) and protein (25 ± 5 v 25 ± 5 %) respectively. The 30 minute DWR post-test was undertaken at the same time of day (± 1 hour) of each participant's pre-test.

3.2.4 Validity of the Deep Water Running Test

To determine the validity of the DWR test, data from all participants were combined (DWR and DWR+RT). Maximal physiological values obtained during DWR and TMW protocols, prior to the aquatic intervention, are presented in Table 12. These are the highest values obtained for each variable during the protocols and are represented as 'peak'. Maximal relative $\dot{V}O_2$, HR and RER were significantly lower for the DWR test compared to the TMW test ($p < 0.01$). Maximal relative $\dot{V}O_2$ during DWR was 19% lower than during TMW. At maximal intensity, HR was 6% lower during DWR than during TMW. This corresponds to a maximal HR 11 beats/min lower in water compared to land.

Table 12. Maximal group responses during deep water running (DWR) and treadmill walking (TMW) graded exercise tests for overweight and obese women

Variable	DWR (n = 20)	TMW (n = 20)	p value
$\dot{V}O_{2\text{peak}}$ (ml/kg/min)	22.5 ± 4.9	27.7 ± 4.7	< 0.01**
HR _{peak} (beats/min)	159 ± 16	170 ± 12	< 0.01**
Respiratory exchange ratio	1.03 ± 0.06	1.10 ± 0.06	< 0.01**
Rating of perceived exertion	17 ± 2	17 ± 1	0.11

** p value column indicates difference between DWR and TMW (p < 0.01)

Data demonstrating validity of the of the DWR protocol in comparison with the TMW protocol are presented in Figures 9 and 10. A significant positive correlation was evident between DWR and TMW for $\dot{V}O_{2\text{peak}}$ (ml/kg/min), $r = 0.70$, $p < 0.01$ (Figure 10), and HR_{peak} (beats/min) $r = 0.65$, $p < 0.01$ (Figure 11).

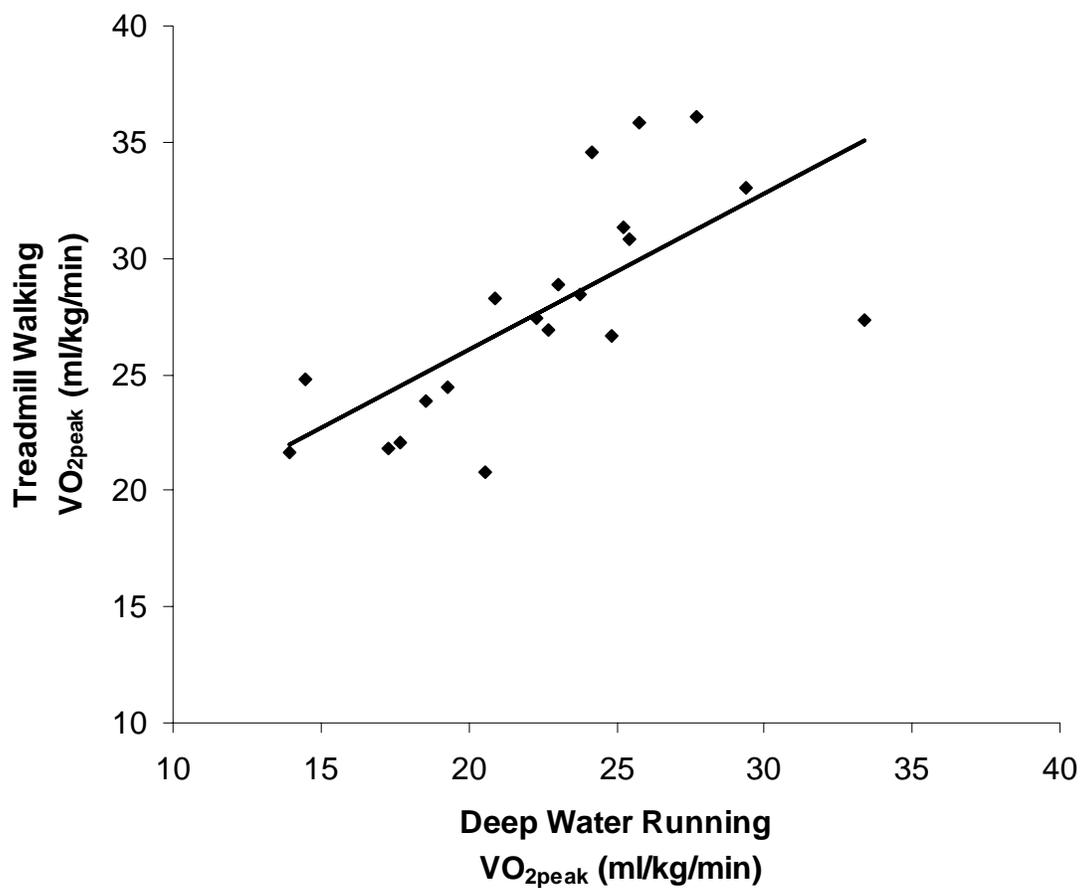


Figure 10. Relationship between deep water running (DWR) and treadmill walking (TMW) protocols for maximal oxygen consumption ($\dot{V}O_{2peak}$) (ml/kg/min) in overweight and obese women ($n = 20$) ($r = 0.70$, $p < 0.01$).

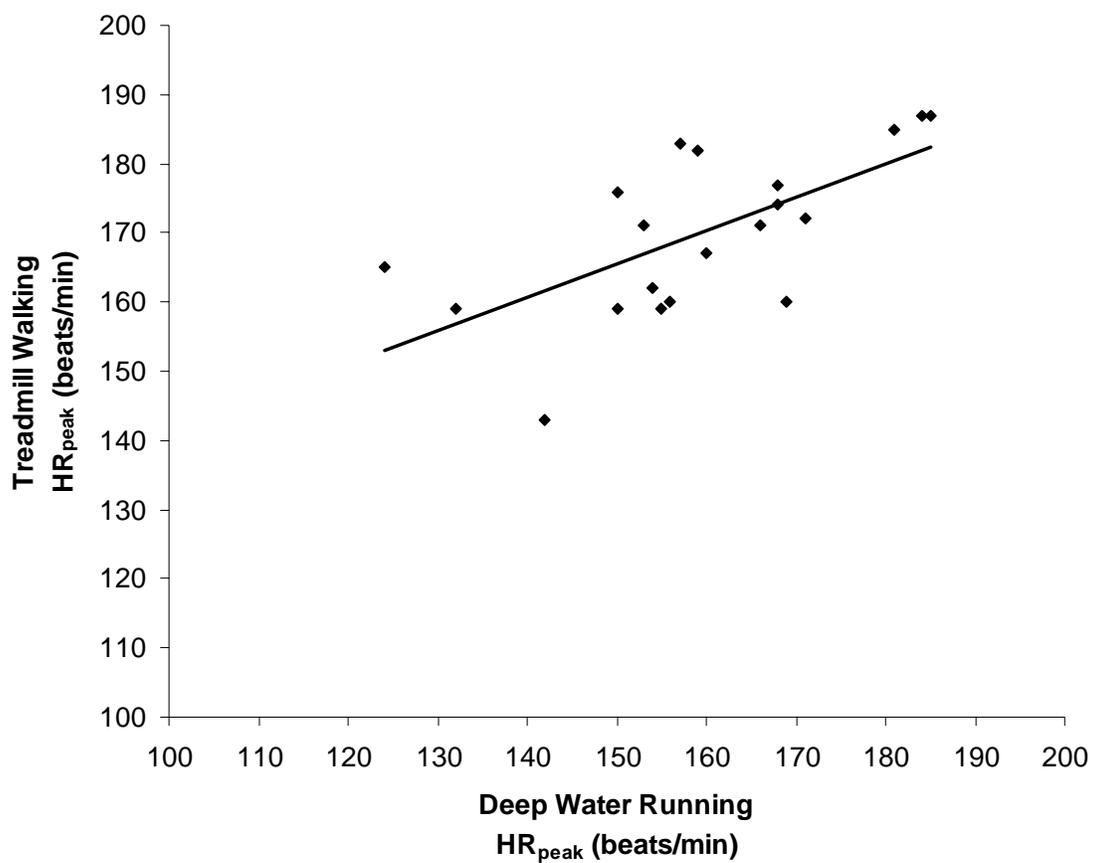


Figure 11. Relationship between deep water running (DWR) and treadmill walking (TMW) protocols for maximal heart rate (HR_{peak}) (beats/min) in overweight and obese women ($n = 20$) ($r = 0.65$, $p < 0.01$).

3.3 Discussion

Past research has largely focused on investigating the effects of land-based exercise training on fat oxidation in normal weight individuals. Consequently there is limited understanding of the effects of chronic aquatic exercise training on fat oxidation in those who are overweight or obese. Therefore, the primary objective of this study was to investigate fat oxidation following aquatic-based exercise training. The aim was to determine whether differences existed between two aquatic-based exercise training programmes; endurance based deep water running (DWR), or endurance based deep water running combined with resistance training (DWR+RT), for changes in exercise fat oxidation and related measures in overweight and obese women.

3.3.1 Metabolism and Substrate Utilisation

It was hypothesised that the combination of DWR+RT would result in a greater increase in exercise fat oxidation than DWR in overweight and obese women; however, this hypothesis was disproven. The aquatic-based exercise intervention had no effect on exercise fat oxidation, as indicated by indirect calorimetry. Fat oxidation responses did not differ with the type of aquatic training programme implemented (DWR or DWR+RT), nor was exercise fat oxidation increased following aquatic training overall. Studies that demonstrate an increase in exercise fat oxidation following training have been undertaken on land. Therefore, in overweight and obese women, it appears that aquatic-based exercise does not provide an adequate training stimulus to result in an increase in fat oxidation rate, or contribution of fat metabolism to EE. The lack of change in exercise fat oxidation rate and contribution of fat metabolism to EE following aquatic based exercise in the present investigation may either confirm the observation that overweight and obese individuals cannot increase exercise fat oxidation rates following exercise training; or provide new evidence that the aquatic exercise programme was not a sufficient stimulus to increase fat oxidation rates or fat contribution; or alternatively, a combination of the two.

To date, no exercise training studies have looked at the effectiveness of chronic aquatic-based exercise training on measures of fat oxidation or overall exercise metabolism, either in normal weight, or overweight and obese individuals. This makes comparison of the present investigation difficult. Therefore studies investigating metabolism during acute aquatic exercise will be discussed.

The physiological responses to water immersion appear to affect substrate utilisation. Earlier research indicates exercise undertaken in water relies more on the anaerobic pathways (Svedenhag & Seger, 1992). A higher RER during equivalent submaximal exercise for DWR compared to TMW is demonstrated in older women (Broman, Quintana, Engardt et al., 2006) and younger trained men (DeMaere & Ruby, 1997; Michaud, Rodriguez-Zayas et al., 1995; Svedenhag & Seger, 1992), resulting in a greater reliance on CHO metabolism during DWR. The greater RER and CHO utilisation, and lower fat oxidation during DWR indicates an increased reliance on anaerobic glycolysis and glycogenolysis, and a decrease in the oxidation of available lipid sources compared to TMW (DeMaere & Ruby, 1997). It is known that FA oxidation decreases at higher intensity exercise, where greater RER and CHO utilisation are present (Romijn et al., 1993). During high exercise intensities catecholamines continue to rise, stimulating lipolysis, however a reduction in adipose tissue blood flow may decrease FFA release from adipose tissue and hence FA oxidation (Romijn et al., 1993). Every attempt was made to maintain a low exercise training intensity, but the physiological effects of water immersion may predominate and hence display features of higher intensity exercise, despite working at a lower exercise intensity. Anaerobic metabolism during water immersion may be the result of a lowered perfusion pressure in the legs due to increased hydrostatic pressure, resulting in the decrease in total muscle blood flow (Svedenhag & Seger, 1992); a feature required for optimal fat oxidation (van Baak, 1999). Consequently, alterations in the pulmonary system and ventilatory effects due to water immersion may also influence substrate utilisation (Becker, 2004). Exercise undertaken in water demonstrates increased central blood volume which leads to a reduction in the partial pressure of oxygen (Becker, 2004). A reduction in the partial pressure of oxygen results in a concomitant increase in the partial pressure of carbon dioxide, and therefore increases in ventilation and exhaled carbon dioxide (Becker,

2004). An increase in carbon dioxide production is normally associated with increased CHO utilisation measured by indirect calorimetry; consequently fat oxidation may be masked during aquatic exercise. If an increase in fat oxidation does occur following aquatic exercise training, the methodological use of indirect calorimetry may prevent any increase to be detected.

Markers of lipolysis, through measurement of plasma FFA and glycerol concentrations, may give an indication of TAG breakdown and thus FFA availability. When aquatic training groups were combined in the present study, similar concentrations of plasma FFA and plasma glycerol were observed during a 30 minute aquatic exercise session, pre and post training. The lack of increase in exercise fat oxidation following aquatic training, when combined group data was analysed, may be reflected by no change in circulating plasma FFA and glycerol concentration during aquatic exercise, and hence TAG release. A positive correlation was identified between a change in plasma glycerol concentration during 30 minutes of aquatic exercise pre to post training, and changes in exercise fat oxidation following aquatic-based exercise training overall. Therefore, based on this relationship between plasma glycerol concentration and exercise fat oxidation, if a significant increase in circulating plasma glycerol was found, indicating TAG breakdown and therefore more available FFA for oxidation, a significant increase in exercise fat oxidation rate may have been observed. To date, no studies have investigated the effects of aquatic exercise training with changes in plasma FFA and glycerol concentrations during acute exercise, therefore no comparison of the current results can be made to previous literature.

Basal levels of plasma FFA and glycerol were also unchanged with aquatic-based exercise training overall, which may reflect a lack of change in resting fat oxidation following aquatic training. However, no correlations were identified between basal levels of plasma FFA and glycerol and resting fat oxidation, or exercise fat oxidation rate. Basal levels of plasma FFA and glycerol may therefore not provide an accurate indication of the relationship between TAG breakdown, via lipolysis, and corresponding availability for resting fat oxidation or exercise fat oxidation rate. The results of FFA and glycerol concentrations in the current investigation provide an initial point for future research

investigating lipolysis, basal levels of FFA and glycerol and changes in fat oxidation following aquatic exercise training.

Differences observed in CHO utilisation, RER and the contribution of fat and CHO to EE following exercise training between DWR and DWR+RT in the current investigation may be due to an altered pattern of muscle recruitment whilst undertaking respective training programmes. During resistance exercise within DWR+RT there is increased recruitment of the smaller muscle mass of the arms, due to specific prescribed upper body movement against the resistance of water. Previous research indicates an increased reliance on CHO utilisation during arm exercise compared to leg exercise at various intensities (Coggan, 1991; Kang, Hoffman, Wendell, Walker, & Hebert, 2004) and higher intensities (Yasuda, Ruby, & Gaskill, 2002). The resistance of water on the submerged body increases as the speed of movement increases, intensifying muscular work (Tsourlou et al., 2006). Therefore, forces acting against the exercising body, specifically the arms, would be greater when performing specific faster movements with a greater surface area; as executed during the resistance component of DWR+RT using body surface area and flotation devices. Greater CHO utilisation due to arm exercise may explain the increase in CHO oxidation following DWR+RT, as CHO is likely the predominant substrate used. The higher CHO oxidation during arm exercise may be due to a lower respiratory capacity of skeletal muscle in the arms (Gollnick, Armstrong, Saubert, Piehl, & Saltin, 1972; Yasuda et al., 2002). On this assumption, the smaller upper extremity muscles may fatigue faster during the resistance component of training, leading to altered substrate utilisation (DeMaere & Ruby, 1997; Wilder & Brennan, 1993; Yasuda et al., 2002). Although DWR and DWR+RT both incorporated upper body movements, the DWR+RT group performed prescribed upper body exercises isolating specific small muscle groups creating additional resistance with flotation dumbbells and greater surface area. Overall, the continuous endurance nature of the DWR training group resulted in an altered pattern of substrate utilisation compared to the intermittent nature of the DWR+RT group. These findings contradict literature stating that resistance exercise combined with endurance based training incurs the same metabolic response as endurance training; as seen in land based exercise (Jurimae et al., 1990). Fiber type propensity may also be a factor contributing to the increase in exercise CHO oxidation

rate seen following DWR+RT. Overweight and obese individuals tend to exhibit greater Type 2 muscle fibers than normal weight individuals (Hickey et al., 1995; Tanner et al., 2002), and since Type 2 muscle fibers have a smaller capacity to oxidise fatty acids, fat oxidation will decrease and concomitantly CHO utilisation will increase (Jeukendrup et al., 1998b). However, this was not observed for the overweight and obese individuals in the DWR group. Differences in substrate utilisation cannot be explained by differences in exercise intensity during training, as HR and hence exercise intensity was controlled between DWR and DWR+RT. Consequently, the greater use of CHO during DWR+RT compared to DWR, may be due to increased specific upper body exercise incorporating the smaller muscle mass of the arms, during the resistance component of DWR+RT.

In this group of overweight and obese women there were no differences in RMR between DWR and DWR+RT, or following aquatic training overall. To date, no studies have investigated the effects of aquatic training on RMR, so no comparison can be made on the effectiveness of the current investigation on RMR. Following land based training however, it is still inconclusive as to whether RMR can be increased (Poehlman, 1989; Ravussin & Bogardus, 1989), and the optimal exercise training programme to elicit increases in RMR has not been determined. It appears that if RMR can be increased, the mode, frequency and intensity of each aquatic training programme, or aquatic training overall, in the current investigation did not provide a sufficient stimulus to result in any increase in RMR.

The timing of the RMR measurement may have had an influence on the results. Measurement of RMR was taken at least 24 hours following an aquatic training session to minimise acute adaptations in RMR. However, any increase in RMR as a result of training is suggested to be transitory, most likely lasting less than 24 hours; therefore most of the effect may be in response to an acute bout of exercise and is only marginally associated with chronic adaptations to exercise training (Wilmore et al., 1998). In addition, as the magnitude of RMR is determined by the level of FFM (Sharp et al., 1992; Speakman & Selman, 2003; Stiegler & Cunliffe, 2006), lack of change in FFM following aquatic training may be the most likely reason for the lack of change in RMR. This was supported by positive correlations between changes in both total body and trunk FFM with RMR, and negative relationship between changes in total body fat percentage and

RMR in the current participants, which is also demonstrated in previous investigations (Nielsen et al., 2000)

Resting measures of fat and CHO oxidation, RER or contribution of fat and CHO during rest were also unchanged following aquatic training in grouped analyses, or between aquatic training groups. Improvement in resting fat oxidation appears to be dependent on caloric restriction in addition to exercise in obese individuals (Solomon et al., 2008). Due to the paucity of research on resting substrate utilisation measures following aquatic training, no comparison can be made with the current investigation. It therefore suggests that in this group of overweight and obese women, the aquatic training programme did not provide an appropriate stimulus to elicit change in measures of resting metabolism.

3.3.2 Cardiovascular Fitness

The aquatic based exercise training intervention failed to induce any significant increase in CV fitness, regardless of whether DWR or DWR+RT was performed. It appears that addition of resistance exercise to an endurance based aquatic exercise programme may elicit a similar CV stimulus to an endurance based DWR programme of equal intensity and EE.

In the current investigation a non significant, increase in $\dot{V}O_{2peak}$ of 2% was demonstrated in the combined groups following aquatic-based exercise training. The lack of significant increase in CV fitness following aquatic exercise training is comparable to results obtained by Quinn et al (1994), and Eyestone et al (1993), who measured CV fitness on land following DWR training. However, the exercise intensity used in these studies was prescribed to maintain CV fitness in trained individuals and may have been insufficient to represent a physiological training stimulus to result in any increase in CV fitness (Reilly, Dowzer et al., 2003). In the present investigation, prescription of DWR based on DWR maximal values may not be considered a high enough training intensity to result in significant increases in CV fitness. Participants in the current investigation were prescribed steady state aquatic exercise. It may be important to increase training intensity over the duration of an exercise intervention to result in an increase in CV fitness. However, as the aim of the present study was to

investigate changes in fat oxidation, intensity was set at a generic steady state level aimed to elicit increases in fat oxidation.

The physiological effects of aquatic based exercise training, appear to be more pronounced in previously sedentary individuals (Reilly, Dowzer et al., 2003). As the present investigation did not comprise a truly sedentary population, the prescribed workload of 71% mode specific HR_{max} , corresponding to 50% of $\dot{V}O_{2peak}$ may not have provided sufficient intensity to improve CV fitness in this recreationally active population. Although this group of overweight and obese women were measured at 'below average' CV fitness for DWR, when standardised with a treadmill $\dot{V}O_{2max}$ test (ACSM, 2006), their non sedentary, recreationally active status, may have attenuated gains in CV fitness. Nevertheless, the prescribed intensity used in the current investigation was sufficient to maintain CV fitness, as 12 weeks of aquatic training did not result in a decrease in CV fitness.

In contrast to the present study, the majority of exercise interventions conducted using DWR have demonstrated increases in CV fitness. A significant improvement in DWR $\dot{V}O_{2max}$ of 20% was demonstrated after eight weeks of progressive, aerobic, interval DWR training in sedentary adults (Michaud, Brennan et al., 1995). Participants trained three days per week at 63-82% treadmill HR_{max} (67-88% DWR HR_{max}), between 16-36 minutes (Michaud, Brennan et al., 1995). Although the intensity of the current investigation at 70% DWR HR_{max} falls within the range of that by Michaud et al (1995), it is at the lower end of the training level, with some participants in the study by Michaud et al (1995) training at higher exercise intensities to that of the current investigation. Furthermore, the recreationally active status of the participants in the current investigation may have influenced the results. A significant increase of 15% in DWR $\dot{V}O_{2peak}$ has been demonstrated following six weeks of progressive DWR in untrained males at 80% of mode specific $\dot{V}O_{2peak}$ (Reilly, Cable, & Dowzer, 2003). The use of a progressive DWR training programme at a higher relative intensity appears to be better at stimulating CV adaptations and increases in CV fitness, compared to the lower intensity prescribed in the current investigation. However, a group of sedentary overweight and obese women were able to increase $\dot{V}O_{2peak}$ significantly following DWR+RT at the same intensity as in the current investigation (70-75% mode specific HR_{max}) (Meredith-

Jones et al., 2009). The non-sedentary nature of the participants in the current investigation may have prevented any increase in $\dot{V}O_{2peak}$. Higher intensity interval DWR at 75% of cycle HR_{max} (85% DWR HR_{max}) has been demonstrated to significantly increase $\dot{V}O_{2peak}$ in older women (Broman, Quintana, Lindberg et al., 2006). Again, the intensity prescribed in the current investigation may not have been high enough to increase CV fitness in a 'recreationally active' group of overweight or obese women. In addition, as the total amount of weekly EE did not meet the current recommendations for the minimum effective dose for improving CV health (ACSM, 1998, 2006), a higher total weekly EE may be required to elicit increases in CV fitness in this group of overweight and obese women.

The lack of increase in CV fitness following aquatic-based exercise training in the present investigation may also correspond to the observation of a lack of increase in exercise fat oxidation following aquatic training. If the aquatic training programme provided a sufficient stimulus to elicit an increase in CV fitness then a concomitant increase in exercise fat oxidation may have been observed, as fitter individuals display greater rates of exercise fat oxidation than sedentary individuals (Klein et al., 1994; Tremblay et al., 1992). However, no correlation was evident between changes in exercise fat oxidation and CV fitness in the current participants. Overall, DWR prescribed at a generic intensity aimed to elicit increases in fat oxidation did not provide a sufficient enough stimulus to demonstrate increases in CV fitness in overweight and obese women, nor increases in fat oxidation, which do not appear to be related in the current population.

3.3.3 Muscular Strength

Aquatic-based exercise training had a significant positive effect on muscular strength. Overall, aquatic-based exercise demonstrated significant increases in both chest (10%) and quadriceps strength (8%), regardless of session structure. These results are similar to those found by Takeshima et al, (2002) who reported increases of 7% and 8% for chest strength and quadriceps strength respectively, in older women following 12 weeks of a shallow water exercise. However, the magnitude of strength change in the present study was lower than that reported by Tsourlou et al, (2006) who reported increases of 26% and 29% for chest strength and quadriceps strength, respectively. The

greater increase in chest and quadriceps strength demonstrated by Tsourlou and colleagues (2006), compared to the present study, may be due to the greater duration of the training programme at 24 weeks, and the progressive nature; where intensity, duration, sets, repetitions and external resistance were increased over the course of the intervention. A group of overweight and obese women also demonstrated greater strength increases, compared to the current investigation, for chest (20%) and quadriceps (33%), following a circuit based DWR programme consisting of both endurance and resistance DWR (Meredith-Jones et al., 2009). The sedentary nature of the overweight and obese women in the study by Meredith-Jones et al (2009) may have resulted in greater strength increases. The DWR+RT component within the current aquatic training intervention complied with ACSM recommendations for resistance exercise for obese individuals, incorporating one set of 3-20 repetitions of 8-10 exercises including all major muscle groups (ACSM, 2006). However, participants undertaking DWR in the present investigation were also able to improve strength, even in the absence of prescribed specific resistance exercises. Therefore, aquatic exercise is an ideal stimulus for strength adaptations due to the resistance of water, as demonstrated in the present study. According to the principles of hydrodynamics, when the velocity of a moving object doubles, the consequent drag force quadruples (Tsourlou et al., 2006). Therefore, the resistive effect of water provides loading during movement of limbs, enhancing muscular tension and providing a training stimulus for strength increases and muscular development.

Alternatively, individuals in the DWR group demonstrated a significantly greater increase in hamstring strength (26%), than following DWR+RT (1%). This could be due to a greater proportion of exercise time devoted to continual lower extremity movement against the resistance of water during DWR with greater activation of the hamstrings; compared to DWR+RT in which a mixture of alternating upper and lower body resistance exercises were performed. As also demonstrated by Meredith-Jones and colleagues (2009), Tsourlou et al, (2006) and Poyhonen et al, (2002), aquatic training has a positive effect on hamstring strength, which can be explained by hydrodynamic principles. In order to optimise the resistance of water, leg movement is recommended to oppose the upward force of buoyancy during the major part of the range of motion (Poyhonen et al.,

2002), a feature of continuous DWR. As no other aquatic investigation to date has directly compared DWR and DWR+RT, comparison with other results is again limited. However, increases in hamstring strength of 11% measured by isometric dynamometer (Tsourlou et al., 2006), and 13% measured by hydraulic-resistance (Takeshima et al., 2002), were demonstrated following shallow water exercise programmes (similar to DWR+RT) in elderly women. Potentially, repetitive foot contact with the bottom of the pool, and subsequent weight bearing muscular contraction while undertaking resistive exercises, may have resulted in greater hamstring strength gains than during DWR+RT in the current investigation. Conversely, increases in hamstring strength of 7% measured with an isokinetic dynamometer (Nm), specific to the current investigation, were demonstrated in healthy women following prescribed lower body aquatic resistance training (Poyhonen et al., 2002). This increase in hamstring strength is lower than that observed following purely endurance based DWR (26%) in the present investigation, indicating the effectiveness of DWR without the need for resistance exercise. Overall, the resistive properties of water make aquatic training an ideal environment to elicit increases in upper and lower body strength while undertaking either endurance based DWR or DWR+RT.

3.3.4 Anthropometry

Both body weight and BMI remained unchanged following aquatic training in these overweight women, indicating no significant effect of the exercise intervention on total body mass. In this investigation, dramatic changes in body weight were avoided during the intervention, as changes in body weight may interfere with metabolism (van Aggel-Leijssen et al., 2002), and consequently any change detected in metabolic measures cannot be solely attributed to training. If weight reduction was the aim, modification to diet may be recommended. However diet, as measured through 24 hour diet records, was unchanged during the course of the investigation. Correlational analysis demonstrated a positive relationship between changes in BMI and total body fat percentage with exercise fat oxidation rate in the current participants, as in previous investigations (Schutz, Tremblay, Weinsier, & Nelson, 1992). If an increase in exercise fat oxidation rate was observed, it may have been due to an increase in body mass or

body fat rather than a training induced increase in fat oxidation rate. Further investigation is required to determine the relationships between body mass and fat, and exercise fat oxidation.

Studies investigating the effects of aquatic exercise training on body composition are scarce. In the present investigation there were no changes in body composition as measured by DXA for percentage total body and trunk fat, total body and trunk FM or total body or trunk FFM either between DWR and DWR+RT groups, or overall following aquatic training. This is in agreement with Wilber et al (1996), who also demonstrated no change in the percentage of body fat following six weeks of DWR. However, subjects in the study by Wilber and colleagues (1996) were trained male runners, and it was concluded that DWR may serve as an effective training alternative to land based running for maintenance of body composition (Wilber et al., 1996). Furthermore, in sedentary, healthy individuals undertaking DWR training for eight weeks there was also no change in percent body fat or body weight (Michaud, Brennan et al., 1995). Aquatic exercise training in the current investigation was not successful in eliciting changes in body composition as measured by DXA, despite 12 weeks of exercise. Significant changes in FM and FFM are reported to be detectable via DXA following 10 weeks (Thomson, Brinkworth, Buckley, Noakes, & Clifton, 2007) and eight weeks (Van Marken Lichtenbelt, Hartgens, Vollaard, Egging, & Kuipers, 2004) of exercise training, indicating that 12 weeks of exercise training in the current investigation was of sufficient duration to change body composition. Decreases in body fat were reported following 12 weeks of a combination of endurance and resistance shallow water exercise in elderly women (Takeshima et al., 2002). However, in the investigation by Takeshima and colleagues (2002), skin fold measurements were used which are more prone to measurement bias compared to DXA. The sedentary nature of the elderly women may have also contributed to the change in body fat, which is more likely in sedentary individuals, rather than the recreationally active individuals from the current investigation.

Increases in muscular strength were demonstrated in the current investigation, despite no change in FFM. In a previous investigation, a significant increase in FFM of 3% accompanied an increase in muscular strength following shallow water, resistance

based exercise in elderly women, after 24 weeks of training (Tsourlou et al., 2006). It is well established that the underlying mechanisms behind increases in strength after initial weeks of resistance training are mostly attributed to adaptive changes in neural activation and thereafter, due to muscular hypertrophy (Poyhonen et al., 2002). Therefore, changes in neural activation may have resulted in increased strength among participants in the aquatic exercise modality, which differs in balance and gravitational influences compared to land. However, the 12 weeks of aquatic exercise training was not a sufficient stimulus to elicit an increase in muscular hypertrophy or changes in FM or FFM.

Despite no detectable changes in body composition or total body weight, aquatic training demonstrated significant losses in abdominal adiposity via WC and WHR. The reduction in WC and WHR following aquatic training illustrates the anthropometrical effectiveness of this mode of exercise, regardless of how aquatic exercise is performed. Both endurance (DWR) and a combination of endurance and resistance training (DWR+RT) were equally beneficial in reducing measures of abdominal adiposity in the current investigation. The reduction in WC, and hence WHR, with no change in body weight, suggests a selective depletion of abdominal fat with aquatic exercise, despite no change in total weight. This observation was further supported by the significant positive correlation between trunk FM and WC in the current investigation. The relationship between trunk FM and WC suggest that loss of centrally located fat may have contributed to the alteration in WC, as centrally located trunk FM measured by DXA has been reported to be a valid and reliable assessment of abdominal obesity (Glickman et al., 2004). However, the two types of aquatic-based exercise training demonstrated differences in the measure of hip circumference, with a greater reduction in hip circumference following DWR+RT. This may be reflected in the pattern of a greater reduction in percent body fat, FM and increase in FFM with DWR+RT compared to DWR, despite these values not attaining statistical significance. The trend for improved body composition in the DWR+RT group is an interesting feature, considering the trend for a increase in CHO oxidation in the DWR+RT group. As CHO oxidation was not involved in the hypothesis of this study, it is recognised that future research may benefit from investigation of CHO oxidation and body composition, particularly with reference to exercise in an aquatic environment

Few studies have assessed changes in waist and hip circumference or WHR following aquatic exercise training. One study reported no change in WHR following 12 weeks of aquatic exercise in a group of normal weight older women (Taunton et al., 1996). However, in a study similar to the current investigation, a group of overweight and obese women significantly reduced WC and WHR following combined endurance and resistance DWR for 12 weeks, three times per week at 70-75% HR_{peak} (Meredith-Jones et al., 2009). The positive change in WC and WHR in the current investigation, and that of Meredith-Jones et al (2009), may be due to the overweight and obese nature of the participants. It could be suggested that the positive reductions in WC and WHR in the current investigation may help offset the metabolic complications associated with a high accumulation of abdominal fat, including; glucose intolerance, hyperinsulinemia, diabetes, hypertension and hyperlipidemia (Despres et al., 1991). Positive alterations in abdominal adiposity occurred in the current investigation, despite no change in fat oxidation. This indicates that positive changes in body composition may occur, irrespective of changes in exercise metabolism following aquatic exercise training.

3.3.5 Lipid profile

In the current investigation, the blood lipid profile remained unchanged following 12 weeks of aquatic exercise training. This is contrary to the positive alterations in LDL and total cholesterol levels reported following 12 weeks of a combination of endurance and resistance shallow water based exercise training in elderly women (Takeshima et al., 2002). However, like the current study, they also reported no improvement in TG or HDL (Takeshima et al., 2002). Exercise training intensity is reported to have an influence on HDL levels. Stein et al. (1990) stated that a minimal exercise intensity of 75% HR_{max} was required to improve HDL in a group of healthy middle aged men. The intensity prescribed in the current investigation was slightly below this minimal level, therefore changes in HDL and possibly LDL, TAG and TC may have been demonstrated if exercise was undertaken at a higher intensity. Consequently, exercising at a generic intensity prescribed for optimal fat oxidation may not provide a great enough stimulus for positive alterations in the lipid profile. However, exercise intensity appears to be less predictive of HDL levels than EE. As weekly EE in the current investigation did not

achieve that recommended for positive alterations in the lipid profile, of 1200 kcal/week (Durstine et al., 2001), changes in cholesterol measures are therefore not expected. Furthermore, a longer training duration may have been required to see larger reductions in LDL, TAG and TC as those reported in the current investigation. Weight loss has also been shown to be a strong predictor of reductions in TC and LDL (Nieman et al., 2002). With no change in weight in the current investigation, it was unlikely that positive changes in TC and LDL would have occurred. An increase in exercise fat oxidation rate is also related to a reduction in LDL cholesterol in the present investigation. Therefore, with no change in exercise fat oxidation rate a reduction in LDL cholesterol was also unlikely.

3.3.6 Validity of the Deep Water Running Test

The positive correlations between maximal responses during the DWR test and the TMW test indicate validity of the DWR protocol as a graded exercise test in overweight women. In agreement with other studies comparing maximal responses of DWR to TMW, the present investigation also demonstrated significantly lower $\dot{V}O_{2peak}$, HR_{peak} and RER during DWR compared to TMW (Broman, Quintana, Engardt et al., 2006; Butts et al., 1991; Frangolias & Rhodes, 1995; Frangolias et al., 2000; Mercer & Jensen, 1997; Michaud, Rodriguez-Zayas et al., 1995; Nakanishi et al., 1999; Reilly, Dowzer et al., 2003; Svedenhag & Seger, 1992; Town & Bradley, 1991). In this study $\dot{V}O_{2peak}$ was 19% lower during DWR than TMW, which is comparable to reports in young men (Brown, Chitwood, Beason, & McLemore, 1996; Frangolias & Rhodes, 1995; Mercer & Jensen, 1997; Nakanishi et al., 1999; Svedenhag & Seger, 1992) and women (Broman, Quintana, Engardt et al., 2006). This indicates that in this sample of middle aged, overweight to obese women, CV responses are similar to other populations during maximal DWR and TMW. Factors such as the exertional force required to overcome viscosity friction of the water medium, different muscle activation patterns (Brown et al., 1998; Svedenhag & Seger, 1992), and CV and pulmonary responses to increased hydrostatic pressure (Becker, 2004; Butts et al., 1991) may contribute to the reduction in relative $\dot{V}O_{2peak}$ during DWR compared to TMW. During water immersion blood

volume is redistributed centrally. This increases venous return, possibly leading to a higher stroke volume and lower HR to maintain cardiac output during exercise undertaken in an aquatic environment (Becker, 2004; Frangolias & Rhodes, 1995). In the current study, a 6% reduction in maximal HR response during DWR compared to TMW was observed and is comparable to values obtained in previous studies of young males (Brown et al., 1996; Frangolias & Rhodes, 1995; Mercer & Jensen, 1997; Nakanishi et al., 1999; Svedenhag & Seger, 1992), young women (Mercer & Jensen, 1997), older women (Broman, Quintana, Engardt et al., 2006) and sedentary subjects (Michaud, Brennan et al., 1995). Heart rate responses are known to be affected by water temperature during exercise (Craig & Dvorak, 1969; Svedenhag & Seger, 1992); however, the water temperature and ambient air temperature of the present study was kept at a thermoneutral level to minimise temperature effects. Furthermore, the lower RER may also reflect the reduced exercise intensity that is able to be achieved at maximum effort during DWR compared to TMW, as demonstrated by the decreased maximal $\dot{V}O_2$ and HR responses. Therefore, the maximal DWR test utilised in the current investigation was considered to accurately reflect maximal responses. Use of the mode specific DWR test is unique in the present investigation, and may be deemed most appropriate to measure maximal responses in an aquatic environment due to the significant differences identified between maximal DWR and TMW.

3.3.7 Summary

There were some differences in substrate utilisation between aquatic exercise training groups; DWR and DWR+RT, following exercise training. Deep water running with the addition of aquatic resistance exercises (DWR+RT), induced an increase in CHO oxidation rate and contribution of CHO to EE due to the physiological nature of resistance training in an aquatic environment. Measures of chest and quadriceps strength, WC and WHR showed significant improvement following aquatic-based exercise when training groups were combined. Traditional DWR demonstrated greater gains in hamstring strength than DWR+RT due to continual resistance provided by water and upward buoyancy during repetitive DWR motion.

However, as examined in this study, exercise fat oxidation, exercise and basal FFA and glycerol concentrations, resting metabolic measures, CV fitness, weight, BMI, body composition and lipid profiles, showed no significant difference after aquatic-based exercise when training groups were combined.

In conclusion, the results of the present investigation demonstrate that aquatic-based exercise training was not effective at increasing fat oxidation in overweight and obese women. Furthermore, as significant differences were evident between maximal responses during DWR and TMW, aquatic exercise training and adaptations may differ to that undertaken on land due to differences in the exercise environment. Therefore, overweight and obese women in the current investigation may either demonstrate an inability to increase fat oxidation following exercise training in general, or failure to increase fat oxidation could be a feature of aquatic-based exercise training.

Chapter 4: Land-Based Exercise Training

4.1 Methods

4.1.1 Participants

A total of 22 overweight and obese Caucasian women were enrolled in the land-based exercise training investigation, which took place approximately eight months following completion of the aquatic-based exercise training investigation. Of the 20 participants that completed the aquatic-based intervention, 18 agreed and were suitable to participate in the land-based intervention. One participant from the DWR group was excluded from participation in the land-based intervention due to weight loss following completion of the aquatic intervention, resulting in a BMI $< 25 \text{ kg/m}^2$. One participant from the DWR+RT group could not participate in the land-based exercise intervention due to poor health. Four new participants were recruited by word of mouth for this study in order to increase sample size number. Again, due to differing definitions of BMI values for “overweight” and “obese” in Maori and Pacific Islanders, only Caucasian participants were studied (Swinburn et al., 1999). After delivery of information sheets for participants (Appendix 8), written consent was obtained from each participant. This study was approved by the University of Otago Human Ethics Committee (Ethics Number 06/044). A screening questionnaire (Appendix 9), obtaining medical and exercise history was completed by each participant, based on the PAR-Q (ACSM, 2006). Exclusion criteria were identical to those used in the aquatic-based investigation and included; BMI $< 25 \text{ kg/m}^2$, a diagnosis of heart disease, pulmonary disease, diabetes, orthopaedic limitations and any other health problem that may interfere with exercise. Participants who reported significant weight change in excess of 4.5 kg in the two months before enrollment were also excluded (Horner et al., 2001). Individuals on medication that may affect metabolism were included if medication had been stable over the previous six months. This included low dose HRT, antidepressant medication and tobacco smoking ($n = 4$). Participants were instructed to do the following – “continue with your original exercise, dietary and medication routines during the course of the intervention.” None of the subjects had participated in a supervised systematic exercise program for at

least six months prior to the land-based intervention, as stated in past research (Volaklis et al., 2007).

4.1.2 Sample Size

Sample size calculation was the same as that used for the aquatic-based exercise training study (Chapter 3) and is as follows. To obtain a power at 0.80 and $\alpha < 0.05$, the paired t-test for pre to post exercise intervention measures in exercise fat oxidation required a total overall sample of nine individuals (Bausell & Li, 2002). Analysis of covariance testing comparing the improvement pre to post intervention in fat oxidation between DWR and DWR+RT required a sample size of 12 per group at 0.80 power and $\alpha < 0.05$ (Bausell & Li, 2002).

4.1.3 Procedure

In this investigation, participants completed a 12 week land-based exercise training intervention. All individuals were assigned to either a land-based endurance (LBE) walking group, or land-based endurance exercise combined with resistance training (LBE+RT). Participants who had completed the aquatic-based exercise intervention continued in the investigation through rolling recruitment and randomisation, and therefore were assigned to the same intervention protocol; additional participants were randomly assigned to LBE or LBE+RT using computer generated random numbers (graphpad, 2005). Testing was undertaken prior to and on completion of the land based exercise intervention. Baseline and post intervention testing included the same outcome measures as in the aquatic investigation; CV fitness, exercise and resting metabolism and substrate utilisation, muscular strength, anthropometry and lipid profile. Baseline testing was undertaken during the four weeks leading up to the intervention, with post testing undertaken within one week of completion of the intervention in participants. In pre-menopausal participants, baseline and post testing for metabolic measures was completed during the follicular phase of the menstrual cycle. All participants continued training until post testing was completed.

4.1.4 Exercise Intervention

Each participant was assigned to one of two land-based exercise training groups:

- 1) Land Based Endurance (LBE)
- 2) Land Based Endurance combined with Resistance Training (LBE+RT)

Participants who had completed the aquatic-based exercise intervention were assigned to the same training group for the land-based exercise intervention i.e. DWR to LBE and DWR+RT to LBE+RT. Assignment to the same training group was required to allow comparison of aquatic-based to land-based training for the final investigation (Chapter 5). Four new participants were randomly assigned to one of the two training groups (two participants to LBE, and two participants to LBE+RT).

LBE: Eleven women (including nine participants who completed the aquatic based exercise training intervention) were enrolled in land-based endurance (LBE) programme which consisted of a supervised walking group. Participants completed a 60 minute walking session, three days per week for 12 weeks. The walks were conducted in a group environment and consisted of walks around the Dunedin area. For the endurance programme, participants walked the same route for each of the three exercise sessions in any single week. Six different routes were used and then repeated to complete the 12 week intervention period. Participants warmed up at a slow walking pace for five minutes, then increased their walking pace until target heart rate (HR) was achieved and maintained for 50 minutes. The session concluded with a five minute cool down phase where participants completed lower body stretches.

LBE+RT: Eleven women (including nine participants who completed the aquatic-based exercise training intervention) were enrolled in the supervised combined endurance and resistance programme which took place in a gymnasium at the School of Physical Education, University of Otago. Participants attended a 60 minute exercise session, three days per week for 12 weeks. Each session began with a five minute endurance based warm up, which consisted of; cycle ergometry, treadmill walking, elliptical trainer, aerobics, stepping or skipping. This was followed by 50 minutes of resistance exercises interspersed with endurance exercise. The combined exercise session consisted of resistance exercises alternated with endurance stations, employing a ratio of

1:2 for RT and LBE respectively. The RT exercise periods utilised resistive exercises for all major muscle groups for 90 seconds. These included exercises which targeted; chest, back, shoulders, triceps, biceps, hip adductors, hip abductors, hip flexors, gluteal muscles, hamstrings, quadriceps, calf muscles, rectus abdominals and oblique abdominals (Appendix 11). Resistance exercises consisted of body-weight resisted activities, dumbbells, barbells or machine based exercises (Body-Solid strength training equipment, Illinois, USA). Participants were instructed on proper technique and emphasis placed on smooth, continuous movements, correct posture and body mechanics for each exercise. The exercise load associated with the resistance training component was rationalised and quantified through the Biodex strength testing component. The load associated with resistance training was originally determined through mimicking the speed of contraction during Biodex strength testing during the resistance training component of the exercise intervention. Exercise intensity and load for the resistance training portion of the LBE+RT program was also determined in the first training session each participant attended. Participants were asked to choose a weight they could comfortably lift for 90 seconds at the same speed undertake during Biodex strength testing. Depending on the body part moved, this equated to approximately 45 repetitions per work interval. This weight was recorded. Participants were then required to increase the weight lifted to maintain their prescribed target HR as the program progressed. The exercise intensity for the resistance training portion of the land-based program was chosen in order to mimic the water-based intervention. The LBE period consisted of endurance exercise and was designed to maintain exercise HR within the training zone. Endurance exercise stations were performed for three minutes at which point a buzzer would sound and participants had 15 seconds to move to a resistance station. The exercise session concluded with a five minute cool down phase where participants performed upper and lower body stretches.

Each exercise session was supervised and instructed by the investigator. To ensure equality between aquatic-based and land-based interventions, participants were instructed to undertake their respective supervised exercise programme at the same moderate intensity of 70% HR_{peak} . This was specific to the mode of training and, for land-based training, was calculated from a pre intervention TMW maximal test as

described in section 4.1.5.1. Participants wore HR monitors during the exercise sessions (Polar A5, Kempe, Finland) to ensure correct intensity during exercise training. Training HR was recorded at each session and an average calculated for each individual over the 12 week period. Emphasis was placed on exercising at the desired training HR; participants were encouraged to walk faster or increase the weight lifted to keep HR in the prescribed training zone. As in the aquatic-based intervention, participants were also instructed to select an exercise intensity corresponding to between 'very light' (RPE 9) to 'fairly light' (RPE 11) on the 6-20 point Borg RPE scale (Borg, 1982). A training log was given to each participant detailing their training HR with information on the days and times of sessions for the 12 week training duration. Details of respective exercise programs were also given to each participant to ensure they had sufficient information to correctly undertake each session. Sessions were available one to two times each day and participants were instructed to have three sessions signed off each week after attendance by the instructor.

Energy expenditure of the exercise sessions was calculated retrospectively using values obtained during the maximal TMW test. Using the Weir (1949) formula, EE was calculated from corresponding values for $\dot{V}O_2$ and CO_2 and the relationship with HR plotted. From the linear trend line, EE was calculated from the average training HR for each individual.

4.1.5 Pre and Post Intervention Outcome Measures

Specific land-based testing was undertaken for CV fitness and exercise substrate utilisation. However, pre and post testing for all other measures; RMR, strength, anthropometry and lipid profile, were identical to those undertaken for the aquatic-based exercise training intervention. For more information on these other measures please refer to the methods section in the aquatic-based exercise training chapter (Chapter 3.1.5).

4.1.5.1 Cardiovascular Fitness

A graded treadmill walking (TMW) $\dot{V}O_{2\max}$ test was conducted in an exercise physiology laboratory at the School of Physical Education, University of Otago on a

motorized treadmill (Quinton Instrument Company, Series 90 Q65, WA, USA) following the Harbor Protocol (Wasserman et al., 2005). This test was identical to that performed prior to the aquatic intervention to determine the validity of the DWR test. The TMW test was continuous and consisted of one minute stages. Participants completed three minutes of warm up at a zero grade walking at a comfortable speed, which was determined during familiarisation with the mouthpiece and head gear. The grade was increased at a constant pre selected amount of 1-1.5% each minute until volitional exhaustion. Continuous respiratory gas analysis using a Sormedics 2900 metabolic cart (Sormedics, California, USA) and HR (Polar A3, Kemple, Finland) were undertaken throughout the test. Participants received strong verbal encouragement throughout the entire test. The test was terminated when the participant could no longer continue and signaled to stop. The highest oxygen uptake and corresponding HR were taken as $\dot{V}O_{2\text{ peak}}$ and HR_{peak} respectively. A RPE was taken on completion of the test using the 6-20 Borg scale (Borg, 1982).

4.1.5.2 Metabolism and Substrate Utilisation

Exercise Metabolism

Testing of exercise metabolism was conducted in the same manner as in the aquatic-based exercise intervention; however, the type of exercise used for testing was specific to the training mode. To determine exercise fat oxidation participants performed a 30 minute treadmill walk session at 60% HR_{peak} in an exercise physiology laboratory at the School of Physical Education, University of Otago. Intensity was calculated from the HR_{peak} obtained during the TMW test. Analysis using indirect calorimetry (Sormedics, California, USA) was performed for one minute, after excluding the first 20 seconds of measurement, at five minute intervals while walking. Gas analyzers were calibrated prior to each test with a known concentration of oxygen and carbon dioxide. Volume was also calibrated prior to each test using a standard 3L syringe. The participant breathed through the mouthpiece while maintaining walking technique. A mean of the five sampling periods for $\dot{V}CO_2$ and $\dot{V}O_2$ (ml/min) was used to calculate fat and CHO oxidation rates according to the non-protein respiratory quotient technique

(Peronnet & Massicotte, 1991). Oxidation rates were converted to determine the contribution of fat and CHO to total EE using the Atwater general conversion factors (Atwater, 1909), as in the aquatic-based exercise training intervention (Section 3.1.5.2). Total EE during the 30 minute steady state exercise session was determined using the Weir equation (Weir, 1949).

From the RER the approximate contribution of substrates to energy metabolism during exercise was determined. Heart rate was monitored continuously using a HR monitor (Polar A3, Kempe, Finland). Participants were asked to keep walking intensity at the prescribed HR, which was monitored by the investigator during the session and recorded during the breath analysis every five minutes. As in the aquatic-based intervention, participants were also instructed to exercise at an RPE of 9 to 11 (Borg, 1982).

Blood was collected by venepuncture from a superficial vein in the antecubital fossa at rest prior to, and on completion of the 30 minute treadmill walk session. A total of 10 ml of blood was collected into EDTA tubes. Following centrifugation, plasma was pipetted into Eppendorf tubes and stored at -80°C until analysis. Samples were analysed for plasma glycerol and total free fatty acids in the Department of Biochemistry, University of Otago, using the same methods as that undertaken in the aquatic study (Section 3.1.5.2).

4.1.6 Standardisation of Testing Procedures

Each participant undertook the 30 minute treadmill walk pre and post intervention test at the same time of the day to avoid diurnal variations that may alter substrate utilisation (Galliven et al, 1997). All metabolic testing, including resting and exercising substrate utilisation and RMR, took place during the follicular phase (days 3-9) of the menstrual cycle in pre menopausal participants to prevent possible elevated estrogen levels promoting lipolytic activity during the luteal phase of menstruation (Ruby & Robergs, 1994). Participants were instructed to record their diet in the 24 hour period prior to the 30 minute treadmill walk pre test (Appendix 5). Women who completed the aquatic intervention replicated their original 24 hour diet and they were required to follow this same diet before undertaking the post intervention 30 minute treadmill test. Analysis

of the 24 hour diet was undertaken with Diet Cruncher for Windows (version 1.6.0, Way Down South Software, Dunedin, New Zealand) to ensure equality in nutrient composition between pre and post testing. Each participant was asked to refrain from eating, consuming caffeine and smoking for at least four hours before each 30 minute treadmill walk pre and post-intervention test.

4.1.7 Statistical Analysis

Group data were described using means and standard deviations (SD). Results were analysed using Statistics Package for Social Sciences (SPSS version 14.0, Chicago, Illinois). Independent t-tests were undertaken to investigate baseline participant characteristics. Analysis of Covariance (ANCOVA) was used to compare the effectiveness of LBE and LBE+RT training on metabolic and physiological parameters. The independent variable was the type of intervention (LBE, LBE+RT) and the primary dependent variable was the post-intervention measure of exercise fat oxidation. The pre-intervention measure of exercise fat oxidation was used as the covariate in the analysis. Secondary dependent variables and covariates included other post-intervention measures and pre-intervention measures respectively. These consisted of; exercise CHO oxidation and RER; resting CHO oxidation, RER and RMR; basal and exercise plasma FFA and glycerol concentrations; $\dot{V}O_{2peak}$; chest, quadriceps and hamstring strength; weight, BMI, hip and waist circumference; total body and trunk fat mass, fat free mass and percent body fat; and total cholesterol, HDL, LDL and TAG. Preliminary checks were conducted to ensure that there was no violation of the assumptions of normality, linearity and homogeneity of variances. Residuals were examined for normality using histograms. If a large studentised residual was identified, this value was removed from the original data set and analyses re-run. If the re-run analysis reached the same statistical conclusion, the original data set was retained for analysis. Linearity and the association between pre and post-intervention scores were visually assessed using scatter plots. Levene's test of equality of error variances was used to check homogeneity of variances. If there was evidence for a difference in follow up scores, controlling for baseline scores, between LBE and LBE+RT training, one-sample t-tests were performed on the change score for each group. When no evidence of a difference existed between the two training groups,

combined analysis was undertaken using paired t-tests to investigate the overall effects of land-based training on metabolic and physiological parameters. Linear regression analysis was used to examine the relationships between change scores in exercise and resting fat metabolism, with change scores in anthropometry, CV fitness, lipid profile and blood measures of lipolysis (change in FFA and glycerol and basal FFA and glycerol). Associations were also examined between change scores in total body and trunk DXA data with anthropometric data, lipid profile and blood measures of lipolysis. The strength of a relationship between two variables was measured using Pearson product-moment correlations and partial correlation where appropriate. Statistical significance for all tests was set at $p < 0.05$.

4.2 Results

4.2.1 *Pre Exercise Training Data*

4.2.1.1 Participant Characteristics

A total of 22 participants were recruited and began participation in the land-based exercise intervention. Of these 22 participants, 19 participants completed the land-based intervention; nine completed LBE, and 10 completed LBE+RT (Figure 12). Three participants were unable to complete 12 weeks of land based training due to work commitments ($n = 2$), and ill health which occurred outside of the training programme ($n = 1$). The land-based exercise intervention was well tolerated with no adverse events or injury as a result of training. Overall, attendance was 98% for LBE and 95% for LBE+RT to the required 36 sessions.

Individuals on low dose HRT, antidepressants or who smoked tobacco did not change their habits over the course of the study. To check for potential confounding from medications and other substances that could impact metabolism, participants were removed from the data set ($n = 4$) and statistical analyses re-run. No confounding or effect modification was present, therefore results were pooled.

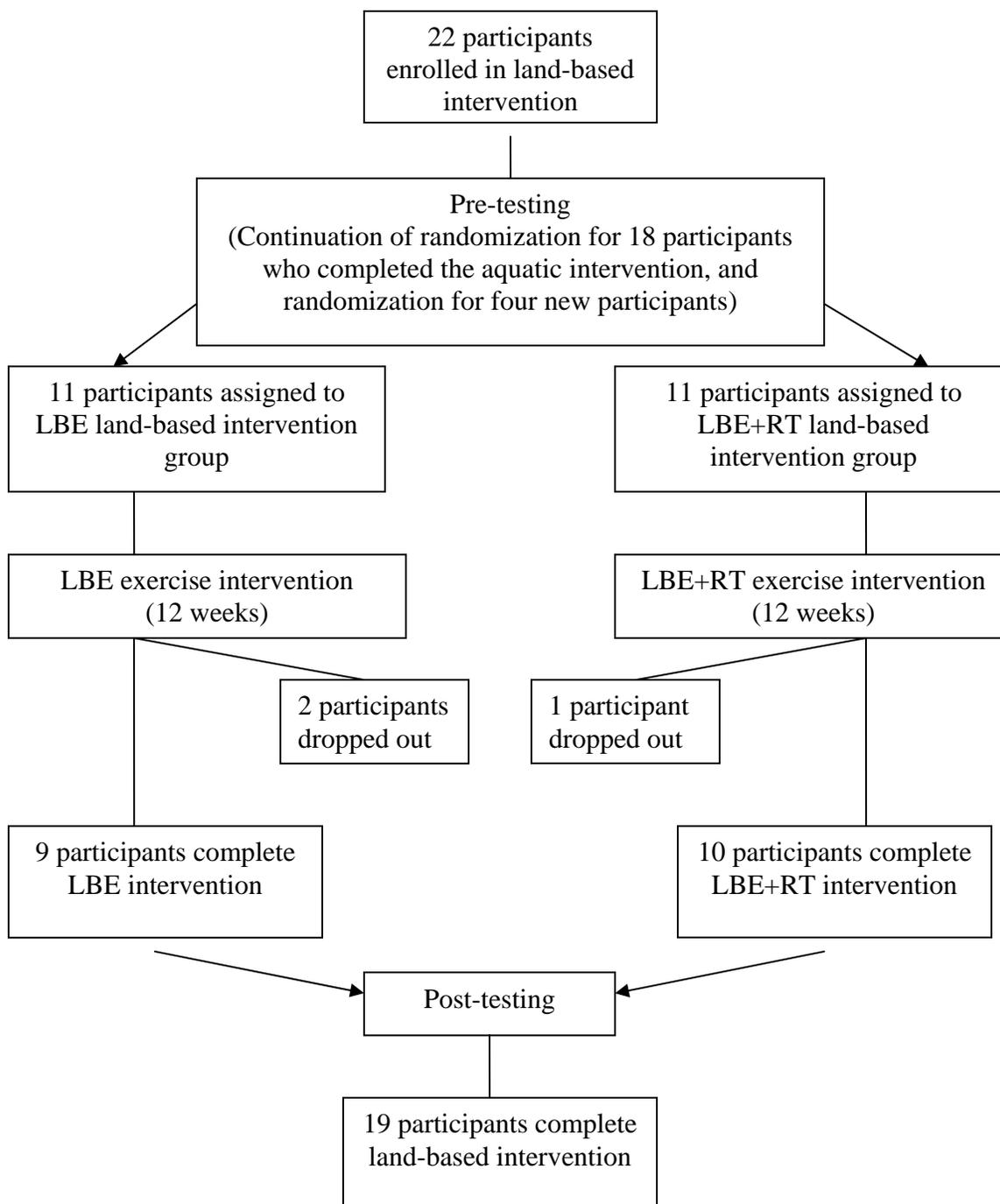


Figure 12. Flow diagram demonstrating the number of participants completing each stage of the land-based exercise intervention.

Table 12 displays baseline characteristics of participants who completed the land-based intervention ($n = 19$). These baseline measures did not differ between participants in LBE and LBE+RT groups ($p > 0.05$) (Table 12).

Of the nine women who completed the LBE intervention, four women were pre-menopausal and five women were post-menopausal. Of the 10 women who completed the LBE+RT intervention, five women were pre-menopausal and five post-menopausal.

The LBE training group had an initial waist circumference of ≥ 88 cm placing them at 'high' risk for Type 2 diabetes, hypertension and cardiovascular disease (ACSM, 2006). The LBE+RT group had an initial waist circumference of ≤ 88 cm which places them at 'increased' risk for type 2 diabetes, hypertension and cardiovascular disease due to their overweight status (ACSM, 2006). According to WHR measures, both intervention groups are ≥ 0.80 , also indicative of significant health risk (McArdle et al., 2001; Williams, Hunter, Kekes-Szabo, Snyder, & Treuth, 1997).

The LBE training group was ranked between the 10th and 20th percentile, and LBE+RT between the 20th and 30th percentile, for fitness using normative values for $\dot{V}O_{2\max}$ (ml/kg/min), with specific reference to age and sex (ACSM, 2006). These percentiles for both LBE and LBE+RT correspond to a 'below average' description of fitness in these groups, for mode specific $\dot{V}O_{2\max}$ testing using a treadmill (ACSM, 2006).

Pre intervention screening identified that 17 of 19 participants were currently recreationally active prior to participation in the land-based exercise intervention. Exercise participation was sporadic, on average, one to two exercise activities per week, and included DWR, swimming, walking, jogging, cycling, gymnasium use and golf.

Table 13. Baseline characteristics of participants who completed the land-based endurance (LBE) and land-based endurance combined with resistance training (LBE+RT) exercise interventions

	LBE (n = 9)	LBE+RT (n = 10)	p value
Age (years)	49 ± 7	49 ± 7	0.82
Weight (kg)	78.5 ± 11.6	78.0 ± 10.5	0.93
Body mass index (kg/m ²)	30.0 ± 3.8	29.4 ± 4.0	0.74
Waist circumference (cm)	90.3 ± 11.7	86.5 ± 6.6	0.39
Hip circumference (cm)	112.4 ± 8.8	107.4 ± 10.6	0.28
Waist to hip ratio	0.80 ± 0.05	0.81 ± 0.05	0.77
TMW $\dot{V}O_{2\text{peak}}$ (ml/kg/min)	26.09 ± 5.36	28.57 ± 5.36	0.33

Data are presented as mean ± SD

Note: TMW = treadmill walk, $\dot{V}O_{2\text{peak}}$ = peak oxygen consumption

4.2.2 Exercise Training Data

4.2.2.1 Training Intensity and Energy Expenditure

When LBE and LBE+RT groups were combined for analyses, HR monitor data indicated participants were exercising at 70% of pre-intervention HR_{peak} as originally instructed. Using the relationship between HR and $\dot{V}O_2$ during the TMW pre test, the corresponding training percentage of $\dot{V}O_{2peak}$ was estimated retrospectively. It was determined that participants were training at 53% of $\dot{V}O_{2peak}$. Training intensity, expressed as % HR_{peak} or % $\dot{V}O_{2peak}$, was not significantly different between LBE and LBE+RT groups ($p > 0.05$) (Table 14). When using RPE as an alternate gauge of exercise intensity, the LBE group reported an average RPE of 11 and the LBE+RT group an average RPE of 11.

Using the Weir (1949) formula, EE was estimated retrospectively from corresponding values for $\dot{V}O_2$ and $\dot{V}CO_2$ obtained during the maximal oxygen consumption test as in the aquatic intervention. The relationship between EE and HR was plotted for values obtained during the TMW pre test. From the linear trend line, EE was calculated using the average training HR for each individual. It was estimated that participants expended an average of $984 \pm 204 \text{ kcal}\cdot\text{week}^{-1}$ ($4119 \pm 852 \text{ kJ}\cdot\text{week}^{-1}$) during the land-based intervention. Amount of weekly EE was not significantly different between LBE and LBE+RT groups ($p > 0.05$) (Table 14).

Table 14. Training intensity and total energy expenditure for land-based endurance (LBE) and land based endurance combined with resistance training (LBE+RT) groups

	LBE (n = 9)	LBE+RT (n = 10)	p value
%HR _{peak}	70 ± 3	71 ± 2	0.47
% $\dot{V}O_{2peak}$	57 ± 4	51 ± 7	0.06
Energy expenditure (kcal.week ⁻¹)	1021 ± 193	950 ± 217	0.47
Energy expenditure (kJ.week ⁻¹)	4275 ± 808	3979 ± 909	0.47

Data are presented as mean ± SD

Note: %HR_{peak} = percentage of heart rate peak, % $\dot{V}O_{2peak}$ = percentage of peak oxygen consumption

4.2.2.2 Pre and Post Intervention Outcome Measures

4.2.2.3 Cardiovascular Fitness

Post intervention $\dot{V}O_{2\text{peak}}$, was not significantly different between LBE and LBE+RT groups when controlling for baseline values (LBE 26.1 ± 5.36 to 27.8 ± 4.2 ml/kg/min; LBE+RT 28.6 ± 5.4 to 31.2 ± 6.3 ml/kg/min, $p = 0.32$). When LBE and LBE+RT groups were combined for analysis to investigate the overall effects of land-based exercise training there was a significant increase in $\dot{V}O_{2\text{peak}}$ ($p < 0.01$) (Figure 13).

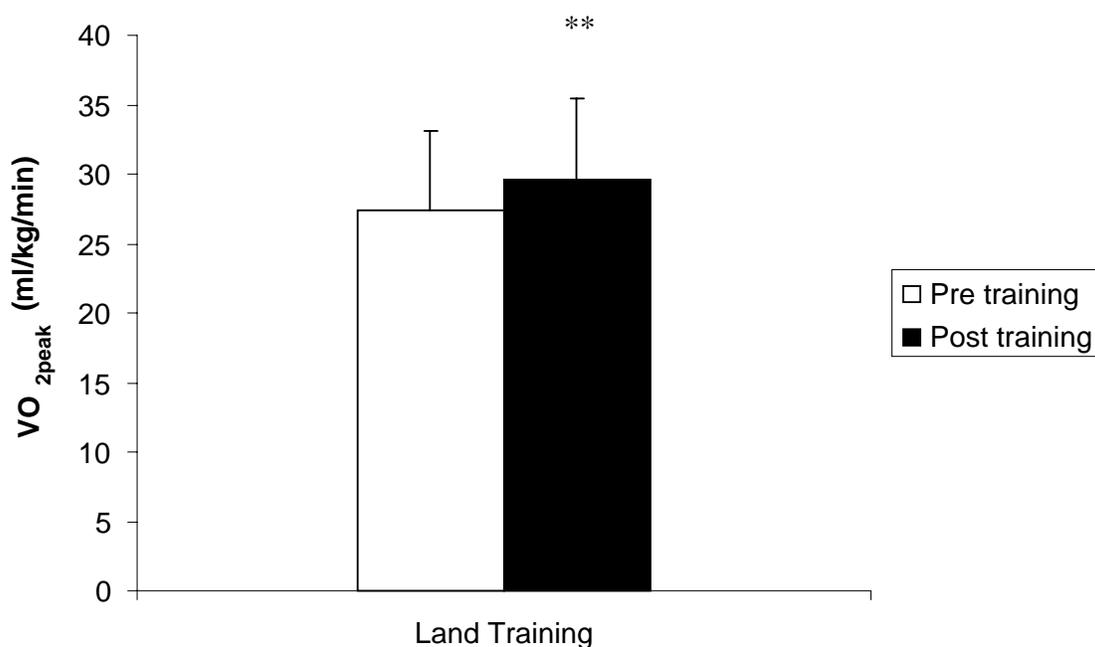


Figure 13. Peak oxygen consumption ($\dot{V}O_{2\text{peak}}$) pre and post land-based exercise training (combined LBE and LBE+RT) in overweight and obese women ($n = 19$).

** $p < 0.01$ significant increase pre to post training

4.2.2.4 Metabolism and Substrate Utilisation

Participants were instructed to complete a 30 minute TMW session at 60% of pre-intervention HR_{peak} to assess exercise fat and CHO oxidation rates, and changes in plasma FFA and glycerol concentrations, with measurement of RER, pre and post intervention. Student's paired t-test within LBE and LBE+RT exercise groups was undertaken to confirm equivalent exercise intensity between pre and post testing. Within group analysis confirmed that exercise was undertaken at the same $\%HR_{peak}$, $\% \dot{V}O_{2peak}$, total EE, HR and $\dot{V}O_2$, at pre and post testing for LBE ($p > 0.05$) (Table 15). In the LBE+RT group, HR and $\%HR_{peak}$ were equivalent at pre and post testing, as prescribed ($p > 0.05$) (Table 15). However, despite equal HR prescription, $\dot{V}O_2$, $\% \dot{V}O_{2peak}$, and total EE were not comparable between pre and post intervention for the LBE+RT group ($p < 0.05$) (Table 15). When land-based exercise training groups were combined, retrospective analysis of HR demonstrated that participants were exercising at 61% HR_{peak} during pre and post intervention. This corresponded to 44% $\dot{V}O_{2peak}$ during pre intervention however, during post intervention this corresponded to 47% $\dot{V}O_{2peak}$ for the 30 minute TMW session.

Table 15. Exercise intensity and energy expenditure during 30 minutes of steady state treadmill walking pre and post land-based exercise intervention for land-based endurance (LBE) and land-based endurance combined with resistance training (LBE+RT) groups

	LBE (n = 9)		p value	LBE+RT (n= 10)		p value
	Pre	Post		Pre	Post	
HR beats/min	100 ± 5	100 ± 5	0.34	106 ± 7	106 ± 7	0.58
%HR _{peak}	60 ± 1	60 ± 1	0.34	62 ± 1	62 ± 1	0.57
$\dot{V}O_2$ (ml/kg/min)	11.6 ± 3.2	12.1 ± 3.0	0.50	12.3 ± 1.8	13.3 ± 1.7	0.01*
$\dot{V}O_2$ (L/min)	0.90 ± 0.25	0.94 ± 0.22	0.38	0.95 ± 0.14	1.03 ± 0.20	0.03*
% $\dot{V}O_{2peak}$	44 ± 7	47 ± 11	0.23	43 ± 4	47 ± 6	0.03*
Total EE (kJ)	540 ± 149	560 ± 127	0.49	570 ± 85	617 ± 117	0.03*

Data are presented as mean ± SD

* p < 0.05 significant difference pre to post training

Note. %HR_{peak} = percentage of heart rate peak, % $\dot{V}O_{2peak}$ = percentage of peak maximal oxygen consumption, $\dot{V}O_2$ = rate of oxygen consumption, HR = heart rate, EE = energy expenditure

There was no significant difference between LBE and LBE+RT groups for the rate of exercise fat oxidation following training ($p > 0.05$) (Table 16). However, land-based training had a positive influence on exercise fat metabolism, with a significant increase in the rate of exercise fat oxidation when training groups were combined ($p < 0.01$) (Figure 14).

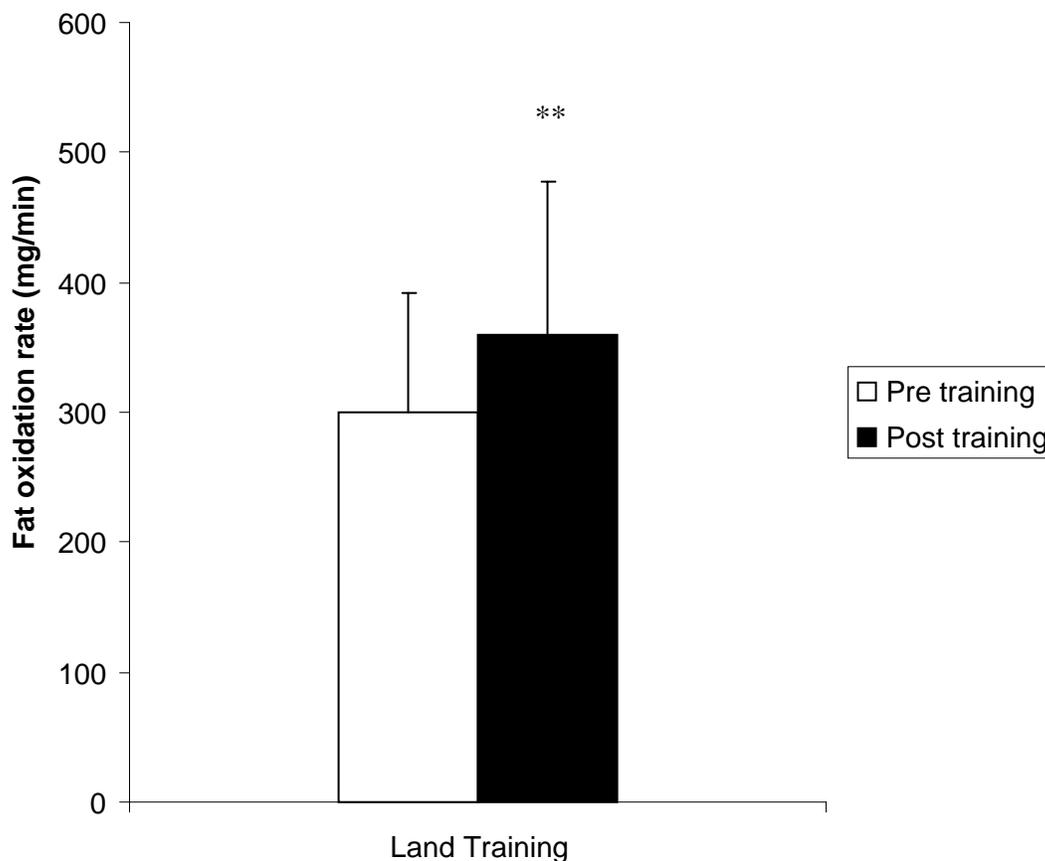


Figure 14. Fat oxidation rate during 30 minutes of steady state treadmill walking pre to post land-based exercise training (combined LBE and LBE+RT) in overweight and obese women ($n = 19$).

Data are presented as mean \pm SD

** $p < 0.01$ significant difference pre to post training

After adjusting for pre intervention scores, there was no evidence of a difference in follow up scores between LBE and LBE+RT groups for exercise CHO oxidation rate ($p = 0.17$) (Table 16). Analysis of combined groups suggested a possible decrease in CHO oxidation rate after land-based training; however, this did not achieve significance (432 ± 224 v 357 ± 227 mg/min, $p = 0.13$).

There was no difference between LBE and LBE+RT groups for changes in RER following training ($p > 0.05$) (Table 16). However, when training groups were combined to determine the effect of land-based exercise training overall, the amount of fat and CHO substrate oxidised during exercise was altered, demonstrated by a significant reduction in RER ($p = 0.04$) (Figure 15). The reduction in RER is indicative of an increase in fat oxidation and decrease in CHO oxidation.

The proportion of fat and CHO used for EE during the steady state TMW session were reported from the RER data. There was no difference between training groups for the percent of fat and CHO contributing to EE during the 30 minute TMW ($p > 0.05$) (Table 16). When LBE and LBE+RT groups were combined for overall analysis, land-based training demonstrated a significant increase in the contribution of fat to EE, and a corresponding significant decrease in the contribution of CHO to EE of 8% ($p = 0.03$) (Figure 15).

Table 16. Exercise and resting metabolism measures pre and post land-based exercise intervention for land-based endurance (LBE) and land-based endurance combined with resistance training (LBE+RT) groups

	LBE (n = 9)			LBE+RT (n = 10)			p value
	Pre	Post	Chg (%)	Pre	Post	Chg (%)	
Exercise fat ox (mg/min)	298 ± 116	366 ± 127	22.8	302 ± 70	353 ± 118	16.9	0.70
Exercise CHO ox (mg/min)	403 ± 216	275 ± 161	-31.8	459 ± 239	430 ± 259	-6.3	0.17
Exercise RER	0.80 ± 0.05	0.77 ± 0.04	-3.8	0.81 ± 0.05	0.79 ± 0.05	-2.5	0.22
% exercise fat oxidation	62 ± 18	74 ± 15	12.0	61 ± 16	66 ± 19	5.0	0.26
% exercise CHO oxidation	38 ± 18	26 ± 15	-12.0	39 ± 16	34 ± 19	-5.0	0.26
RMR (kcal/day)	1309 ± 425	1441 ± 339	10.1	1367 ± 238	1481 ± 360	8.3	0.95
Resting fat ox (mg/min)	63.22 ± 25.81	71.72 ± 26.08	13.4	57.71 ± 22.45	67.58 ± 26.17	17.1	0.85
Resting CHO ox (mg/min)	82.38 ± 68.19	85.32 ± 50.28	3.6	107.01 ± 61.86	103.17 ± 42.24	-3.6	0.71
Resting RER	0.79 ± 0.06	0.79 ± 0.05	0.0	0.82 ± 0.06	0.81 ± 0.04	-1.2	0.68
% resting fat oxidation	67 ± 21	65 ± 17	-2.0	56 ± 20	59 ± 14	3.0	0.70
% resting CHO oxidation	33 ± 21	35 ± 17	2.0	44 ± 20	41 ± 14	-3.0	0.70

Data are presented as mean ± SD

p value column indicates difference between training groups

Note. Chg = change, Ox = oxidation, CHO = carbohydrate, RER = respiratory exchange ratio, RMR = resting metabolic rate

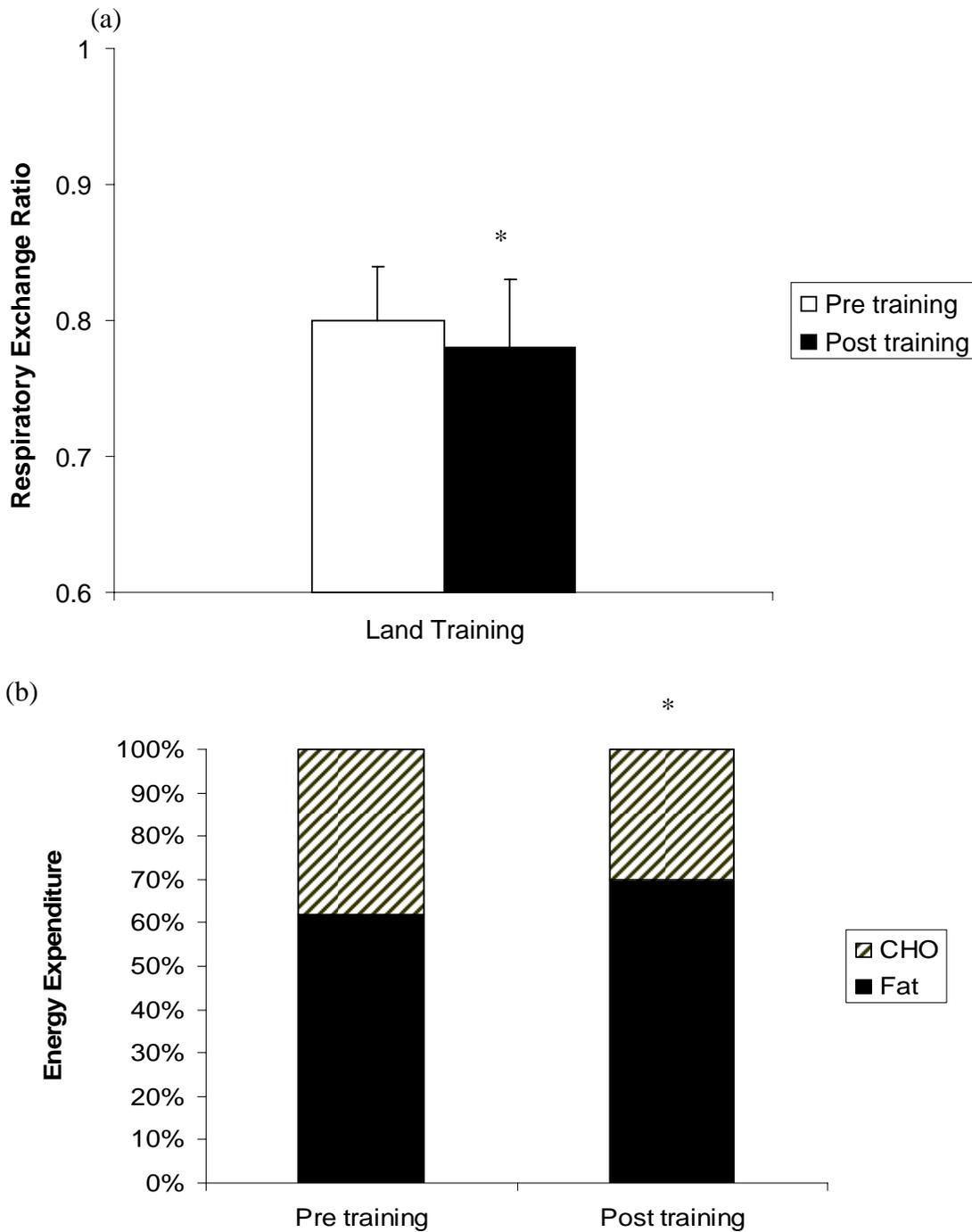


Figure 15. (a) Respiratory exchange ratio (RER), and (b) contribution of fat and CHO to energy expenditure during 30 minutes of treadmill walking pre and post land-based exercise training (combined LBE and LBE+RT, n = 19).

* $p < 0.05$ significant difference pre to post training

Blood samples before and after the 30 minute TMW steady state session were obtained from 18 out of 19 participants who completed the land-based intervention. An ANCOVA was used to compare LBE (n = 9) to LBE+RT (n = 9) groups for plasma FFA and plasma glycerol concentrations. There was no significant difference between LBE and LBE+RT groups for post intervention release of plasma FFA or plasma glycerol during a single land-based exercise session following training ($p > 0.05$) (Table 17). There was no significant difference between LBE and LBE+RT groups for changes in the basal concentration of plasma FFA after respective land-based interventions ($p > 0.05$) (Table 17). However, the change in basal concentration of plasma glycerol was significantly different between LBE and LBE+RT groups after training ($p < 0.05$) (Table 17). The LBE training group demonstrated an increase in basal plasma glycerol concentration ($32 \pm 68 \mu\text{mol/L}$, $p = 0.20$), whereas the LBE+RT group demonstrated a decrease in basal plasma glycerol concentration ($-28 \pm 53 \mu\text{mol/L}$, $p = 0.15$), following respective land-based interventions.

Training groups were combined to determine the overall effects of land-based training on plasma FFA and plasma glycerol concentrations. Student's paired t-test showed that land-based training did not significantly influence the release of plasma FFA (289 ± 163 and $311 \pm 223 \mu\text{mol/L}$, $p = 0.65$) or plasma glycerol (70 ± 50 and $76 \pm 54 \mu\text{mol/L}$, $p = 0.62$) after the 30 minute TMW session pre to post training. The concentration of FFA increased significantly after the 30 minute TMW for both pre intervention status (417 ± 223 to $706 \pm 280 \mu\text{mol/L}$, $p < 0.001$) and post intervention status (446 ± 229 to $757 \pm 341 \mu\text{mol/L}$, $p < 0.001$). The concentration of glycerol increased significantly after the 30 minute TMW at pre intervention (122 ± 40 to $191 \pm 57 \mu\text{mol/L}$, $p < 0.001$), and post intervention (124 ± 59 to $200 \pm 80 \mu\text{mol/L}$, $p < 0.001$). When LBE and LBE+RT groups were combined, similar basal plasma FFA concentrations (417 ± 223 v $446 \pm 229 \mu\text{mol/L}$, $p = 0.65$) and basal plasma glycerol concentrations (122 ± 40 v $124 \pm 59 \mu\text{mol/L}$, $p = 0.91$) were seen at pre and post intervention, respectively.

Table 17. Basal concentrations of free fatty acid and glycerol and difference in free fatty acid and glycerol concentrations during 30 minutes of treadmill walking, pre and post land based exercise training for land based endurance (LBE) and land based endurance combined with resistance training (LBE+RT) groups

	LBE (n = 9)		LBE+RT (n = 9)		p value
	Pre	Post	Pre	Post	
Basal FFA ($\mu\text{mol/L}$)	406 \pm 253	534 \pm 265	429 \pm 203	357 \pm 153	0.08
Basal glycerol ($\mu\text{mol/L}$)	124 \pm 38	156 \pm 66	120 \pm 55	92 \pm 29	0.02*
ΔFFA ($\mu\text{mol/L}$)	328	347	249	276	0.85
$\Delta\text{Glycerol}$ ($\mu\text{mol/L}$)	83	77	56	76	0.63

* $p < 0.05$ significant difference between training groups

Note. ΔFFA = change in free fatty acid concentration from pre to post 30 minute DWR exercise testing, $\Delta\text{Glycerol}$ = change in glycerol concentration from pre to post 30 minute DWR exercise testing

There was no evidence of a difference in follow up scores, controlling for baseline scores in RMR (kcal/day) between LBE and LBE+RT groups after training ($p > 0.05$) (Table 16). Furthermore, there was no significant change in RMR following land-based training when LBE and LBE+RT groups were combined for analysis (1339 ± 331 to 1462 ± 341 kcal/day, $p = 0.12$).

There was also no difference between LBE and LBE+RT groups for changes in resting fat oxidation rate, resting CHO oxidation rate, resting RER or the contribution of fat and CHO during the RMR measurement ($p > 0.05$) (Table 16). Additionally, when LBE and LBE+RT groups were combined for analyses to assess effects of the land-based training intervention over time there were no significant changes in resting fat oxidation rate (60.33 ± 23.58 to 69.54 ± 25.48 mg/min, $p = 0.18$), resting CHO oxidation rate (95.34 ± 64.34 to 94.71 ± 45.82 mg/min, $p = 0.96$), resting RER (0.81 ± 0.06 v 0.81 ± 0.04 , $p = 0.75$) or contribution of fat (61 ± 21 to 62 ± 16 %, $p = 0.81$) or CHO (39 ± 21 to 38 ± 16 %, $p = 0.81$) during the RMR measurement.

4.2.2.5 Muscular Strength

Analysis of covariance showed no difference between LBE and LBE+RT for changes in chest strength, quadriceps strength or hamstring strength ($p > 0.05$, Table 18); therefore groups were combined for analysis. Overall, paired t-testing on combined groups demonstrated land-based training had no significant effect on chest strength (206 ± 41 to 220 ± 44 Nm, $p = 0.05$), quadriceps strength (104 ± 28 to 105 ± 26 Nm, $p = 0.81$), or hamstring strength (71 ± 17 to 75 ± 14 Nm, $p = 0.08$).

Table 18. Strength measures pre and post land-based exercise training for land-based endurance (LBE) and land-based endurance combined with resistance training (LBE+RT) groups

	LBE (n = 9)			LBE+RT (n = 10)			p value
	Pre	Post	Chg (%)	Pre	Post	Chg (%)	
Chest strength (Nm)	199 ± 42	215 ± 46	8.0	213 ± 40	224 ± 44	5.2	0.82
Quadriceps strength (Nm)	100 ± 26	102 ± 21	2.0	107 ± 31	108 ± 31	0.9	0.82
Hamstring strength (Nm)	71 ± 15	74 ± 15	4.2	71 ± 19	76 ± 14	7.0	0.75

Data are presented as mean ± SD
p value column indicates difference between training groups

Note: Chg (%) = percentage change

4.2.2.6 Anthropometry

No significant difference between LBE and LBE+RT groups were evident for changes in weight or BMI ($p > 0.05$) (Table 19). Neither weight nor BMI changed significantly following land-based training when LBE and LBE+RT groups were combined (78.2 ± 10.7 to 78.1 ± 10.9 kg, $p = 0.77$; 29.7 ± 3.8 to 29.7 ± 3.9 kg/m², $p = 0.76$).

Analysis of covariance demonstrated no difference between LBE and LBE+RT groups for changes in waist or hip circumference, or WHR ($p > 0.05$) (Table 19). On further investigation there was a significant reduction in WC following land-based training when groups were combined ($p = 0.02$) (Figure 16). There was also a reduction in hip circumference (-0.76 ± 1.70 cm, $p = 0.07$) and WHR (-0.01 ± 0.02 , $p = 0.07$) following training when LBE and LBE+RT groups were combined, but this did not reach statistical significance.

There was evidence of a significant difference between LBE and LBE+RT for changes in total body fat percentage ($p = 0.04$) and changes in trunk fat percentage ($p = 0.03$) (Table 20). Participants in the LBE group demonstrated an increase in total body fat percentage ($0.4 \pm 0.9\%$, $p = 0.14$) and trunk fat percentage ($0.7 \pm 1.1\%$, $p = 0.09$), while participants in the LBE+RT group demonstrated a decrease in total body fat percentage ($-0.9 \pm 1.4\%$, $p = 0.07$) and trunk fat percentage ($-1.3 \pm 2.2\%$, $p = 0.10$). Conversely, there was no significant difference between LBE and LBE+RT for changes in both total body and trunk FM or FFM ($p > 0.05$) (Table 20). For further investigation, LBE and LBE+RT groups were combined to determine the overall effect of land-based training across time on total body and trunk FM and FFM. There were no significant changes in total body FM (34.42 ± 8.53 to 34.0 ± 8.6 kg, $p = 0.24$) or trunk FM (17.22 ± 4.69 to 16.81 ± 4.72 kg, $p = 0.47$) following land-based training. There were also no significant changes in total body FFM (40.38 ± 4.67 to 40.37 ± 4.53 kg, $p = 0.99$) or trunk FFM (20.35 ± 2.5 to 20.09 ± 2.3 kg, $p = 0.24$) upon completion of land-based training.

Table 19. Anthropometric data pre and post land-based exercise training for land-based endurance (LBE) and land-based endurance combined with resistance training (LBE+RT) groups

	LBE (n = 9)			LBE+RT (n = 10)			p value
	Pre	Post	Chg (%)	Pre	Post	Chg (%)	
Weight (kg)	78.5 ± 11.6	78.8 ± 11.2	0.4	78.0 ± 10.5	77.4 ± 11.3	-0.8	0.31
Body mass index (kg/m ²)	30.0 ± 3.8	30.2 ± 3.5	0.7	29.4 ± 4.0	29.2 ± 4.3	-0.7	0.32
Waist (cm)	90.3 ± 11.7	88.3 ± 9.2	-2.2	86.5 ± 6.6	85.4 ± 8.1	-1.3	0.73
Hip (cm)	112.4 ± 8.8	111.7 ± 8.5	-0.6	107.4 ± 10.6	106.6 ± 10.6	-0.7	0.81
Waist to hip ratio	0.80 ± 0.05	0.79 ± 0.04	-1.3	0.81 ± 0.05	0.80 ± 0.04	-1.2	0.34

Data are presented as mean ± SD
p significant difference between training groups
Note: Chg (%) = percentage change

Table 20. Dual-energy x-ray absorptiometry data pre and post land-based exercise training for land-based endurance (LBE) and land-based endurance combined with resistance training (LBE+RT) groups

	LBE (n = 9)			LBE+RT (n = 10)			p value
	Pre	Post	Chg (%)	Pre	Post	Chg (%)	
Total fat percentage (%)	45 ± 5	45 ± 5	0.0	43 ± 7	42 ± 7	-1.0	0.04*
Total fat mass (kg)	34.9 ± 7.8	35.2 ± 7.3	0.9	34.0 ± 9.5	32.9 ± 9.9	-3.2	0.08
Total fat free mass (kg)	40.2 ± 5.8	39.9 ± 5.8	-0.7	40.5 ± 3.7	40.8 ± 3.3	0.7	0.38
Trunk fat percentage (%)	45 ± 7	46 ± 7	1.0	44 ± 6	42 ± 7	-2.0	0.03*
Trunk fat mass (kg)	17.7 ± 5.0	17.9 ± 4.4	1.1	16.8 ± 4.6	15.9 ± 5.0	-5.4	0.05
Trunk fat free mass (kg)	20.5 ± 3.2	20.1 ± 3.1	-2.0	20.2 ± 1.9	20.0 ± 1.5	-1.0	0.87

Data are presented as mean ± SD

* p < 0.05 significant difference between training groups

Note: Chg (%) = percentage change

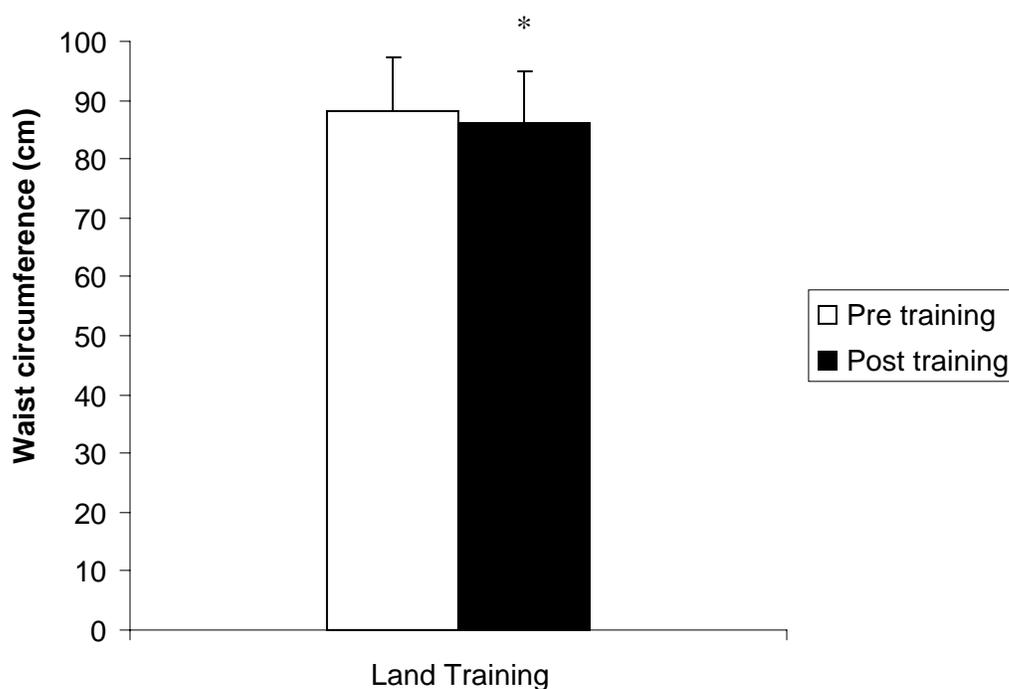


Figure 16. Waist circumference pre to post land-based exercise training (combined LBE and LBE+RT) in overweight and obese women (n = 19).

* $p < 0.05$ significant difference pre to post training

4.2.2.7 Lipid Profile

Between group analyses demonstrated no evidence of a difference between LBE and LBE+RT for HDL, LDL, TAG, or total cholesterol ($p > 0.05$, Table 21).

Consequently, LBE and LBE+RT groups were combined to determine the overall effect of land-based training across time on the lipid profile. There were no significant changes in HDL (1.34 ± 0.20 to 1.31 ± 0.22 mmol/L, $p = 0.38$), LDL (3.10 ± 0.73 to 3.20 ± 0.72 mmol/L, $p = 0.37$), TAG (1.17 ± 0.58 to 1.08 ± 0.33 mmol/L, $p = 0.28$) or total cholesterol (4.97 ± 0.75 to 5.00 ± 0.72 mmol/L, $p = 0.85$).

Table 21. Lipid profile pre and post land-based exercise training for land-based endurance (LBE) and land-based endurance combined with resistance training (LBE+RT) groups

	LBE (n = 9)			LBE+RT (n = 10)			p value
	Pre	Post	Chg (%)	Pre	Post	Chg (%)	
HDL (mmol/L)	1.33 ± 0.28	1.31 ± 0.27	-1.5	1.34 ± 0.12	1.30 ± 0.17	-3.0	0.79
LDL (mmol/L)	2.88 ± 0.89	3.08 ± 0.71	6.9	3.30 ± 0.53	3.30 ± 0.75	0.0	0.62
TAG (mmol/L)	1.32 ± 0.73	1.07 ± 0.41	-18.9	1.03 ± 0.40	1.08 ± 0.27	4.9	0.10
Total Cholesterol (mmol/L)	4.82 ± 0.94	4.89 ± 0.67	1.5	5.11 ± 0.55	5.10 ± 0.78	-0.2	0.97

Data are presented as mean ± SD

p value column indicates difference between groups

Note. Chg (%) = percentage change, HDL = high density lipoprotein, LDL = low density lipoprotein, TAG = triglyceride

4.2.2.8 Correlation Analyses

Data for LBE and LBE+RT groups were combined to investigate relationships between fat oxidation measures and outcome variables following land-based exercise training overall. Significant positive correlations were evident between exercise fat oxidation rate and changes in both basal FFA concentration ($r = 0.71$, $p < 0.01$) and glycerol concentration ($r = 0.50$, $p = 0.03$) (Table 22). Partial correlation was used to explore the relationship between change scores for exercise fat oxidation rate with both changes in basal FFA and glycerol concentrations, while controlling for change scores for total body FM, trunk FM and BMI. There remained a significant positive correlation between exercise fat oxidation rate and basal FFA, controlling for total body FM ($r = 0.69$, $p < 0.01$), trunk FM ($r = 0.63$, $p < 0.01$) and BMI ($r = 0.67$, $p < 0.01$). This suggests that controlling for total body FM, trunk FM or BMI had very little effect on the strength of the relationship between these two variables. However, the positive significant relationship was no longer evident between exercise fat oxidation rate and basal glycerol, when controlling for total body FM ($r = 0.45$, $p = 0.07$), trunk FM ($r = 0.33$, $p = 0.18$) or BMI ($r = 0.44$, $p = 0.08$). It appears that changes in body composition, including total body and trunk fat, and BMI had an effect on the relationship between changes in exercise fat oxidation rate and basal glycerol levels following land-based training.

There was no relationship between changes in RMR with DXA data ($p > 0.05$) (Table 22).

Combined data were also used to investigate relationships between DXA data with anthropometric and blood variables following land-based exercise training. Significant positive associations were identified between change in BMI and changes in total body fat percentage ($r = 0.61$, $p < 0.01$), trunk fat percentage ($r = 0.55$, $p = 0.02$), total body FM ($r = 0.88$, $p < 0.01$) and trunk FM ($r = 0.79$, $p < 0.01$) (Table 23). The change in total body FM was positively correlated with changes in hip circumference following land based training ($r = 0.62$, $p < 0.01$) (Table 23). Both total body and trunk FM demonstrated a similar significant positive association with HDL ($r = 0.53$, $p = 0.02$) (Table 23). Changes in trunk FM were positively related to changes in basal levels of

FFA ($r = 0.50$, $p = 0.04$) and glycerol ($r = 0.55$, $p = 0.02$); however, total body FM was not significantly associated with these variables ($p > 0.05$) (Table 23).

Table 22. Pearson correlation coefficients (r) and significance for change scores in exercise and resting fat oxidation rate and resting metabolic rate, with change scores in cardiovascular fitness, body composition, lipid profile, and FFA and glycerol concentrations following land-based exercise training (LBE plus LBE+RT).

	Exercise Fat Oxidation Rate (mg/min)	Resting Fat Oxidation Rate (mg/min)	Resting Metabolic Rate (kcal/day)
	Pearson r	Pearson r	Pearson r
n = 19			
TMW VO _{2peak} (L/min)	-0.38	-0.14	N/A
Body Mass Index (kg/m ²)	0.33	-0.28	N/A
Waist (cm)	0.40	-0.36	N/A
Total fat (%)	0.28	0.07	0.16
Total FM (kg)	0.26	-0.14	-0.04
Total FFM (kg)	-0.04	-0.40	-0.41
Trunk fat (%)	0.37	0.23	0.29
Trunk FM (kg)	0.46	-0.05	0.13
Trunk FFM (kg)	0.08	-0.46	-0.33
HDL (mmol/L)	0.05	-0.18	N/A
LDL (mmol/L)	0.04	-0.06	N/A
TAG (mmol/L)	-0.01	0.08	N/A
TC (mmol/L)	0.04	-0.07	N/A
n = 18			
ΔFFA (μmol/L)	0.35	0.27	N/A
ΔGlycerol (μmol/L)	0.46	0.40	N/A
Basal FFA (μmol/L)	0.71**	-0.28	N/A
Basal Glycerol (μmol/L)	0.50*	-0.35	N/A

* p < 0.05, ** p < 0.01; *Note.* FM = fat mass, FFM = fat free mass, HDL = high density lipoprotein, LDL = low density lipoprotein, TAG = triacylglycerol, TC = total cholesterol, FFA = free fatty acid, N/A = not applicable for that measure

Table 23. Pearson correlation coefficients (r) and significance for change scores in total body and trunk DXA data with anthropometric data, lipid profile, and FFA and glycerol data, following land-based exercise training (LBE plus LBE+RT).

	Total fat (%)	Total FM (kg)	Total FFM (kg)	Trunk fat (%)	Trunk FM (kg)	Trunk FFM (kg)
	Pearson r	Pearson r	Pearson r	Pearson r	Pearson r	Pearson r
n = 19						
BMI (kg/m ²)	0.61**	0.88**	0.29	0.55*	0.79**	0.33
Waist (cm)	0.14	0.31	0.32	0.14	0.40	0.43
Hip (cm)	0.31	0.62**	0.44	0.20	0.44	0.39
WHR	0.01	0.02	0.08	0.09	0.24	0.26
HDL (mmol/L)	0.34	0.53*	0.20	0.33	0.53*	0.22
LDL (mmol/L)	0.17	0.28	0.07	0.22	0.31	0.09
TAG (mmol/L)	-0.28	-0.26	0.30	-0.13	-0.06	0.16
TC (mmol/L)	0.16	0.31	0.20	0.24	0.39	0.18
n = 18						
Δ FFA (μmol/L)	-0.02	-0.06	-0.13	0.03	0.11	0.09
Δ Glycerol (μmol/L)	-0.06	-0.13	-0.18	0.01	-0.03	-0.15
Basal FFA (μmol/L)	0.17	0.29	0.24	0.30	0.50*	0.30
Basal Glycerol (μmol/L)	0.25	0.38	0.27	0.36	0.55*	0.34

* p < 0.05, ** p < 0.01

Note. BMI = body mass index, WHR = waist to hip ratio, FFM = fat free mass, HDL = high density lipoprotein, LDL = low density lipoprotein, TAG = triacylglycerol, FFA = free fatty acid

4.2.3 Standardisation of Testing Procedures

Of the 19 participants who completed the land-based exercise training intervention, eight women were still menstruating. These participants were tested during the follicular phase of their menstrual cycle, on average at day six, for both the 30 minute TMW and RMR test.

All participants completed the 24 hour diet record and reported that they refrained from eating at least four hours prior to the 30 minute TMW session. Nutrient composition was again maintained during the 24 hours leading up to the 30 minute TMW session between pre and post intervention for fat (14 ± 6 v 14 ± 5 %), CHO (60 ± 8 v 61 ± 7 %) and protein (26 ± 6 v 26 ± 6 %) respectively. The 30 minute TMW session post test was undertaken at the same time of day (± 1 hour) of each participant's pre test session.

4.2 Discussion

According to the literature, increases in exercise fat oxidation following land-based exercise training in overweight or obese individuals remains inconclusive. Therefore, the purpose of this study was to determine whether fat oxidation can be increased following a land-based exercise training programme similar to that performed in the aquatic-based exercise training study (Chapter 3). Specifically, comparison of a land-based endurance (LBE) programme to a land-based endurance combined with resistance training (LBE+RT) programme was investigated in overweight and obese women.

4.2.1 Metabolism and Substrate Utilisation

The significant increase in exercise fat oxidation rate exhibited in overweight and obese women in the present study following land-based exercise training overall is consistent with results exhibited in women of normal body weight (Friedlander et al., 1998; Horowitz et al., 2000; Sial et al., 1998). Therefore, the outcome from the present investigation suggests that excess adiposity does not prevent training induced increases in fat oxidation during exercise.

The results of the present investigation are also comparable to studies of overweight and obese individuals demonstrating increased exercise fat oxidation rate following low intensity, land-based endurance training (Dumortier et al., 2003; van Aggel-Leijssen et al., 2001; van Aggel-Leijssen et al., 2002). The 20% increase in the rate of exercise fat oxidation in the present study is similar to the 19% increase in relative fat oxidation, observed in obese women following land based endurance training at 40% $\dot{V}O_{2max}$, three times per week for 12 weeks (van Aggel-Leijssen et al., 2001). Alternatively, Dumortier and colleagues (2003) reported a much greater increase in the rate of exercise fat oxidation (52%) in obese men and women who trained at an individualised intensity of maximal fat oxidation. Conversely, the increase in exercise fat oxidation in the current investigation is in contrast to the study by Kanaley and colleagues (2001), who failed to demonstrate an increase in exercise fat oxidation in obese women following 16 weeks of endurance training. The lack of change in exercise

fat oxidation rate in the study by Kanaley et al (2001) could be explained by the higher training exercise intensity ($70\% \dot{V}O_{2\text{peak}}$), compared to the lower intensity exercise performed in the current investigation ($53\% \dot{V}O_{2\text{peak}}$). Therefore, these data suggest that low, and not high intensity exercise is recommended for land-based training, with individualised prescription at the maximal fat oxidation point increasing fat oxidation to a greater extent than generic exercise prescription.

It was hypothesised that the combination of LBE+RT would result in a greater increase in exercise fat oxidation than LBE in overweight and obese women; however, this hypothesis was disproven. Both LBE and LBE+RT groups demonstrated similar increases in exercise fat oxidation following respective training programmes, indicating that increases in fat oxidation can occur regardless of exercise structure. Reasons exist as to why both training groups demonstrated similar responses. To ensure similar exercise intensity and EE between land-based exercise training sessions, LBE and LBE+RT were prescribed at the same relative percentage of HR_{peak} and at equivalent duration. Furthermore, early investigations estimate the energy cost of combined endurance and resistance training to be approximately 6 kcal/min, similar to the oxygen consumption of walking on a flat surface (Gettman & Pollock, 1981). Therefore, the LBE and LBE+RT groups trained at the same exercise intensity and EE, and any difference in fat oxidation changes between the two training session structures would have been due to addition of resistance exercise to an endurance based programme. The equivalent exercise training intensity may have been the predominant influence resulting in equivalent increases in exercise fat oxidation between LBE and LBE+RT, rather than the type of land-based exercise prescription.

As no studies to date have investigated exercise fat oxidation following land-based endurance training compared to a combination of land-based endurance and resistance training in overweight or obese individuals, the lack of difference between LBE and LBE+RT in the present study cannot be compared to previous research. Independent exercise effects of endurance and resistance training may be used to highlight effects of combined endurance and resistance training in the current population. While low intensity endurance training demonstrates increases in fat oxidation rate (van Aggel-Leijssen et al., 2002), equivocal results exist for substrate utilisation following

chronic resistance training (Ballor et al., 1996; Treuth et al., 1995). However, in the current investigation when lower intensity resistance training is combined with endurance based exercise, the metabolic stimulus favors fat oxidation, similar to that seen with endurance training.

As fat oxidation is dependent on exercise intensity (De Feo et al., 2003; Jeukendrup & Wallis, 2005), it may be possible that changes in fat oxidation could be influenced by the differing intensity and EE demonstrated in the LBE+RT group during the exercise metabolism measurement pre to post intervention. Despite prescribing and achieving equivalent intensity based on HR and %HR_{peak}; $\dot{V}O_2$, % $\dot{V}O_{2peak}$ and EE were higher in the post intervention test compared to pre test for the LBE+RT group. Although there was variation in testing intensity, LBE and LBE+RT groups exhibited similar increases in exercise fat oxidation rate, and increases in the proportion of fat contributing to EE.

Despite a training induced shift in fat oxidation in the present investigation, there was no change in lipolysis, indicated by changes in plasma FFA and glycerol concentrations, following land based exercise. The lack of a significant change in exercise lipolysis following land-based exercise training, even with a significant increase in fat oxidation, is in agreement with studies conducted in normal weight individuals (Sial et al., 1998; Talanian, Galloway, Heigenhauser, Bonen, & Spriet, 2007). As a similar rate of lipolysis was evident pre and post training, the increase in fat oxidation following land based exercise training may be related to an increased percentage of released FFA oxidised, a greater contribution from IMTG stores, or both (Klein et al., 1994). Furthermore, the increase in fat oxidation may be a result of adaptive changes within skeletal muscle itself (Sial et al., 1998). Training induced adaptations to skeletal muscle include; an increase in capillarisation, increases in oxidative and mitochondrial enzymes and increases in mitochondria size and number (Saltin & Astrand, 1993; Sial et al., 1998). There have also been reports of increased fatty acid transporters, resulting in increased capacity to oxidise fatty acids (Saltin & Astrand, 1993; Sial et al., 1998). The results from the present investigation are in accordance with Sial et al (1998), and Klein, Coyle and Wolfe (1994), who established that overweight and obese women can increase exercise fat oxidation rates following land-based exercise training, without a significant

change in lipolysis. Therefore, it appears fat oxidation may be increased in overweight and obese women without any significant alterations in plasma FFA and glycerol concentrations.

The concentrations of plasma glycerol and FFA were also measured in a basal state. Basal plasma FFA concentrations were similar at baseline and following land based training in the current investigation, in agreement with previous cross sectional data (Klein et al., 1994). The availability of FFA in plasma is important to maintain delivery of FFA to working muscle for oxidation (Spriet, 2002). However, the correct balance between FFA availability and oxidation is required, as a continuously high level of plasma FFA in a resting state can lead to metabolic complications associated with obesity (Coppack et al., 1994; Horowitz, 2001; Phillips, Caddy et al., 1996). The combined group analysis in the present study verified a positive association between changes in basal FFA and changes in exercise fat oxidation rate. This positive association indicates that in the current investigation an increase in exercise fat oxidation rate was related to a corresponding increase in basal FFA concentration. Available FFA's are taken up from the bloodstream to be oxidised, facilitating lipid turnover and may prevent metabolic complications from occurring (Coppack et al., 1994; Horowitz, 2001; Phillips, Caddy et al., 1996). In the present study this relationship existed independent of changes in body composition.

In the present investigation however, body composition, and body fat distribution, affected the relationship between changes in basal plasma glycerol concentration and exercise fat oxidation rate. When centrally located trunk fat was measured by DXA, the influence of body fat distribution negated the positive association between changes in exercise fat oxidation rate and basal glycerol levels. In support of this observation, past investigations indicate that body fat distribution is known to influence basal levels of glycerol, FFA and fat oxidation (Jensen, 2008; Kanaley, Cryer, & Jensen, 1993; Nicklas, Goldberg, Bunyard, & Poehlman, 1995; van Aggel-Leijssen et al., 2001).

Trunk fat and basal FFA and glycerol concentrations were also positively correlated in the current investigation. This positive association between trunk fat and basal FFA and glycerol concentrations further support the observation of body fat distribution and its effects on fat metabolism. Body fat from the abdominal region is

recognized as being more lipolytic than adipose tissue from the gluteal-femoral regions in women (Jensen et al., 1989; van Aggel-Leijssen et al., 2001). The greater proportion of centrally located fat in the trunk region therefore, leads to overall greater resting FFA mobilisation and higher concentrations of circulating basal FFA and glycerol levels (Coppack et al., 1994; Jensen et al., 1989). The higher concentration of FFA in the blood, if not oxidised, can result in metabolic abnormalities associated with obesity (Coppack et al., 1994; Horowitz, 2001; Phillips, Caddy et al., 1996). Despite displaying a relationship between higher trunk fat mass and basal FFA concentrations, the overweight and obese women in the current investigation were able to achieve significant increases in exercise fat oxidation rate following land-based training, perhaps compensating for this impairment. This was further supported by the aforementioned relationship identified between basal FFA concentration and exercise fat oxidation rate.

Although training induced alterations in exercise fat oxidation were found in overweight and obese women in the present study, exercise training did not affect resting metabolic measures. The lack of change in RMR in the current investigation is comparable to that of Schulz et al. (1991), and Wilmore et al. (1998), who also reported no effect of land-based endurance training on RMR in individuals of normal weight. Conversely, in obese individuals, RMR can increase following land-based endurance training (Tremblay, 1986). In another investigation, RMR increased following combined endurance and resistance exercise in overweight women, even with weight loss (Svendsen et al., 1993). In the study by Svendsen and colleagues (1993) however, the 12 week exercise programme increased in intensity and duration, ending with a training duration of 90 minutes at above 70% $\dot{V}O_{2peak}$, three times per week. The greater intensity, duration and thus EE may have been more effective in eliciting increases in RMR (Svendsen et al., 1993), compared to the lower intensity and duration, and hence EE in the present investigation. Therefore, a threshold of exercise intensity may be necessary to increase RMR (Poehlman, 1989), and the low intensity training utilised in the present investigation for maximal fat oxidation may not be sufficient to induce changes in RMR.

Although RMR increased by 9% in the present investigation, this did not achieve statistical significance. However, two studies utilising smaller sample sizes than used

here, have significantly increased RMR following exercise training (Pratley et al., 1994; Ryan et al., 1995). Both studies utilised resistance training and 16 week interventions to effect an 8% increase in RMR in healthy men ($n = 13$) (Pratley et al., 1994), and a 4% increase in RMR in postmenopausal women ($n = 15$) (Ryan et al., 1995). The effectiveness of these studies may be related to the use of purely resistance training, and a significant increase in FFM (Pratley et al., 1994; Ryan et al., 1995). In the present investigation, the combination of endurance and resistance exercise did not result in a significant increase in total body FFM, despite decreases in FM and body fat %. Similar to the current investigation, Byrne and Wilmore (2001) failed to demonstrate a significant increase in RMR, following combined endurance and resistance training, despite a significant increase in FFM in obese women. Furthermore, there was no significant relationship between changes in FFM and RMR in the current investigation. Schmitz and colleagues suggest that even if increases in FFM are evident, the day to day variability in RMR may be too large to detect statistically significant changes (Schmitz et al., 2003).

The present investigation failed to demonstrate a significant increase in resting fat oxidation, despite a significant increase in exercise fat oxidation following land-based training. There are currently no other reports of changes in resting substrate utilisation following endurance combined with resistance land based exercise training. As there were no differences between LBE and LBE+RT groups for changes in resting measures of fat and CHO oxidation or RER, comparisons with endurance land-based training studies can be made. In agreement with the current investigation, Kanaley and colleagues (2001) reported no significant increase in resting fat oxidation in obese women following land based endurance training at 70% $\dot{V}O_{2max}$. Furthermore, van Aggel-Leijssen and colleagues (2001) failed to demonstrate any increase in resting fat oxidation, represented by RER, in obese women following low intensity training (40% $\dot{V}O_{2max}$). As in the present investigation, there was no increase in resting fat oxidation in the study by van Aggel-Leijssen et al (2001), despite a significant increase in exercise fat oxidation following training. However, Goodpaster et al (2003), found a significant 15% increase in resting fat oxidation following 16 weeks of endurance training (four to six times per week at 60-70% HR_{max} ,) in obese individuals. Although this was also accompanied by a significant loss of body weight, the authors determined that the

increase in resting fat oxidation was not associated with overall loss of adiposity, but with the exercise intervention itself (Goodpaster et al., 2003). Therefore, longer duration training interventions and more frequent training sessions may favour increases in resting fat oxidation in overweight and obese individuals.

4.2.2 Cardiovascular Fitness

The increase in exercise fat oxidation rate following land-based training may be related to the increase in CV fitness observed in the present investigation. Individuals with higher CV fitness display greater rates of exercise fat oxidation than sedentary individuals (Klein et al., 1994; Tremblay et al., 1992). Therefore, the land-based exercise training programme in the current study provided a sufficient stimulus to elicit an increase in CV fitness, with a concomitant increase in exercise fat oxidation. Participants in the present study demonstrated significant increases in exercise fat oxidation and CV fitness; findings which concur with previous investigations in overweight women (Dumortier et al., 2003; van Aggel-Leijssen et al., 2002). Overall, land-based exercise training prescribed at an intensity aimed to elicit increases in fat oxidation provided a sufficient stimulus to increase CV fitness and fat oxidation in these overweight and obese women. However, changes in CV fitness and exercise fat oxidation rate do not appear to be related in the current population as no association was found between the two.

Conversely, the improvement in CV fitness may have confounded the relationship with post training exercise fat oxidation. The exercise bout during post training was performed at the same absolute intensity as pre training, also a feature of previous exercise interventions (Sial et al., 1998). As participants increased their CV fitness, they were exercising at a lower intensity during the post test. This lower relative intensity may have resulted in higher fat oxidation as fat utilisation increases at lower exercise intensities (Brooks, 1997). Therefore, the increase in fat oxidation following land-based training may be due to the training induced physiological changes that occur within the muscle tissue (Saltin & Astrand, 1993; Sial et al., 1998) and/or an increase in CV fitness resulting in a lower relative intensity at an equivalent absolute intensity.

As increases in exercise fat oxidation were demonstrated to occur concomitantly with increases in CV fitness, successful exercise prescription at the correct intensity to

elicit increases in CV fitness may incorporate positive adaptations in both fitness and substrate utilisation.

Combined group analysis indicated that land-based exercise training at 70% HR_{peak} (53% $\dot{V}O_{2peak}$) elicited a significant 8% improvement in CV fitness. The efficacy of LBE and LBE+RT to demonstrate equivalent increases in CV fitness is in agreement with previous research on endurance combined with resistance training in overweight and obese individuals (Maiorana et al., 2002; Svendsen et al., 1993; Wallace et al., 1997). Furthermore, an equivalent exercise training intensity was prescribed in the current investigation indicating similar CV challenge and responses between LBE and LBE+RT.

Early investigations of resistance exercise demonstrate a 5% increase in $\dot{V}O_{2max}$, compared to 15% to 25% with endurance based programmes (Gettman & Pollock, 1981). Therefore, in the current investigation, the combination of endurance with resistance exercise allowed participants to challenge the CV system to a greater extent than demonstrated with resistance exercise only.

The typical training response in $\dot{V}O_{2max}$ is about 20% in sedentary adults, with less of an increase in athletes (Shephard, 1984). As physiological effects appear to be more pronounced in previously sedentary individuals (Reilly, Dowzer et al., 2003), the lower increase in $\dot{V}O_{2peak}$ demonstrated in overweight and obese women in the current investigation (8%) may be due to the non-sedentary nature of the participants prior to the beginning of the investigation. However, the average baseline CV fitness level of the participants in this study was still classified as 'below average' fitness with reference to age and sex as identified by the ACSM (ACSM, 2006). On completion of the intervention, CV fitness levels in this cohort fell within the 'average' description of fitness (ACSM, 2006). As a dose response relationship is apparent between exercise intensity and the magnitude of $\dot{V}O_{2max}$ improvement (Asikainen et al., 2002), a greater increase in CV fitness may have been observed if participants were instructed to train at a higher exercise intensity. Nevertheless, the results from the current investigation suggest that land-based exercise training at an intensity prescribed for maximal fat oxidation can provide sufficient stimulus to increase CV fitness.

4.2.3 Muscular Strength

Overall, when groups were combined, land-based training was not effective at increasing upper or lower body strength. The lack of response was similar between LBE and LBE+RT, with both training programmes unable to provide sufficient stimulus to increase chest, quadriceps or hamstring strength. The added resistance exercise to an endurance programme in the LBE+RT group should have lead to a greater increase in strength than the LBE, which consisted of walking only. The lack of a significant difference between LBE and LBE+RT groups is in contrast to previous studies in overweight individuals who demonstrate greater chest (Wallace et al., 1997), quadriceps (Pierson et al., 2001; Wallace et al., 1997), and hamstring strength (Pierson et al., 2001), following combined endurance and resistance training, compared to endurance training alone. The lack of change in lower body strength is also in contrast to Takeshima et al (2004), who demonstrated significant increases in quadriceps strength (9-52%) and hamstring strength (14-76%) in older adults undertaking concurrent endurance and resistance training. Although the LBE+RT group performed exercises that were targeted specifically for the upper and lower body, the mechanical stress placed on the body was insufficient to result in significantly greater strength increases compared to endurance training. The non-sedentary status of the women in the present investigation may have confounded the results for changes in upper and lower body strength. The aforementioned studies demonstrating significant improvements in muscular strength following combined endurance and resistance training were undertaken on sedentary, older, individuals (Pierson et al., 2001; Takeshima et al., 2004; Wallace et al., 1997). Higher intensity resistance training may have been required during LBE+RT to elicit an increase in upper and lower body strength over and above that of the LBE group.

4.2.4 Anthropometry

The DXA scans performed in the current investigation suggest that significantly different physiological adaptations in body composition occurred between LBE and LBE+RT groups. The addition of resistance exercise in LBE+RT resulted in a 2.3%

reduction in total body fat percentage and 3.2% reduction in trunk fat percentage, whereas following LBE training there was a slight increase in both percentage total body fat and trunk fat. This difference for total body and trunk fat percentage was significantly different between LBE and LBE+RT groups. These results are in agreement with earlier research demonstrating significant decreases in percentage body fat of -0.8% to -2.9%, with no change in total body weight following combined endurance and resistance training (Gettman & Pollock, 1981). A similar pattern was reported by Wallace and colleagues (1997), with a significantly greater decrease in body fat percentage following combined endurance and resistance training compared to solely endurance training, with no change in total body weight in obese men. Therefore, favorable alterations in body composition may occur following combined endurance and resistance training compared to endurance only training, despite no change in BMI.

Positive alterations in abdominal adiposity were demonstrated following land-based training with a significant reduction in WC (-1.7%), regardless of session structure. The lack of a significant difference between LBE and LBE+RT suggests that both types of land-based training were equally effective at decreasing metabolic risk associated with abdominal adiposity in overweight and obese women, despite no change in BMI (ACSM, 2006). This result is in agreement with Dumortier et al (2003), who demonstrated a significant reduction in WC in obese individuals following endurance exercise training prescribed at an intensity of individualised maximal fat oxidation. The greater reduction in WC of -4.2% observed in the study by Dumortier and colleagues (2003), might suggest individualised exercise prescription to target increases in exercise fat oxidation may further improve this marker of abdominal obesity. Combined group analyses also revealed reductions in hip circumference (-0.7%) and WHR (-1.0%), although these did not achieve significance. The lack of a significant change in WHR and BMI in the current investigation are similar to that of van Aggel-Leijssen et al (2001), and Wallace and colleagues (1997) who found no change in WHR or BMI in obese women following 12 weeks of low intensity endurance training or obese men following both endurance and combined endurance and resistance exercise training, respectively. Conversely, Maiorana et al (2002), did find a significant reduction in WHR (-1.3%), but no change in BMI in overweight individuals following combined endurance and resistance exercise

training. More importantly however, the significant decrease in WC in the current investigation may be more representative of positive alterations in abdominal fat distribution than WHR, as WC has been found to be significantly better at predicting regional fat distribution (Schneider et al., 2007; Taylor, Keil, Gold, Williams, & Goulding, 1998; Weerarathna, Lekamwasam, & Rodrigo, 2008). Waist circumference is also more strongly associated with CV risk factors for metabolic syndrome, dyslipidemia and Type 2 diabetes than WHR (Schneider et al., 2007).

4.2.5 Lipid profile

There were no alterations in HDL, LDL, TAG or TC following LBE or LBE+RT in the present investigation. These results are comparable to those from Maiorana et al (2002), who found no significant changes in lipid variables after combined endurance and resistance training in overweight individuals exercising at 70% HR_{peak} . Additionally, Dumortier and colleagues (2003) failed to exhibit significant changes in lipid profiles of obese subjects when training at the level of individualised maximal fat oxidation (Dumortier et al., 2003). Therefore, despite achieving increases in fat oxidation, changes in fat oxidation rate do not appear to influence the lipid profile. Conversely, endurance training at 55% $\dot{V}O_{2max}$ for 24 weeks (Lamarche et al., 1992) or 56 weeks (Despres et al., 1991) achieved positive alterations in lipid profiles in obese women who had borderline high baseline levels based on National Cholesterol Education Program standards (Despres et al., 1991; Lamarche et al., 1992; NCEP, 2001). The overweight and obese women in the present investigation had normal baseline lipid profiles (NCEP, 2001), thus a ceiling effect may have mitigated improvement in these measures (Santiago et al., 1995). Past research has suggested that lack of change in lipid profiles following exercise training, may be attributed to 'normal' baseline lipid profiles (Santiago et al., 1995). Consequently, positive changes in the lipid profile are more likely to occur when individuals display an unfavorable baseline level, as positive changes in the lipid profile are related to adverse initial levels (Ready et al., 1996), and may not be related to improvements in exercise fat oxidation rate.

The lack of a difference between LBE and LBE+RT for changes in the lipid profile is in contrast to that of Wallace and colleagues (1997), who found greater positive

changes in HDL and TAG concentrations following combined endurance and resistance training compared to endurance training in obese men. However, the volume of the combined endurance and resistance programme was twice that of the endurance training programme resulting in greater overall EE (Wallace et al., 1997). Consequently, a threshold of EE appears necessary to elicit increases in HDL and TAG (Wallace et al., 1997). An exercise EE of approximately 1200-2200 kcal/week has been suggested to increase HDL levels and decrease TAG levels (Durstine et al., 2001). The overall exercise EE in the present investigation (984 kcal/week) was insufficient to positively alter HDL, TAG levels, or any other measure of the lipid profile. It is most likely that exercise induced changes in the lipid profile are the result of the interaction between exercise intensity, frequency, duration of each training session and the length of the exercise period (Kokkinos & Fernhall, 1999). As well as being related to the baseline lipid concentration, it is possible that a threshold exists for training intensity, frequency, and duration to elicit positive changes in the lipid profile (Halbert et al., 1999). In the current investigation, it is apparent that the threshold required to induce change in normal lipid profiles was not met. Therefore, features of the current exercise training intervention prescribed to increase fat oxidation; including total EE, exercise intensity, frequency and duration of training, were not appropriate to result in positive alterations in lipid profile. In addition, a significant increase in exercise fat oxidation rate following training does not appear to positively alter plasma lipid levels in overweight or obese women.

4.2.6 Summary

The results of the present study indicate that overweight and obese women are capable of increasing exercise fat oxidation rate and increasing the contribution of fat toward exercise EE following land-based exercise training. As examined in this study, the overall effect of land-based training was an increase in exercise fat oxidation, irrespective of the addition of resistance training to endurance training, or change in FFA and glycerol concentration. This was further demonstrated by a reduction in RER, and an increase in fat contribution and decrease in CHO contribution toward exercise EE. No differences existed between LBE and LBE+RT groups, with positive overall effects

evident for CV fitness and WC. However, the addition of a resistance component resulted in significantly greater changes in percentage total body and trunk fat than endurance only training. Furthermore, body fat distribution appears to influence lipolysis and fat oxidation relationships in the cohort of women in the present study. No significant differences were demonstrated between training groups for changes in CHO oxidation, basal FFA and glycerol concentration, RMR measures, upper or lower body strength, BMI, hip circumference, WHR or lipid profile.

Chapter 5: Comparison between Aquatic and Land Based Exercise Training

5.1 Methods

5.1.1 Participants and Procedure

In order to make comparisons between aquatic-based and land-based exercise training for fat oxidation, only those participants who completed both the aquatic and land-based exercise interventions (Chapter 3 and 4) were used in analyses. Accordingly, results from a total of 17 participants were included in this comparative investigation.

Participants completed a 12 week aquatic-based exercise training intervention (Section 3.1.4) followed eight months later, by a 12 week land based exercise training intervention (Section 4.1.4). The washout period of eight months was considered adequate to reduce the likelihood of carry over effects from the aquatic exercise training intervention. Two months of washout has been reported to be sufficient time to induce significant detraining (Mujika & Padilla, 2001). Each participant was either assigned to the mode specific endurance training group (DWR and LBE, n = 9), or the combined endurance plus resistance training group (DWR+RT and LBE+RT, n = 8). Participants were originally randomised into training groups for the aquatic intervention, and continued with their randomisation by being reassigned to the same training group for the land-based intervention. Emphasis was placed on participants training in the same group for each exercise modality. Section 3.1 contains the methodology for the aquatic-based exercise training intervention. Section 4.1 contains the methodology for the land-based exercise training intervention.

5.1.2 Pre and Post Intervention Outcome Measures

Testing of fat oxidation and related measures were undertaken prior to and on completion of the aquatic and land-based exercise interventions. Pre and post intervention testing included measures of CV fitness, exercise and resting metabolism, muscular strength, anthropometry and lipid profiles. Each pre and post test was conducted in exactly the same manner during aquatic and land-based interventions.

Therefore, accurate comparisons of the training responses could be made between exercise modalities to determine the effectiveness of aquatic training compared to land-based training. Section 3.1.5 outlines pre and post intervention testing for the aquatic exercise intervention. Section 4.1.5 outlines pre and post intervention testing for the land-based exercise intervention.

5.1.3 Standardisation

Each participant undertook the mode specific 30 minute steady state exercise session measuring substrate utilisation (DWR and TMW) at the same time of the day to avoid diurnal variations that may alter substrate selection. Participants were asked to replicate their original 24 hour diet from aquatic intervention testing for land-based intervention testing to avoid possible alterations in substrate utilisation due to diet (Christensen & Hansen, 1939). Analysis of the 24 hour diet was undertaken with Diet Cruncher for Windows (version 1.6.0) to ensure equality in nutrient composition between aquatic and land-based exercise interventions.

5.1.4 Statistical Analysis

Group data were described using means and standard deviations (SD). Results were analysed using Statistics Package for Social Sciences (SPSS version 14.0, Chicago, Illinois). Analyses of exercise interventions were undertaken on combined group data i.e. DWR and DWR+RT (aquatic-based exercise training) compared to LBE and LBE+RT (land-based exercise training), so comparisons could be made between exercise modes on training responses for fat oxidation and related measures. Paired t-tests were conducted to compare pre intervention outcome variables for participants at the time of the aquatic-based exercise intervention, to the time of the land-based exercise intervention. Analysis of covariance was undertaken to compare the effectiveness of aquatic-based exercise training to land-based exercise training for metabolic and physiological training adaptations. Analysis of covariance was chosen to control for differences in baseline variables between aquatic and land-based exercise interventions, while comparing post intervention values for each modality of exercise training. Consequently, the most accurate comparison between aquatic and land-based exercise training on fat oxidation

and related adaptations in the same group of individuals can be made. The independent variable was the mode of intervention (Aquatic or Land) and the primary dependent variable was the post-intervention measure of exercise fat oxidation. The pre-intervention measure of exercise fat oxidation was used as the covariate in the analysis. Secondary dependent variables and covariates included other post-intervention measures and pre-intervention measures respectively. These consisted of; exercise CHO oxidation and RER; resting CHO oxidation, RER and RMR; basal and exercise plasma FFA and glycerol concentrations; $\dot{V}O_{2peak}$; chest, quadriceps and hamstring strength; weight, BMI, hip and waist circumference; total body and trunk fat mass, fat free mass and percent body fat; and total cholesterol, HDL, LDL and TAG. Participant identification was entered as a random factor to allow for comparison of paired individuals. Due to testing in differing physiological environments for $\dot{V}O_{2peak}$ and exercise metabolism, baseline scores could not accurately be controlled as differences may occur as a result of the environment in which the test was undertaken (Phillips, Legge, & Jones, 2008). Therefore, repeated measures analysis of variance (ANOVA) with two factors of Aquatic-Land and Pre-Post was undertaken to compare aquatic and land-based training responses for $\dot{V}O_{2peak}$, exercise fat and CHO oxidation rates, exercise RER, contribution of fat and CHO to exercise EE, and plasma FFA and glycerol concentration changes during exercise, allowing for potential differences in baseline scores due to the effects of the testing environment. A Students paired t-test was undertaken within aquatic and land-based training, pre to post intervention on all variables. Statistical significance for all tests was set at $p < 0.05$.

Preliminary checks were conducted to ensure that there was no violation of the assumptions of normality, linearity, homogeneity of variances and regression slopes. Residuals were examined for normality using histograms. If a large studentised residual was identified, this value was removed and analyses re-run. If the re-run analysis reached the same statistical conclusion, the original data set was used for analysis. Linearity and the associations between pre and post test scores were visually assessed using scatter plots. Levene's test of equality of error variances was used to check homogeneity of variances.

5.2 Results

5.2.1 Pre Exercise Training Data

5.2.1.1 Participant Characteristics

Seventeen participants completed both the aquatic and land-based exercise training interventions. From these 17 participants, nine completed the endurance component of training (DWR and LBE), and eight completed combined endurance and resistance training (DWR+RT and LBE+RT). A flow diagram depicting movement of participants through both the aquatic and land-based intervention is depicted in Figure 17. Baseline variables of participants who completed both the aquatic and land-based exercise interventions are displayed in Table 24. Age, mode specific CV fitness ($\dot{V}O_{2peak}$ ml/kg/min), WC, WHR, and hamstring strength variables were significantly different at baseline between aquatic and land-based exercise training interventions ($p < 0.05$). All remaining baseline variables were comparable prior to aquatic and land-based exercise training; indicating an adequate wash out period following aquatic exercise training, and/or similarity between responses during aquatic and land-based exercise environments.

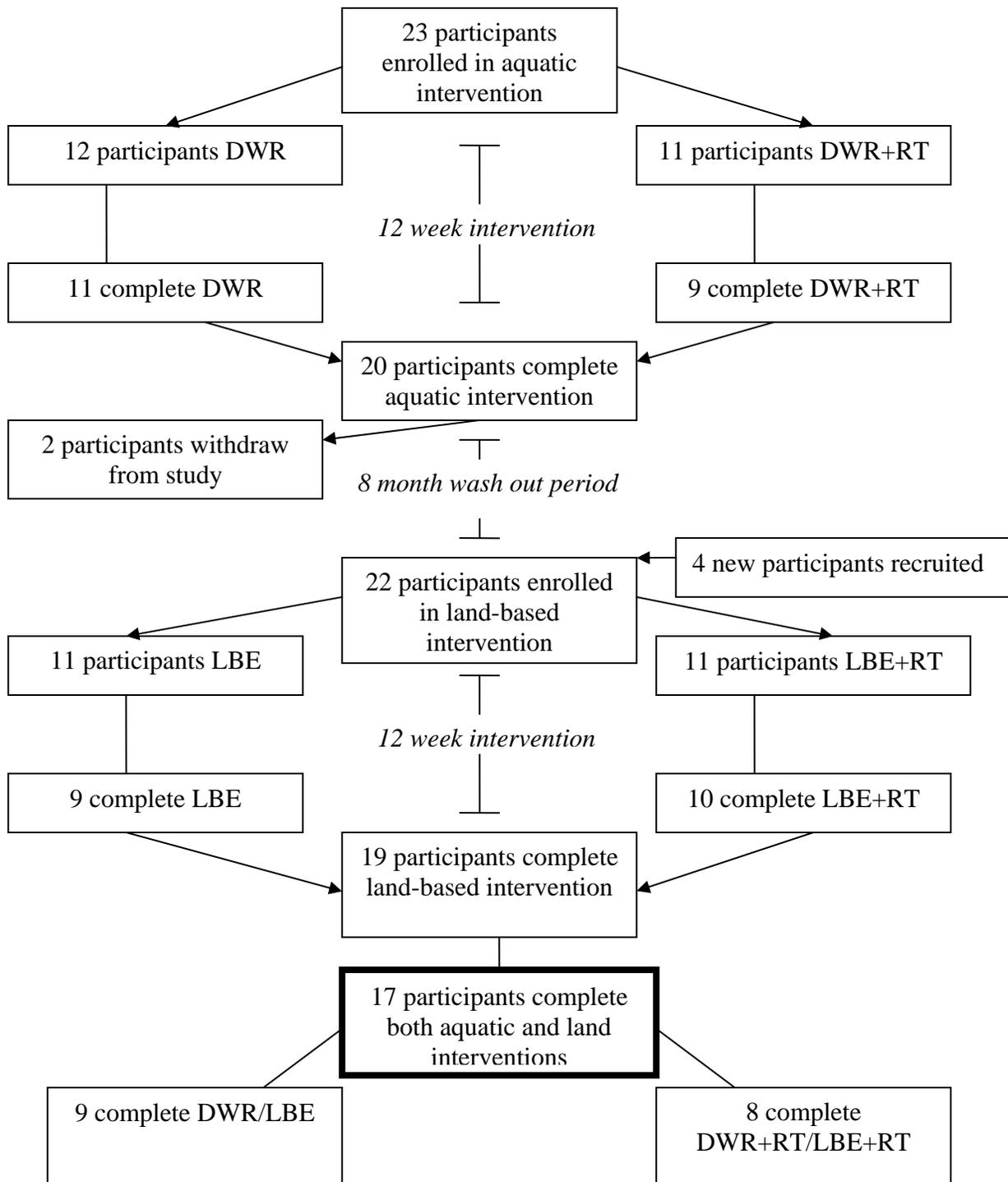


Figure 17. Flow diagram demonstrating the number of participants who completed both aquatic and land-based exercise interventions.

Table 24. Pre intervention outcome measures for female participants prior to aquatic-based exercise training and land-based exercise training interventions

	Aquatic (n = 17)	Land (n = 17)	p value
Age (years)	48 ± 7	49 ± 7	< 0.01**
Mode specific $\dot{V}O_{2peak}$ (ml/kg/min)	22.15 ± 4.89	27.49 ± 5.32	< 0.01**
Exercise fat oxidation rate (mg/min)	264.56 ± 109.45	309.87 ± 90.86	0.09
Exercise CHO oxidation rate (mg/min)	371.24 ± 257.05	411.42 ± 212.08	0.57
Exercise respiratory exchange ratio	0.80 ± 0.07	0.80 ± 0.04	0.76
% exercise fat oxidation	63 ± 24	63 ± 16	0.95
% exercise CHO oxidation	37 ± 24	37 ± 16	0.95
Δ FFA concentration (μ mol/L)	180 ± 221	324 ± 144	0.07
Δ Glycerol concentration (μ mol/L)	55 ± 52	84 ± 50	0.21
Basal FFA concentration (μ mol/L)	539 ± 257	418 ± 211	0.23
Basal glycerol concentration (μ mol/L)	125 ± 56	115 ± 35	0.56
Resting metabolic rate (kcal/day)	1416 ± 312	1359 ± 317	0.52
Resting fat oxidation rate (mg/min)	65.75 ± 17.87	64.16 ± 21.74	0.80
Resting CHO oxidation rate (mg/min)	95.72 ± 39.41	89.29 ± 57.89	0.61
Resting respiratory exchange ratio	0.81 ± 0.04	0.80 ± 0.05	0.64
% resting fat oxidation	61 ± 13	64 ± 19	0.59
% resting CHO oxidation	39 ± 13	36 ± 19	0.59
Chest strength (Nm)	198 ± 59	207 ± 42	0.32
Quadriceps strength (Nm)	101 ± 30	106 ± 29	0.20
Hamstring strength (Nm)	66 ± 20	72 ± 17	0.04*
Weight (kg)	77.6 ± 10.3	78.1 ± 11.2	0.45

Body mass index (kg/m ²)	29.5 ± 3.5	29.7 ± 4.0	0.61
Waist circumference (cm)	90.9 ± 8.5	88.0 ± 9.8	< 0.01**
Hip circumference (cm)	109.6 ± 9.1	109.9 ± 10.2	0.66
Waist to hip ratio	0.83 ± 0.05	0.80 ± 0.05	< 0.01**
Total body fat percentage	44 ± 5	44 ± 6	0.87
Total fat mass (kg)	34.0 ± 7.5	34.2 ± 8.7	0.74
Total fat free mass (kg)	40.2 ± 4.8	40.4 ± 4.9	0.42
Trunk fat percentage	44 ± 5	44 ± 6	0.88
Trunk fat mass (kg)	16.6 ± 4.0	17.0 ± 4.8	0.36
Trunk fat free mass (kg)	20.0 ± 2.6	20.3 ± 2.7	0.06
HDL (mmol/L)	1.34 ± 0.23	1.35 ± 0.21	0.79
LDL (mmol/L)	3.22 ± 0.79	3.09 ± 0.77	0.10
TAG (mmol/L)	1.08 ± 0.71	1.16 ± 0.61	0.26
Total cholesterol (mmol/L)	5.09 ± 0.77	4.97 ± 0.80	0.34

Data are presented as mean ± SD

* p < 0.05 **p < 0.01 significant difference between baseline measures prior to aquatic and land-based training

Note. $\dot{V}O_{2peak}$ = peak oxygen consumption, CHO = carbohydrate, HDL = high density lipoprotein, LDL = low density lipoprotein, TAG = triacylglycerol

5.2.2 Exercise Training Data

5.2.2.1 Training Intensity and Energy Expenditure

To allow for comparison between exercise training modes, relative training intensity was kept consistent between aquatic and land-based interventions for %HR_{peak} and estimated % $\dot{V}O_{2peak}$ ($p > 0.05$, Table 25). This was based upon the mode specific maximal exercise test carried out for each modality (DWR test for aquatic-based exercise intervention, and TMW test for land-based exercise intervention). However, when expressed as absolute values, there were significant differences during aquatic and land-based exercise training for HR ($p < 0.05$), and estimated mode specific absolute and relative oxygen consumption ($p < 0.001$) (Table 25). Weekly estimated EE was also significantly different between aquatic and land-based exercise interventions ($p < 0.05$) (Table 25). When using RPE as an alternate gauge of exercise intensity, participants reported an RPE of 11 during both aquatic and land-based interventions. Attendance to aquatic-based exercise training was 100%, with 98% attendance during land-based exercise training.

Table 25. Mode specific exercise training intensity during aquatic and land-based exercise interventions

	Aquatic (n = 17)	Land (n = 17)	p value
%HR _{peak}	73 ± 8	71 ± 3	0.24
% $\dot{V}O_{2peak}$	53 ± 15	54 ± 8	0.75
HR beats/min	114 ± 14	121 ± 8	0.04*
$\dot{V}O_2$ (L/min)	0.86 ± 0.22	1.14 ± 0.22	< 0.01**
$\dot{V}O_2$ (ml/kg/min)	11.1 ± 2.8	14.6 ± 2.4	< 0.01**
EE (kcal/week)	744 ± 204	989 ± 186	< 0.01**
EE (kJ/week)	3114 ± 854	4140 ± 779	< 0.01**

Data are presented as mean ± SD

* p < 0.05 significant difference between aquatic and land-based training

**p < 0.01 significant difference between aquatic and land-based training

Note. %HR_{peak} = percentage of heart rate peak, % $\dot{V}O_{2peak}$ = percentage of peak maximal oxygen consumption, $\dot{V}O_2$ = rate of oxygen consumption, HR = heart rate, EE = energy expenditure

5.2.2.2 Pre and Post Intervention Outcome Measures

5.2.2.3 Cardiovascular Fitness

There was no evidence of a difference in the effectiveness of aquatic and land-based training to elicit changes in relative peak oxygen consumption ($\dot{V}O_{2\text{peak}}$) as demonstrated by ANOVA ($p = 0.15$). However, when analysed independently, a significant increase in mode specific $\dot{V}O_{2\text{peak}}$ following land-based training (27.5 ± 5.3 to 29.9 ± 5.5 ml/kg/min; $p = 0.001$) was demonstrated, with no significant increase following aquatic exercise training (22.2 ± 4.9 to 22.4 ± 4.2 ml/kg/min; $p = 0.79$). The difference in the changes in $\dot{V}O_{2\text{peak}}$ between aquatic and land-based training was not great enough to demonstrate significant variation between the two training modes in their ability to alter CV fitness.

5.2.2.4 Metabolism and Substrate Utilisation

In the original interventions participant's performed 30 minutes of mode specific steady state exercise pre and post intervention, in which measurements of metabolism and substrate utilisation were undertaken. Participants were instructed to complete each 30 minute exercise test at 60% of pre intervention HR_{peak} . Paired t testing within exercise modes confirmed that exercise was undertaken at the same HR, % HR_{peak} , $\dot{V}O_2$ and total EE at pre and post testing for both aquatic and land-based exercise interventions ($p > 0.05$) (Table 26). Despite achieving comparable HR, % HR_{peak} , $\dot{V}O_2$ and total EE; % $\dot{V}O_{2\text{peak}}$ was significantly higher during the post intervention land based test ($p < 0.05$) (Table 26).

Table 26. Exercise intensity and energy expenditure during 30 minutes of steady state mode specific exercise pre and post aquatic and-land based interventions

	Aquatic (n = 17)		P value	Land (n = 17)		P value
	Pre	Post		Pre	Post	
HR beats/min	100 ± 9	100 ± 10	0.80	104 ± 6	104 ± 6	0.29
%HR _{peak}	64 ± 4	64 ± 4	0.67	61 ± 1	61 ± 1	0.29
$\dot{V}O_2$ (ml/kg/min)	10.4 ± 2.7	10.7 ± 2.5	0.31	12.0 ± 2.6	12.8 ± 2.4	0.08
$\dot{V}O_2$ (L/min)	0.81 ± 0.23	0.82 ± 0.23	0.56	0.93 ± 0.20	0.99 ± 0.21	0.07
% $\dot{V}O_{2peak}$	49 ± 12	50 ± 11	0.55	44 ± 6	47 ± 9	0.04*
Total EE (kJ)	485 ± 137	493 ± 142	0.55	557 ± 122	588 ± 127	0.10

Data are presented as mean ± SD

* p < 0.05 Significant difference pre to post training

Note. %HR_{peak} = percentage of heart rate peak, % $\dot{V}O_{2peak}$ = percentage of peak maximal oxygen consumption, $\dot{V}O_2$ = rate of oxygen consumption, HR = heart rate, EE = energy expenditure

In the study participants, aquatic and land-based exercise training elicited significantly different responses for changes in fat oxidation during exercise ($p = 0.03$, Figure 18). There was a significant increase in exercise fat oxidation rate following land-based training ($p < 0.01$) however, no change was observed following aquatic-based exercise training ($p = 0.97$).

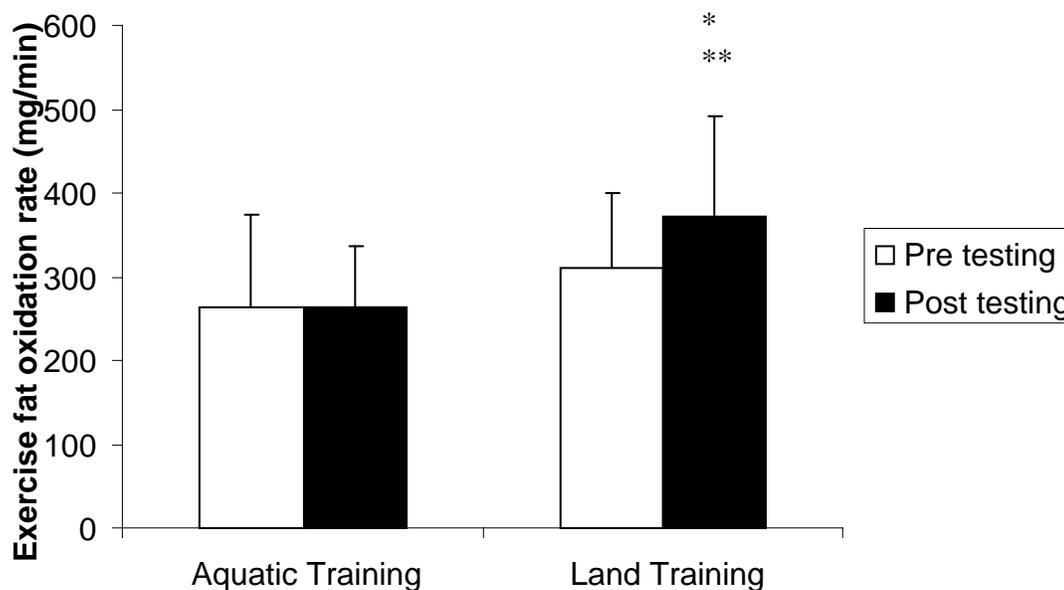


Figure 18. Exercise fat oxidation rate pre and post aquatic and land-based exercise training in overweight and obese women ($n = 17$).

* Indicates significant difference between training modes ($p < 0.05$)

** Indicates significant difference within training mode ($p < 0.01$)

Alternatively, there were no differences between aquatic and land-based training modes for exercise CHO oxidation rate ($p > 0.05$), with no significant change following either aquatic or land training ($p > 0.05$) (Table 27).

Similarly, there was no evidence of a difference between aquatic and land-based exercise training pertaining to exercise RER ($p > 0.05$) (Table 27). However, a significant reduction in exercise RER following land based exercise training ($p = 0.03$),

indicating greater fat oxidation rate. No significant change in exercise RER following aquatic-based exercise training was observed ($p > 0.05$) (Table 27).

The pattern observed for exercise RER was also apparent when expressed as the contribution of fat and CHO to total EE during the steady state mode specific exercise session. There were no differences between aquatic and land-based training for percentage contribution of fat, and CHO to total EE ($p > 0.05$) (Table 27). However, a significant increase in the contribution of fat toward exercise EE, and a corresponding decrease in CHO of 10% was noted following land-based exercise training ($p = 0.04$) (Table 27). Conversely, there was no significant change in fat or CHO contribution to EE following aquatic-based exercise training ($p = 0.92$) (Table 27).

There was no difference in follow up measures of RMR between aquatic and land-based training ($p > 0.05$) (Table 27). There were also no significant differences between aquatic and land-based training for post intervention resting fat oxidation rate, resting CHO oxidation rate, resting RER or contributions of fat and CHO during the RMR measurement ($p > 0.05$) (Table 27). Furthermore, neither aquatic nor land-based exercise training altered resting metabolism measures. Paired t-testing demonstrated no significant change from pre to post intervention for RMR, resting fat oxidation rate, resting CHO oxidation rate, resting RER, or the contribution of fat or CHO during the RMR measurement following either mode of training ($p > 0.05$).

Table 27. Exercise and resting metabolic measures pre and post aquatic-based exercise training and land-based exercise training

Variable	Aquatic (n = 17)			Land (n = 17)			p value
	Pre	Post	Chg (%)	Pre	Post	Chg (%)	
Exercise CHO oxidation rate (mg/min)	371 ± 257	390 ± 222	4.9	411 ± 212	320 ± 197	-22	0.16
Exercise RER	0.80 ± 0.07	0.80 ± 0.04	-0.1	0.80 ± 0.04	0.77 ± 0.04*	-3.8	0.15
% exercise fat oxidation	63 ± 24	62 ± 16	-1.0	63 ± 16	73 ± 16*	10.0	0.11
% exercise CHO oxidation	37 ± 24	38 ± 16	1.0	37 ± 16	27 ± 16*	-10.0	0.11
RMR (kcal/day)	1416 ± 312	1352 ± 221	-4.5	1359 ± 317	1476 ± 355	8.6	0.15
Resting fat oxidation rate (mg/min)	66 ± 18	61 ± 21	-7.6	64 ± 22	70 ± 26	9.4	0.22
Resting CHO oxidation rate (mg/min)	96 ± 39	97 ± 49	1.0	89 ± 58	95 ± 48	6.7	0.92
Resting RER	0.81 ± 0.04	0.81 ± 0.06	0.9	0.80 ± 0.05	0.80 ± 0.44	0.5	0.44
% resting fat oxidation	61 ± 13	59 ± 20	-2.0	64 ± 19	62 ± 16	-2.0	0.47
% exercise CHO oxidation	39 ± 13	41 ± 20	2.0	36 ± 19	38 ± 16	2.0	0.47

Data are presented as mean ± SD

* Indicates significant difference pre to post intervention ($p < 0.05$), p value column indicates difference between training modes

Note. Chg = change CHO = carbohydrate, RER = respiratory exchange ratio, RMR = resting metabolic rate

Due to the inability to obtain complete blood samples from every participant during the 30 minute steady state exercise session in the aquatic and land based studies, data from 13 participants were used for analysis of plasma FFA and glycerol concentrations during exercise. There were significant increases in plasma FFA concentration during mode specific exercise at the time of the aquatic-based exercise intervention at pre training (539 ± 257 to 719 ± 187 $\mu\text{mol/L}$; $p = 0.01$) and post training (475 ± 155 to 576 ± 191 $\mu\text{mol/L}$; $p = 0.04$), and also at the time of the land-based exercise intervention pre (418 ± 211 to 759 ± 296 $\mu\text{mol/L}$; $p < 0.001$) and post intervention (483 ± 247 to 823 ± 347 $\mu\text{mol/L}$; $p < .001$). There were also significant increases in plasma glycerol concentration during mode specific exercise at the time of the aquatic-based exercise intervention pre training (125 ± 56 to 180 ± 47 $\mu\text{mol/L}$; $p = 0.003$) and post training (117 ± 45 to 161 ± 49 $\mu\text{mol/L}$; $p = 0.009$), and also at the time of the land-based exercise intervention pre (115 ± 35 to 199 ± 63 $\mu\text{mol/L}$; $p < 0.001$) and post intervention (134 ± 66 to 208 ± 81 $\mu\text{mol/L}$; $p < 0.001$). Analysis of variance, demonstrated no significant difference between aquatic and land-based exercise training in the release of plasma FFA or plasma glycerol during mode specific exercise ($p > 0.05$) (Table 28). There was no significant change in plasma FFA concentration with acute mode specific exercise following either aquatic ($p = 0.20$) or land-based exercise training ($p = 0.98$), or in plasma glycerol concentration with acute mode specific exercise following either aquatic ($p = 0.55$) or land-based exercise training ($p = 0.51$) (Table 28).

Analysis of covariance also revealed no significant difference between aquatic and land-based training for changes in basal concentrations of plasma FFA and glycerol, measured prior to the exercise session, after respective interventions ($p > 0.05$) (Table 28). There was no significant change in basal plasma FFA concentrations following either aquatic ($p = 0.29$) or land based exercise training ($p = 0.35$), or in basal plasma glycerol concentration following either aquatic ($p = 0.31$) or land-based exercise training ($p = 0.29$) (Table 28).

Table 28. Basal free fatty acid and glycerol concentrations, and changes in plasma free fatty acid and glycerol concentrations during 30 minutes of mode specific exercise, pre and post aquatic-based exercise training and land-based exercise training.

	Aquatic (n = 13)		Land (n = 13)		p value
	Pre	Post	Pre	Post	
Basal FFA ($\mu\text{mol/L}$)	539 \pm 257	475 \pm 155	418 \pm 211	483 \pm 247	0.58
Basal Glycerol ($\mu\text{mol/L}$)	125 \pm 56	117 \pm 45	115 \pm 35	134 \pm 66	0.20
ΔFFA ($\mu\text{mol/L}$)	180	101	342	340	0.33
$\Delta\text{Glycerol}$ ($\mu\text{mol/L}$)	55	44	84	74	0.98

p value column indicates difference between training modes

Note. ΔFFA = change in free fatty acid concentration from pre to post 30 minute DWR exercise testing, $\Delta\text{Glycerol}$ = change in glycerol concentration from pre to post 30 minute DWR exercise testing, FFA = free fatty acid

5.2.2.5 Muscular Strength

There was no evidence of a difference in post intervention scores for chest strength when controlling for baseline scores, between aquatic and land-based exercise training modalities ($p > 0.05$) (Table 29). Furthermore, neither aquatic nor land-based training modes significantly altered chest strength ($p > 0.05$) (Table 29).

In this group of participants, a significant increase in quadriceps and hamstring strength was evident following aquatic-based exercise training ($p = 0.01$), with no change following land-based exercise training ($p > 0.05$) (Table 29). However, the increase in lower body strength following aquatic-based training was not large enough to be statistically different from post intervention scores between exercise modes ($p > 0.05$) (Table 29).

Table 29. Strength data pre and post aquatic based exercise training and land based exercise training

	Aquatic (n = 17)			Land (n = 17)			p value
	Pre	Post	Chg (%)	Pre	Post	Chg (%)	
Chest strength (Nm)	198 ± 59	214 ± 44	8.0	207 ± 42	221 ± 45	6.8	0.29
Quadriceps strength (Nm)	101 ± 30	110 ± 27*	8.9	106 ± 29	107 ± 26	0.9	0.07
Hamstring strength (Nm)	66 ± 20	74 ± 13*	12	72 ± 17	75 ± 15	4.2	0.75

Data are presented as mean ± SD

*p < 0.05 significant difference pre to post intervention

p value column indicates difference between training modes

Note. Chg (%) = percentage change

5.2.2.6 Anthropometry

When controlling for baseline values, no differences existed between exercise training modalities for post intervention measures of weight or BMI ($p > 0.05$) (Table 30). Overall, there were no significant alterations in weight or BMI following aquatic or land-based exercise training ($p > 0.05$) (Table 30).

Both aquatic and land-based exercise training induced similar significant decreases in WC ($p < 0.05$), with no significant difference between the two training modalities when controlling for baseline data ($p > 0.05$) (Table 30). Following aquatic-based exercise training there was a significant reduction in hip circumference ($p < 0.05$), with no change following land-based exercise training ($p > 0.05$); however this was not large enough to result in a significant difference between training modes ($p > 0.05$) (Table 30). In addition, aquatic-based exercise training demonstrated a significant reduction in WHR ($p < 0.05$), with no significant change following land-based exercise training ($p > 0.05$); however this difference was also not large enough to achieve significance between exercise training modes ($p > 0.05$) (Table 30).

Finally, aquatic and land-based exercise training demonstrated equivalent responses for changes in total body and trunk; fat percentage, FM, and FFM ($p > 0.05$) (Table 31). This corresponded to no significant change in these variables following either aquatic or land-based exercise training ($p > 0.05$) (Table 31).

Table 30. Anthropometric data pre and post aquatic-based exercise training and land-based exercise training

	Aquatic (n = 17)			Land (n = 17)			p value
	Pre	Post	Chg (%)	Pre	Post	Chg (%)	
Weight (kg)	77.6 ± 10.3	76.6 ± 9.6	-1.3	78.1 ± 11.2	77.8 ± 11.3	-0.4	0.12
BMI (kg/m ²)	29.5 ± 3.5	29.2 ± 3.3	-1.0	29.7 ± 4.0	29.6 ± 4.0	-0.3	0.18
Waist (cm)	90.9 ± 8.5	88.3 ± 7.9*	-2.9	88.0 ± 9.8	86.4 ± 9.0*	-1.8	0.85
Hip (cm)	109.6 ± 9.1	108.0 ± 7.4*	-1.5	109.9 ± 10.2	109.0 ± 10.2	-0.8	0.36
WHR	0.83 ± 0.05	0.82 ± 0.04*	-1.3	0.80 ± 0.05	0.79 ± 0.04	-1.3	0.09

Data are presented as mean ± SD

* p < 0.05 significant difference pre to post intervention

p value column indicates difference between training modes

Note. Chg (%) = percentage change, BMI = body mass index, WHR = waist to hip ratio

Table 31. Body composition data pre and post aquatic-based exercise training and land-based exercise training

	Aquatic (n = 17)			Land (n = 17)			p value
	Pre	Post	Chg (%)	Pre	Post	Chg (%)	
Total fat (%)	44.0 ± 5.0	43.0 ± 5.0	-1.0	44.0 ± 6	43.0 ± 6.0	-1.0	0.80
Total FM (kg)	34.0 ± 7.5	33.2 ± 6.7	-2.4	34.2 ± 8.7	33.7 ± 8.7	-1.5	0.56
Total FFM (kg)	40.2 ± 4.8	40.4 ± 4.9	0.5	40.4 ± 4.9	40.4 ± 4.8	0.1	0.97
Trunk fat (%)	44.0 ± 5.0	43.0 ± 5.0	-1.0	44.0 ± 6	44.0 ± 7.0	0.0	0.67
Trunk FM (kg)	16.6 ± 4.0	16.2 ± 3.7	-2.4	17.0 ± 4.8	16.6 ± 4.8	-2.4	0.60
Trunk FFM (kg)	20.0 ± 2.6	20.2 ± 2.6	1.0	20.3 ± 2.7	20.1 ± 2.5	-1.0	0.72

Data are presented as mean ± SD

p value column indicates difference between training modes

Note. Chg (%) = percentage change, FM= fat mass, FFM = fat free mass

5.2.2.7 Lipid Profile

No significant differences were evident between aquatic and land-based exercise training modalities for measures of the lipid profile, ($p > 0.05$) (Table 32). Furthermore, when training modes were analysed separately, there were no significant changes in HDL, LDL, TAG or TC following aquatic or land-based training ($p > 0.05$) (Table 32).

Table 32. Lipid profile pre and post aquatic-based exercise training and land-based exercise training

	Aquatic (n = 17)			Land (n = 17)			p value
	Pre	Post	Chg (%)	Pre	Post	Chg (%)	
HDL (mmol/L)	1.34 ± 0.23	1.33 ± 0.26	-0.7	1.35 ± 0.21	1.31 ± 0.21	-2.9	0.63
LDL (mmol/L)	3.22 ± 0.79	3.15 ± 0.85	-2.2	3.09 ± 0.77	3.22 ± 0.73	4.2	0.94
TAG (mmol/L)	1.08 ± 0.71	1.02 ± 0.46	-5.6	1.16 ± 0.61	1.06 ± 0.33	-8.6	0.95
TC (mmol/L)	5.06 ± 0.77	4.95 ± 0.84	-2.2	4.97 ± 0.80	5.02 ± 0.73	1.0	0.61

Data are presented as mean ± SD

p value column indicates difference between training modes

Note. Chg (%) = percentage change, HDL = high density lipoprotein, LDL = low density lipoprotein, TAG = triacylglycerol, TC = total cholesterol

5.2.3 Standardisation

All 17 participants completed and replicated the 24 hour diet record between aquatic and land-based exercise interventions prior to the 30 minute steady state exercise sessions, measuring substrate utilisation. Nutrient composition was maintained, with no significant differences between pre testing for aquatic and land-based interventions, and post testing for aquatic and land-based interventions ($p > 0.05$) (Table 33).

Table 33. Percentage of nutrient composition consumed 24 hours prior to 30 minutes of mode specific exercise at pre and post testing for aquatic based and land based exercise interventions

	Aquatic (n = 17)	Land (n = 17)	p value
Pre testing			
Fat %	13 ± 6	15 ± 6	0.09
CHO %	61 ± 8	60 ± 9	0.27
Protein %	25 ± 5	25 ± 6	0.95
Post testing			
Fat %	13 ± 6	14 ± 5	0.22
CHO %	61 ± 7	61 ± 7	0.69
Protein %	25 ± 5	25 ± 5	0.62

p value column indicates difference between training modes

5.3 Discussion

The present investigation has reported on the metabolic and physiological effects of aquatic-based exercise training and land-based exercise training in overweight and obese women, with a specific focus on fat oxidation. Metabolic responses from participants who completed both the aquatic and land-based exercise interventions were compared, to determine the overall effectiveness of these training modalities on fat oxidation and related measures.

5.3.1 Metabolism and Substrate Utilisation

It was hypothesised that both low intensity aquatic and land-based exercise training would result in an increase in exercise fat oxidation in overweight and obese women; with aquatic-based exercise training resulting in less of an increase in exercise fat oxidation than land-based exercise training. This hypothesis was partially supported in that land-based exercise training was successful for increasing exercise fat oxidation rate and the contribution of fat oxidation to exercise EE, in overweight and obese women, however aquatic-based exercise training had no effect on these parameters. Thus, differences were observed between aquatic and land-based exercise training modalities for alterations in the rate of fat oxidation during exercise. Moreover, the present results provide a preliminary finding; that aquatic-based exercise training does not stimulate the same increase in exercise fat oxidation as seen with land based exercise training in overweight and obese women (Dumortier et al., 2003; van Aggel-Leijssen et al., 2001; van Aggel-Leijssen et al., 2002). However, further research with a larger sample of overweight and obese women needs to be conducted in order to verify this. Furthermore, the possible confounding effects of pulmonary changes and accuracy of indirect calorimetry measurement for exercise fat oxidation in an aquatic environment require further investigation. The lack of increase in exercise fat oxidation following aquatic-based exercise training may be a result of the aquatic-based exercise training environment, rather than the inability of this population to demonstrate increases in exercise fat oxidation following exercise training.

As no studies to date have investigated changes in substrate utilisation following aquatic compared to land-based chronic exercise training; responses from an acute bout of aquatic compared to land-based exercise may provide insight, as alluded to in chapter 3. Acute responses during aquatic and land-based exercise may lead to training adaptations specific to each modality when chronic exercise training is undertaken. Consequently, substrate utilisation appears to differ between acute running on land and in water when oxygen consumption is similar (DeMaere & Ruby, 1997). A greater reliance on CHO metabolism, measured by a higher RER, is demonstrated during equivalent submaximal acute exercise using DWR compared to TMW (Broman, Quintana, Engardt et al., 2006; DeMaere & Ruby, 1997; Michaud, Rodriguez-Zayas et al., 1995; Svedenhag & Seger, 1992). Furthermore, greater CHO oxidation rates and lower fat oxidation rates have been reported during submaximal DWR compared to treadmill running at the same intensity (DeMaere & Ruby, 1997). It is possible that aquatic-based exercise may be anaerobic, as a consequence of lowered perfusion pressure in the lower body resulting from an increase in hydrostatic pressure due to water immersion (Svedenhag & Seger, 1992). A lower perfusion pressure in the lower body therefore results in a decrease in total muscle blood flow (Svedenhag & Seger, 1992), which is essential for optimal fat oxidation (van Baak, 1999). The greater reliance on CHO metabolism during submaximal acute DWR indicates lower fat oxidation and therefore less adaptation for fat oxidation during aquatic compared to land-based exercise training.

Alterations in the pulmonary system, and ventilatory effects due to the external hydrostatic pressure associated with water immersion, may also influence substrate utilisation compared to when exercise is undertaken on land (Becker, 2004). The reduction in the partial pressure of oxygen due to water immersion results in a parallel increase in the partial pressure of CO₂, and therefore increases in ventilation and expired CO₂ (Becker, 2004). This increase in CO₂ production is associated with increased CHO utilisation measured by indirect calorimetry (Becker, 2004; Christensen & Hansen, 1939; Jeukendrup et al., 1998a). Consequently, any possible increase in fat oxidation rate may be masked following aquatic based exercise training when measured with indirect calorimetry during water immersion. If an increase in fat oxidation rate does occur following aquatic-based exercise training, the methodological use of indirect calorimetry

may prevent any increase to be detected. Retrospective analysis was therefore undertaken to investigate respiration rate throughout the measurement of substrate oxidation via indirect calorimetry, during mode specific exercise. Paired t-testing indicated a significantly greater respiration rate ($p = 0.004$) during aquatic-based exercise (28 ± 6 breaths per minute) compared to land-based exercise (25 ± 4 breaths per minute), at equivalent mode specific intensities. This confirms the physiological effects of water immersion on ventilation and hence indirect calorimetry when compared to land-based measurement. Thus, in an aquatic environment, infusions of labeled fatty acids may provide a more accurate indication of oxidation of stored or circulating fatty acid sources (Coppack et al., 1994; Horowitz, 2001).

An altered pattern of muscle recruitment between aquatic and land-based exercise may also explain differences in substrate utilisation among the two modalities (DeMaere & Ruby, 1997; Kilding et al., 2007). Ground reaction forces encountered during exercise on land are removed in water. Therefore, in an aquatic modality, work undertaken by the lower extremity muscles is replaced by greater use of upper extremity muscles to overcome the resistance of water (Michaud, Brennan et al., 1995; Reilly, Dowzer et al., 2003). Water being more viscous than air, causes an increased resistance to movement (di Prampero, 1986; Wilder & Brennan, 2004). On this assumption, the upper extremity muscles, relatively untrained against resistance, may fatigue faster, leading to altered substrate utilisation compared to land exercise (DeMaere & Ruby, 1997). Therefore, both the external hydrostatic pressure, and altered muscle recruitment encountered in an aquatic environment, may result in increased anaerobic metabolism during submaximal DWR compared to equivalent land-based exercise (DeMaere & Ruby, 1997; Svedenhag & Seger, 1992).

It has been suggested by other authors that differences in fat oxidation rates between weight-bearing and non weight-bearing exercise are caused by comparison of two different exercise intensities, rather than two different exercise modes (Achten, Venables, & Jeukendrup, 2003). Therefore, while it appears that low, and not high, intensity exercise training demonstrates increases in fat oxidation in overweight and obese individuals (van Aggel-Leijssen et al., 2002), there may be a threshold where exercise intensity is too low to result in any adaptation to increase fat oxidation. Due to

physiological differences caused by the exercise environments between aquatic and land-based exercise modalities, there may be variations in each individual's maximal fat oxidation point for each mode, and also the generic intensity for prescribed exercise to increase fat oxidation. The intensity prescribed and displayed during the aquatic intervention may have been further away from each individual's maximal fat oxidation point, or too low, in comparison the intensity of the land-based exercise intervention.

Variations in the exercise intensity between pre and post testing for substrate utilisation may have also affected fat oxidation changes between aquatic and land-based exercise training modalities. Substrate utilisation is dependent on exercise intensity (De Feo et al., 2003; Jeukendrup & Wallis, 2005), therefore fat oxidation may be influenced by the differing intensity at pre and post testing during the exercise metabolism measurement for the land-based training intervention. Although equivalent intensity based on HR, %HR_{peak}, $\dot{V}O_2$ and EE was prescribed; relative intensity, expressed as % $\dot{V}O_{2peak}$, was higher during the land-based intervention post test. This difference in exercise intensity may have been influenced by the increase in CV fitness observed following land based exercise training. However, in a study by Friedlander and colleagues (1998), participants performed post testing at the same absolute workload and relative intensity following exercise training, and demonstrated significant decreases in RER following both absolute and relative intensities. Participants also demonstrated a significant increase in CV fitness (Friedlander et al., 1998), as in the current investigation. The authors suggest that women can increase their reliance on fat oxidation following endurance training, regardless of whether exercise is normalised to either absolute or relative power outputs (Friedlander et al., 1998). Therefore, the variation in relative exercise intensity during the post test in the current investigation may not be of concern, as absolute exercise intensity was equivalent between tests during the land-based exercise intervention. Nevertheless, the differing relative intensity needs to be taken into consideration when interpreting the results of substrate utilisation for the land-based investigation.

Differences in the temperature in which aquatic and land-based exercise are performed may influence training responses in substrate utilisation. However, exercise was undertaken at a thermoneutral temperature for dynamic exercise in each modality. A

thermoneutral temperature is considered 26-29°C in water (Craig & Dvorak, 1969) and between 21-26°C on land (Wilmore & Costill, 2004). As both exercise training modalities were undertaken at thermoneutral temperature, metabolic responses should not have been influenced by differences in temperature. Therefore, variations in substrate utilisation were likely to be related solely to features of the exercise training modalities.

To date, no literature exists comparing the lipolytic responses (via concentrations of plasma FFA and glycerol) between aquatic and land-based exercise training. Therefore, data are not available for comparison with the current results. In the current investigation, significant increases in plasma FFA and glycerol concentrations were demonstrated during aquatic and land-based exercise pre and post training, indicating similar responses in lipolysis regardless of exercise modality. In agreement with the current investigation, Talanian and colleagues (2007) and Sial and colleagues (1998) also demonstrated significant glycerol release during acute land-based exercise, pre and post training. In both investigations, participants were of normal weight, indicating that the overweight and obese nature of the current participants did not affect glycerol response (Sial et al., 1998; Talanian et al., 2007). However, in contrast to the present investigation, Talanian and colleagues (2007) and Sial and colleagues (1998) did not demonstrate any significant increase in plasma FFA after land-based exercise. It is possible that the normal weight status of the participants in the study by Talanian et al (2007) and Sial et al (1998) resulted in regulated lipid turnover during exercise where FFA concentration was matched with fat oxidation rate. A reduced lipid turnover may have been present in the overweight and obese participants in the current investigation as fat oxidation rate was not able to match FFA concentration. The timing of the measurement of plasma FFA and glycerol relative to the initiation of the exercise session may also influence results (Mittendorfer et al., 2004). Mittendorfer and colleagues (2004) evidenced a decrease in plasma FFA concentration over the first 20-30 minutes of exercise with a progressive increase thereafter. In their study, lean, overweight and obese men all displayed equivalent patterns of plasma FFA release (Mittendorfer et al., 2004). Therefore the response in plasma FFA and glycerol concentration in participants from the current investigation is comparable between exercise training modalities, and may or may not be influenced by the overweight and obese nature of the individuals.

Resting metabolism measures were unaltered following both aquatic and land-based exercise training modalities. Therefore, training at an intensity aimed at increasing fat oxidation did not provide sufficient stimulus for alterations in RMR, resting fat and CHO oxidation rates, or resting RER in either aquatic or land-based mediums. In agreement with the current investigation, previous land-based exercise training investigations have also demonstrated no change in resting substrate utilisation in overweight and obese individuals (Kanaley et al., 2001; van Aggel-Leijssen et al., 2001; van Aggel-Leijssen et al., 2002). However, no study to date has investigated metabolic responses following aquatic based exercise training. The direction and magnitude of the effects of land-based exercise training on RMR are inconclusive, and it has been proposed that a threshold of exercise intensity and EE may exist in order for increases in RMR to occur (Poehlman, 1989). However, the current exercise prescription, utilising low intensity exercise training, does not appear to successfully meet the threshold required to elicit changes in RMR or resting metabolism, either in an aquatic or land-based exercise training modality.

As both aquatic and land-based exercise training modalities did not change resting metabolism measures, resting metabolic responses may not be solely affected by modality of exercise, but may be more dependent on the type of training programme or individual genetic ability to respond to adaptation. A training programme of higher intensity, targeted for development of FFM, may be required to elicit increases in resting metabolic measures, rather than a training programme targeted to increase exercise fat oxidation. Both aquatic and land-based training failed to demonstrate any significant increase in total body FFM, which may also explain the lack of change in RMR with either modality of exercise training.

Overall, the lack of change in fat oxidation measures following aquatic exercise training compared to land-based exercise training may be due to either; features of an aquatic DWR environment, or differences in the training intensity and EE compared to that undertaken on land. However, the differences observed in intensity between pre and post testing for substrate utilisation also needs to be taken into account when interpreting these results.

5.3.2 Cardiovascular Fitness

Overall, there was no difference between aquatic and land-based exercise training for changes in mode specific CV fitness. The result of the current investigation does not agree with other randomized controlled trials comparing aquatic to land based exercise training (Assis et al., 2006; Rhodes et al., 1995; Taunton et al., 1996) or cross over study designs (Davidson & McNaughton, 2000). In past investigations, similar significant increases in CV fitness were observed following both aquatic and land-based exercise training (Assis et al., 2006; Davidson & McNaughton, 2000; Rhodes et al., 1995; Taunton et al., 1996). However, in the current investigation, when analysed independently, land-based exercise training demonstrated a significant increase in CV fitness, but no increase was seen following aquatic-based exercise training.

There are differences between earlier comparative studies (Assis et al., 2006; Davidson & McNaughton, 2000; Rhodes et al., 1995; Taunton et al., 1996), and the current investigation, that may explain the increase in CV fitness demonstrated in the present study following land-based training, compared to aquatic training. The current investigation is unique in the way mode specific maximal exercise tests were conducted and used for mode specific exercise prescription. Previous studies comparing changes in CV fitness following aquatic and land-based exercise training have prescribed aquatic training using results from land based maximal exercise tests (Assis et al., 2006; Rhodes et al., 1995; Taunton et al., 1996). As demonstrated in the present investigation, $\dot{V}O_{2peak}$ is lower in water compared to on land, resulting in lower oxygen consumption at the same relative intensity when undertaking aquatic exercise. Therefore, increases in aquatic CV fitness may be more easily observed in previous investigations, due to the higher overall intensity performed in water when prescribed using land-based maximal data (Assis et al., 2006; Rhodes et al., 1995; Taunton et al., 1996). Some investigators suggest that DWR training be performed at a relatively higher intensity level compared to land-based training (Quinn et al., 1994). For example, in elderly women an exercise intensity of 60% of $\dot{V}O_{2max}$ on a treadmill corresponds to 85% of $\dot{V}O_{2peak}$ during DWR (Broman, Quintana, Engardt et al., 2006). Therefore, in order to increase aquatic-based CV fitness, and potentially fat oxidation rate, training intensity when undertaking DWR

must be higher than land-based training intensity (Broman, Quintana, Lindberg et al., 2006).

As individuals with higher CV fitness display greater rates of exercise fat oxidation than sedentary individuals (Klein et al., 1994; Tremblay et al., 1992), the significant difference in absolute exercise intensity, between aquatic and land-based exercise training regimes may have affected both changes in CV fitness and substrate utilisation. Although relative intensity was similar, absolute intensity was different between aquatic and land-based exercise modalities. The magnified effects of buoyancy, and compensatory movements carried out to reduce water resistance during DWR, may reduce the workload to a point that peak CV responses similar to those seen on land are not possible in the water (Silvers, Rutledge, & Dolny, 2007). In the current investigation, the use of mode specific exercise prescription did not allow CV adaptations or changes in fat oxidation to occur following aquatic training, despite prescription of equivalent relative intensity to land-based exercise.

Improvements in CV fitness, and thus fat oxidation rates, may have also been dependent on total EE of exercise training. The greater weekly EE demonstrated with land-based exercise training may have been over an individual threshold needed to elicit adaptations in CV fitness, whereas aquatic training may have been below this threshold (ACSM, 1998, 2006). Therefore, prescription of aquatic exercise training may need to incorporate a higher intensity level, and thus greater EE, in order for an increase in CV fitness, and possibly fat oxidation rate, equivalent to land-based training, to be observed.

5.3.3 Muscular Strength

Both aquatic and land-based exercise training modalities were unsuccessful in significantly improving chest strength. There was also no difference between aquatic and land-based exercise training for changes in lower body strength. Similar to the current investigation, previous studies comparing aquatic and-land based exercise training modalities have also demonstrated no difference between training modes for overall strength changes, with no significant improvements after training, within or between groups (Rhodes et al., 1995; Taunton et al., 1996). However, similar increases in total muscular strength have been reported in a study comparing aquatic to land-based training

in overweight individuals (Volaklis et al., 2007). Combined endurance and resistance training demonstrated comparable increases in whole body muscular strength of 12.8% following aquatic exercise training, and 12.9% following land-based exercise training (Volaklis et al., 2007). Muscular strength was measured as the sum of 1RM bench press, pull down, seated row, peck-deck, leg extension and hamstring curl (Volaklis et al., 2007). As some exercise programmes, but not others, demonstrate similar increases in muscular strength when comparing aquatic to land-based exercise training, it seems that an increase in muscular strength may be more dependent on the type of exercise programme implemented, rather than exercise training modality.

Although there was no difference in quadriceps or hamstring strength between aquatic and land-based exercise training, aquatic-based exercise training independently increased lower body strength. This indicates favorable alterations in lower body strength following an aquatic-based exercise training modality. The pattern of greater increases in lower body strength following aquatic-based exercise training may be due to the resistance of water, making aquatic-based exercise an ideal stimulus for strength adaptations. The resistive effects of water provide loading during any movement of limbs, enhancing muscular tension and providing a continual training stimulus for strength increases and muscular development (Tsourlou et al., 2006; Wilder & Brennan, 2004). Greater strength increases are observed in the lower body with aquatic training due to greater density and pressure of water, and therefore resistance, as water depth increases (Bates & Hanson, 1996; Becker, 2004). In addition, the greater lower body surface area of overweight and obese participants may increase the resistance of water with movement. Whereas, on land, to achieve muscular strength training adaptations, higher intensity resistance training may be required; this was not a feature of the current land-based training programme. Overall, an exercise programme targeted to increase fat oxidation may not provide adequate stimulus to result in increased strength, regardless of the exercise modality. However, there is some evidence that aquatic-based exercise training may favor increases in lower body strength due to features of an aquatic exercise environment.

5.3.4 Anthropometry

Aquatic and land-based exercise training programmes did not alter total body weight. Due to the positive relationship between changes in body mass and substrate utilisation, independent to adaptations from an exercise intervention (Pasma, Westerterp-Plantenga, & Saris, 1999), changes in total body weight was not a focus of the current interventions. A lack of change in body weight has also been demonstrated within aquatic and land-based training groups, and between training groups in older women (Taunton et al., 1996).

Both aquatic and land-based training were equally effective in reducing WC. Previous studies comparing aquatic to land-based exercise training do not specifically measure WC; consequently, results can not be compared to past literature. A reduction in WC in overweight and obese individuals may reduce development of metabolic complications associated with a high accumulation of abdominal fat (Despres et al., 1991). Therefore, regardless of the exercise modality, both aquatic and land-based training proved to be beneficial in eliciting positive health effects through markers of abdominal obesity.

In the present investigation, there was no difference between aquatic and land-based exercise training for hip circumference or WHR. In agreement, Taunton and colleagues (1996) also failed to demonstrate any change in hip circumference or WHR between aquatic and land-based exercise training in elderly women. However, in the current investigation there were significant reductions in hip circumference and WHR within aquatic-based exercise training, with no change following land-based training, indicating an aquatic training environment favorable for anthropometric changes.

Neither aquatic nor land-based training were effective in altering body composition measured by DXA. There was no change in either total body or trunk measures for fat percentage, FFM or FM, following both modes of training. The failure to influence body composition, measured via DXA, in the present investigation is in agreement with Taunton and colleagues (1996), who also demonstrated no change in body composition, measured by skin folds, either within or between aquatic and land-based training groups. The lack of change in body composition was evident in both the current investigation and that of Taunton et al (1996), despite differences in methodology between the two investigations. Differences in methodology for body composition

include limitations with skin fold measurements (Heyward, 1998; Wagner & Heyward, 1999), and high precision with DXA (Mazess et al., 1990b; Wagner & Heyward, 1999). However, Volaklis and colleagues (2007) demonstrated significant reductions in the sum of skin folds, with no difference between combined endurance and resistance training in aquatic or land-based modalities. Although differences exist in measurement technique compared to the present investigation, the positive alterations in body composition may be a feature of the exercise programme prescribed by Volaklis et al (2007). Volaklis and colleagues (2007) prescribed a greater frequency of training sessions and a longer training intervention, despite similar exercise intensities. Similar positive changes in body composition may have been observed in the current investigation if training was prescribed at a greater frequency and duration of training. The non sedentary nature of the current study population may also require more stimuli for changes in body composition to occur, compared to the inactive participants in the aforementioned study (Volaklis et al., 2007).

5.3.5 Lipid Profile

No effect of training modality was observed when assessing lipid parameters. The result of the present study is in contrast to Volaklis and colleagues (2007) who demonstrated equivalent significant reductions in TC (-4.4%, -3.3%) and TAG (-10.2%, -11.8%) following exercise training in both aquatic and land-based modalities, respectively. Despite prescribing a similar intensity to that used in the present study (50-80% treadmill HR_{max}), the exercise programme by Volaklis and colleagues (2007) resulted in a greater volume of exercise than the current investigation. However, training EE was not calculated in the study by Volaklis and colleagues (2007), therefore direct comparison based on EE cannot be made. The presence of coronary artery disease and the sedentary nature of participants in their study, combined with baseline lipid levels that were further away from normative values (NCEP, 2001) may have allowed a greater improvement in TC and TAG to be observed. Similar to the current investigation however, Volaklis and colleagues (2007) failed to demonstrate any significant change in LDL or HDL, either within or between exercise training modalities. Therefore, exercise modes may have little effect on lipid profile changes. It may be the features of exercise

programmes themselves that determine whether changes will occur in blood lipid profiles. Furthermore, the threshold of exercise prescription required to elicit positive changes in LDL and HDL does not appear to be met in either the current investigation or that by Volaklis and colleagues (2007). Consequently, the present study's exercise prescription aimed at increasing exercise fat oxidation, does not appear to provide sufficient stimulus to positively alter the lipid profile in either an aquatic or land-based exercise modality.

5.3.6 Summary

Comparisons between two different treatment groups using the same individuals most often follow the cross over design model (Jones & Kenward, 2003). However, in the case of the present investigation the implementation of a pure cross over design method was not possible when comparing aquatic to land-based exercise training. The present investigation is strengthened in that each individual served as their own control between aquatic and land-based investigations, limiting threats to internal validity.

Overweight and obese women in the current investigation had significant increases in exercise fat oxidation rate, and contribution of fat to exercise EE following land-based exercise training, however they did not display any increase in exercise fat oxidation as a result of aquatic-based exercise training. This indicates that the overweight and obese nature of participants did not prevent any increase in exercise fat oxidation to occur. This feature provides positive implications for overweight and obese individuals who may have an impaired ability to oxidise fat during exercise (Blaak & Saris, 2002; Kanaley et al., 2001; Perez-Martin et al., 2001; Thyfault, Richmond et al., 2004), as reduced lipid turnover is thought to underpin adverse health implications (Corcoran et al., 2007). Moreover, a lack of increase in exercise fat oxidation may be a feature of the aquatic-based exercise training modality, or may relate to appropriate prescription of exercise intensity.

In the current investigation, there is evidence that CV fitness may be stimulated to a greater extent with land-based training, potentially explaining the increase in fat oxidation. However, differences in absolute exercise intensity and EE, due to mode specific exercise prescription at equivalent relative intensities, make it difficult to reach a

definite conclusion. An aquatic-based exercise modality demonstrated positive alterations in lower body strength, hip circumference and WHR, which were not observed following land-based exercise training. Nevertheless, both aquatic and land-based exercise training were equally effective in reducing the metabolically at risk marker of WC in overweight and obese women. No significant differences exist either between or within aquatic and land-based exercise training modalities, as examined in this study, for exercise CHO oxidation, resting metabolic measures, exercise or basal FFA and glycerol concentrations, chest strength, body weight, BMI, DXA measures of body composition, or lipid profiles in the present investigation.

Overall, metabolic and physiological differences exist between chronic exercise undertaken in aquatic and land-based modalities in overweight and obese women. Therefore, exercise prescription in an overweight and obese population should be targeted specifically at the preferred adaptation. In this case, prescription of land-based exercise is recommended for overweight and obese women to increase exercise fat oxidation.

Chapter 6: Summary of Exercise Training Modalities

6.1 Conclusion

The results of the aquatic-based exercise training intervention demonstrated that there was no difference between DWR and DWR+RT for exercise fat oxidation responses, and aquatic exercise overall was not effective at increasing fat oxidation in this group of overweight and obese women. However, land-based exercise training may enhance exercise fat oxidation during exercise in overweight and obese women, with no difference in responses evident between LBE and LBE+RT. Overall, when aquatic and land-based exercise training modalities were compared, differences in exercise fat oxidation were demonstrated, with an increase in fat oxidation following land-based training and not aquatic-based training. In conclusion, a training-induced shift in substrate oxidation can occur in overweight and obese women; however this response appears limited to exercise training on land.

6.2 Strengths and Limitations

The current investigation displayed key strengths, along with a number of limitations that must be acknowledged. The major strength of the present study was the contribution to the body of literature investigating training responses and substrate utilisation. To date, no research has been published on the effects of an aquatic exercise training intervention on substrate utilisation. Furthermore, no research has been published that investigates the effects of combined endurance and resistance training on substrate utilisation in an aquatic or land-based exercise modality.

As the current investigation utilised a randomised trial, this strengthened the study design, thus limiting threats to internal validity. If there was a possibility that randomisation failed, this was controlled for with a covariate analysis.

The current investigation was also strengthened by the low rates of attrition and high rates of attendance in both the aquatic and land-based training interventions. Effort was made on behalf of the researchers to motivate and encourage continued participation during the course of the aquatic and land-based training regimes. Both of the exercise

modalities chosen were well received, reflected by 87% adherence and 100% attendance rates during the 12 weeks of aquatic-based exercise training and pre and post testing, and 86% adherence and 97% attendance throughout 12 weeks of land-based exercise training and pre and post testing. Furthermore, the group exercise environment may have been a positive aspect for continued exercise training for some of the women.

An additional strength of this investigation was using the precise measure of mode specific maximal exercise testing for CV fitness; in water or on land, and exercise training intensity was prescribed at a percentage of this mode specific peak. Past aquatic interventions have prescribed exercise intensity from land-based maximal testing (Broman, Quintana, Lindberg et al., 2006; Eyestone et al., 1993; Michaud, Brennan et al., 1995; Quinn et al., 1994). It is well documented that maximal responses between aquatic and land based exercise differ (Butts et al., 1991; Michaud, Rodriguez-Zayas et al., 1995; Svedenhag & Seger, 1992), and are therefore, mode specific. Thus, mode specific exercise training intensities were accurately prescribed in the current investigation.

Several limitations exist in the present investigation. Namely; whether endurance based exercise training was 'purely' endurance training, a lack of power in the sample size, large variability in some results, difference in training and testing exercise intensities, no blinding of participants or the researcher, and the lack of training diaries and a control group.

Whether the endurance training component included in the current investigation was classified as 'purely' endurance training, may provide insight into the lack of significant difference in outcome measures between exercise training groups during aquatic and land-based exercise training interventions. In the aquatic training investigation, the DWR endurance component incorporated variations of traditional DWR technique. The variations of DWR technique were performed against the resistance of water, as in traditional DWR. Thus, due to the resistive properties of water, the endurance DWR element cannot be exclusively classed as 'purely' endurance training. However, the DWR+RT component differed substantially from the DWR component in the execution of movement. During DWR+RT, the addition of specific exercises, incorporating flotation devices, greatly increased the resistance against water by involving a larger surface area, thereby providing more potential to present a greater

resistance training effect. As muscular strength increases were comparable between DWR and DWR+RT in the aquatic-based exercise intervention, the resistive properties of water provided a resistive stimulus regardless of whether specific resistance training exercises are prescribed. It may also be that the resistance forces were in fact comparable for RT and DWR components, as while the surface area moving through the water would be possibly greater in RT movements, the velocity of this movement may be less than the endurance training movements. Similarly, the endurance component of the land-based training investigation may also not be completely 'purely' endurance based, due to the hilly terrain encountered during exercise sessions in the LBE. However, as in the DWR+RT programme, the LBE+RT incorporated specific resistance exercises against a load, also providing a greater resistance training stimulus. It may be argued that the resistance component of training, both in an aquatic and land based exercise modality, was undertaken for a lengthy duration and subsequently low intensity, and load, to allow for resistance training strength adaptations. Therefore, it may be that the two training regimes were too similar to elicit sufficiently large difference between groups in each exercise modality without a large sample size. As exercise training EE was retrospectively estimated, accurate measurement of exercise EE during each specific training regime is also not available. The potential lack of training difference between DWR and DWR+RT, and LBE and LBE+RT in this investigation may provide one reason for the disproven hypotheses for the aquatic-based and land-based interventions.

During both aquatic and land-based exercise training investigations however, significant differences between training groups within each exercise modality were evident in some outcome measures. There may have been an inability to detect any significant difference in outcome measures such as; resting metabolism, CV fitness, chest strength, quadriceps strength, WC, WHR and lipid profiles between endurance and endurance combined with resistance training groups due to a combination of the training sessions being too similar, and the small sample size. The small sample size could inflate the rate of type II error and the ability to detect differences between endurance and the endurance combined with resistance training groups if they did exist. It is also recognised that the low sample size restricted room for drop outs, a factor that needs to be taken into consideration for any exercise intervention.

Measures of variability used in this study were the variance (mean) and SD. High variability was evident in some outcome measures, such as exercise and resting substrate utilisation via indirect calorimetry, and FFA and glycerol concentrations, both for overall exercise training modalities and between training groups for each modality. Large SD's may have masked finding a significant difference in statistical analysis. For example, the lack of increase in exercise fat oxidation following aquatic-based exercise training overall may be influenced by the high variability in these measures. There may have also been systematic errors in measurement of fat oxidation via indirect calorimetry. These systematic errors may have resulted from biases introduced by instrumental method or human factors; producing large variability in any indirect calorimetry measurement. However, random and systematic measurement errors were controlled through calibration of indirect calorimetry equipment prior to each measurement, and standardisation of pre and post testing. In addition, due to the pulmonary and ventilatory effects of water immersion on indirect calorimetry, this method may have been inferior for accurate measurement of fat oxidation, specifically in an aquatic environment. The measurement of RER and oxidation rate via indirect calorimetry is based on the assumption that the exchange of O₂ and CO₂ measured at the lungs reflects the actual gas exchange at the cellular level (Simonson & DeFronzo, 1990). However, during water immersion, the partial pressure of oxygen is decreased and the partial pressure of carbon dioxide increased (Becker, 2004), potentially influencing indirect calorimetry results and thus calculations of oxidation. Notably, as the current investigation is the first study of its kind to investigate the question of fat oxidation in overweight and obese women in an aquatic setting, this study needs to be replicated to determine if this is the case.

Although exercise training and testing were undertaken at different intensities, this should not have impacted on exercise fat oxidation observed following land-based exercise training. Exercise training intensity was originally prescribed equal to the intensity of the 30 minute steady state exercise session in which substrate utilisation was measured. However, during the aquatic-based exercise intervention, participants were required to exercise at a slightly higher intensity than originally prescribed to prevent a hypothermic response in the water; which was 71% HR_{peak} (50% $\dot{V}O_{2peak}$) rather than 64% HR_{peak} (49% $\dot{V}O_{2peak}$). Different exercise training and testing intensities were

present in the aquatic-based exercise investigation and therefore had to be replicated during the land-based investigation. Land-based exercise training occurred at 70% HR_{peak} (54% $\dot{V}O_{2peak}$), and the 30 minute TMW session to measure substrate utilisation at 61% HR_{peak} (44% $\dot{V}O_{2peak}$). The variation in training and testing intensities exhibited in the present investigation are in accordance with that of Amati and colleagues (2008) who prescribed exercise training at 75% $\dot{V}O_{2peak}$ and undertook metabolic testing at 50% $\dot{V}O_{2peak}$. Amati et al (2008) also demonstrated an increase in exercise fat oxidation, notwithstanding differing training and testing intensities, in overweight and obese individuals. Conversely, despite exercise training and testing at the same absolute intensity of 70% $\dot{V}O_{2peak}$ Kanaley and colleagues (2001) found no increase in fat oxidation in sedentary obese women. Therefore, training and testing at the same relative intensity may not be essential for alterations in fat oxidation to occur.

In this investigation, neither the participants nor the investigator was blinded. However, the research technician who assisted data collection was blinded, minimising measurement bias. Due to the nature of exercise training, it was not possible to blind participants to their treatment group. Furthermore, as the investigator was required to undertake measurement of participants, and implementation and supervision of exercise interventions, they could not be blinded to participant selection. Many of the outcome measures in this study were precise and objective measures, making blinding less important.

Physical activity levels and diet remained unchanged throughout the two exercise interventions; however training diaries were not implemented. Prior to participation and during each exercise intervention, participants were advised to continue with their normal exercise levels and dietary patterns, and weekly verbal confirmation was provided and received between participants and the investigator to ensure original physical activity levels and diet were maintained during the intervention periods. As additional exercise was not recorded it may be possible for unreported additional exercise to be undertaken outside the scope of the investigation, also providing a reason for lack of differences between training groups. The use of physical activity and nutrition diaries would have strengthened the study design; however it is difficult to accurately assess physical activity levels and diet during any exercise intervention.

In both the aquatic and land-based exercise interventions there was no non-exercising control group. In retrospect, including a parallel control group may have been a more suitable study design. However, when this study was designed it was determined from the existing literature that the two exercise groups would be dissimilar enough to elicit different physiological responses. Moreover, the purpose of the investigation was to compare endurance, to endurance combined with resistance training, within and between modalities, where each participant served as their own control. However, a non-exercising control group would have strengthened this study design, allowing the assessment of natural variation of fat oxidation in overweight and obese women. Accordingly, the current study provides preliminary data for future research on the effects of aquatic-based exercise and combined endurance and resistance training on fat oxidation in overweight and obese individuals.

6.3 Practical Implications

The results of the present investigation provide practical implications associated with optimal exercise prescription for both aquatic and land-based exercise training modalities. Aquatic exercise may not be recommended in overweight and obese women to improve rates of fat oxidation, or the contribution of fat oxidation to total EE. However, aquatic-based exercise training demonstrated positive alterations in markers of abdominal adiposity, which may result in improved health related indices in overweight and obese individuals. Furthermore, increases in upper and lower body strength can be achieved through DWR exercise alone due to the resistive nature of water, without addition of resistance exercise, simplifying aquatic-based exercise prescription. Overall, positive alterations in abdominal adiposity and strength occurred following aquatic-based exercise training in overweight women, irrespective of session structure.

Attendance to the aquatic and land-based exercise training intervention was high in the current study sample, signifying that aquatic-based and land-based exercise was well received and tolerated by overweight and obese individuals. No injuries occurred as a result of aquatic-based or land-based exercise training, indicating these modes of exercise training can be performed in supervised settings, with a high degree of safety. Therefore, in overweight and obese individuals, land-based exercise can be considered

safe; and for those who find weight bearing exercise difficult, aquatic-based exercise training can be considered an acceptable and safe alternative for exercise prescription.

From the results of the land-based exercise training investigation, exercise prescription of approximately 70% HR_{max} may be advised to increase the rate of fat oxidation during exercise and increase the contribution of fat to total exercise EE in overweight and obese women. As the structure of land-based exercise did not influence the increase in exercise fat oxidation, increase in CV fitness, or decrease in markers of abdominal obesity in the current study, the type of land-based exercise may be prescribed based on individual preference and is not limited to specific training facilities. However, further investigation, using a larger sample size of overweight and obese women is required to confirm this finding. Alternatively, to positively alter body composition, the addition of resistance training to an endurance land-based programme is recommended, as this type of training was more effective in reducing body fat percentage compared to endurance training alone.

Both aquatic and land-based exercise training was equally effective in positively altering abdominal body fat, enabling the exerciser to choose between exercise modalities based on personal preference. However, as aquatic-based exercise training demonstrated patterns of improvement in lower body strength, hip circumference and WHR more so than following land-based training, to obtain overall physiological improvements, a combination of both aquatic and land-based exercise may be recommended.

In conclusion, if the goal of exercise prescription is to increase fat oxidation and the contribution of fat oxidation to exercise EE in overweight and obese women, based on the results from the present investigation, land-based exercise training would be prescribed before aquatic-based exercise training.

6.4 Recommendations for Future Research

As the incidence of obesity continues to rise internationally, obesity research has become increasingly important. While the results from the present investigation provide an initial point of research investigating aquatic-based exercise training and fat oxidation in overweight and obese individuals, there are areas identified by the current study that require further investigation.

Whole body fat oxidation, via indirect calorimetry, was measured in the current investigation. To provide a more in-depth understanding of substrate utilisation, further examination of plasma and non-plasma fatty acid oxidation using isotopic labeling could be undertaken in future studies. Specifically, investigation into fatty acid oxidation in an aquatic environment will allow for a better understanding of metabolism and substrate utilisation associated with exercise undertaken in water, an area in that further investigation is required, and may be limited with purely indirect calorimetry measurement. Furthermore, due to the significant differences between aquatic exercise training groups in CHO oxidation following training, additional investigation is warranted into CHO metabolism in an aquatic environment.

Overweight and obese individuals demonstrate greater metabolic and physiological adaptations to exercise prescribed at an individualised level of maximal fat oxidation (Dumortier et al., 2003; Dumortier et al., 2002; Venables & Jeukendrup, 2008). Future research could be undertaken in the current sample of overweight and obese women to determine each individual's optimal fat oxidation exercise intensity. Comparison of exercise fat oxidation changes could be made at the generic level of 70% HR_{peak} to that of individualised prescription. If there is significant difference between individualised and generic exercise prescription, guidelines may recommend prior testing to determine individual levels of fat oxidation in order to stimulate the greatest use of this energy substrate. However, if changes in fat oxidation are similar between individualised prescription and generic exercise prescription, generic exercise prescription may be recommended, and is more easily prescribed. This concept has also been suggested recently by other authors (Brun, Jean, Ghanassia, Flavier, & Mercier, 2007).

To date, there has been no quantification of the work imposed during DWR, an area which needs further investigation. There is no methodology to determine work load in weight-supported activity in water, when calculated as the force applied, times the distance moved. Once work performed during aquatic exercise is determined, more accurate comparison can be made with land based exercise by matching of equivalent work rates. Furthermore, as training EE was estimated retrospectively for both aquatic and land-based exercise, future research should include a more accurate measure of EE, such as EE via portable indirect calorimetry or doubly labeled water. It may be

interesting to compare an estimation of EE using data from the average of two maximal exercise tests (pre and post intervention), to that of an actual measurement of EE. Future investigations would therefore require equivalent EE and work rates between exercise modalities.

Due to differences between aquatic-based and land-based exercise training for alterations in exercise fat oxidation rates, future research is critical to explain why aquatic exercise training did not elicit changes in fat oxidation. The current investigation indicates differences between aquatic and land-based exercise modalities for substrate utilisation, which may be physiological or methodological, specifically due to measurement using indirect calorimetry or a small sample size, an area that requires further investigation.

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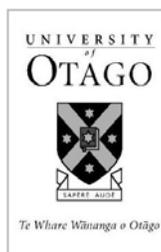
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Appendix A: Recruitment advertisement for aquatic-based exercise training study



Women Aqua Joggers Wanted

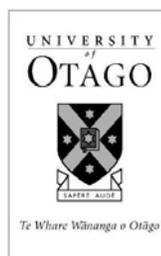
We are undertaking a study to investigate whether aqua jog training can increase your ability to use fat as a fuel during exercise.

For more information please contact:

Vicky Phillips, School of Physical
Education. Phone 479 8946
Or Dr Lynnette Jones, School of Physical
Education. Phone 479 8962

This study has been approved by the
University of Otago Human Ethics
Committee

Appendix B: Participant information sheets for aquatic-based exercise training study



*Fat metabolism following endurance and resistance type aquatic exercise training
in overweight women*

INFORMATION SHEET FOR PARTICIPANTS

Thank you for your interest in this project. Please read this information sheet carefully before deciding whether or not to participate. If you decide to participate we thank you. If you decide not to take part there will be no disadvantage to you of any kind and we thank you for considering our request.

What is the Aim of the Project?

The aim of the project is to compare the responses of a four month endurance based and resistance based aqua jog exercise programme on fat metabolism in overweight women.

What Type of Participants are being sought?

- Women volunteers who are identified as being overweight and having impaired fat oxidation.
- Experience with aqua jogging
- Aged between 25-70 years

You will be asked to complete a screening questionnaire to obtain information on your medical and exercise history prior to entry into the study. You may be excluded if you do not meet the age, exercise or body composition requirements, have a history of heart disease, diabetes, orthopaedic problems, are taking hormone replacement therapy or have any health problem which may interfere with exercise, regular use of any medications known to affect metabolism, or significant weight fluctuations within the six months prior to participation in this study. Women who meet one or more of the exclusion criteria set out above may not participate in this project, because in the opinion of the researchers and the University of Otago Human Ethics Committee, it may involve an unacceptable risk to them.

What will Participants be Asked to Do?

- Should you agree to take part in this project, you will be asked to complete the screening questionnaire. If you meet the entry criteria for admission into the study, you will be assigned to either the endurance aerobic aqua jog or resistance strength aqua jog exercise group.
- You will be asked to participate in the exercise programme for 16 weeks, 3 days per week at the local swimming pool. The entry will be subsidised. We will run exercise sessions in groups at various times of the day to best suit your time schedule. Session duration will average 60 minutes. You will be wearing an aqua jog belt at all times during the session, which will be provided. The endurance aerobic aqua jog session will involve continuous aqua jogging up and down the pool. The resistance strength aqua jog session will involve the use of aquatic equipment to perform resisted exercises for all the major muscle groups. Both exercise sessions will be performed at a moderate exercise intensity of 60% of your maximum heart rate.
- As a requirement we will ask you to undertake the following procedures before and after the exercise programme so we can get an indication of the effectiveness of the aqua jog sessions on these measures.
 1. At one visit we will take body composition measures including waist and hip circumference, weight and height and body density. You will be submerged under the water for a short time to determine your body density, known as underwater weighing. This is a standard procedure which requires you to expel all the air from your lungs, while you are seated on a scale under the water. This allows us to determine the amount of body fat and fat free mass you have. Following this you will be asked to perform a maximal graded deep water running test in the swimming flume at the School of Physical Education, University of Otago. This will give us a measure of your aerobic fitness. It will require gradual increases in aqua jog intensity until you decide that you have reached maximum effort. The test will be supervised at all times. You will be breathing through a mouthpiece attached to the metabolic cart while aqua jogging and will be wearing a heart rate monitor.
 2. At another visit you will be asked to undertake a maximal graded exercise test on a treadmill. Speed will be gradually increased until you decide that you have reached your maximum effort. Continuous breath analysis and heart rate will be undertaken throughout the exercise test. We will compare your maximum fitness measure during the maximum aqua jog to that during the maximum treadmill test.
 3. You will also be asked to meet in the morning after an overnight fast of at least 10 hours. Following a 15 minute rest period where you will be sitting quietly, you will breathe through a mouthpiece attached to a metabolic cart for a further 15 minutes. This equipment measures the oxygen and carbon dioxide content of each breath. A measure of your resting metabolic rate will be calculated using this information which provides an estimate of how many calories you would burn if you were resting for 24 hours. The proportion of fat and carbohydrate you are using as a fuel at rest, and your rate of fat oxidation at rest will also be calculated. A person qualified in taking blood

will obtain a small blood sample from your arm. This will be analysed to provide a lipid profile which will measure total cholesterol, high density lipoprotein, low density lipoprotein and triacylglycerol.

4. At a final visit you will be asked to perform an aqua jog session of 30 minutes duration which will also be undertaken in the swimming flume at the School of Physical Education, University of Otago. You will be asked to wear a heart rate monitor throughout the exercise session and aqua jog at a moderate intensity of 60% of your maximum heart rate. Every five minutes you will be asked to breathe into the mouthpiece for one minute while you are still exercising. A small blood sample will be taken from your arm before and after the test. This will be used to analyse glycerol and fatty acids which give an indication of fat use. You will also be asked to perform an upper body and lower body exercise on land to determine your strength.

- Participants who are menstruating will need to monitor their menstrual cycle closely, as the two aqua jog tests will be conducted between days three and nine following the onset of your menstrual cycle.
- As the food that you eat may affect the results, we will ask you to record what you eat and drink over the 24 hour period prior to each aqua jog test. We would also ask that you do not eat anything in the four hour period before each exercise session and the exercise test. Water may be consumed as needed.
- Research staff with first aid certification will be present at all times throughout the exercise sessions and exercise test. All testing areas are body safe protected. Participants considered at medical risk will not be considered for entry into the study. Your heart rate will be continually monitored and any signs of exercise distress will be reason to terminate the exercise session or exercise test.
- There may be minimal discomfort during the blood sampling and the use of the mouthpiece used for the indirect calorimetry measures. However, we intend to use an individual qualified in collecting blood samples and the mouthpieces are available in different sizes, to ensure the study experience is not too uncomfortable.

Can Participants Change their Mind and Withdraw from the Project?

You may withdraw from participation in the project at any time and without any disadvantage to yourself of any kind.

What Data or Information will be Collected and What Use will be Made of it?

We will obtain information on body composition, fitness, strength, fat and carbohydrate oxidation, resting metabolic rate and lipid profiles. This information will only be obtained for research and no individual data which could be associated with an individual will be released. The results of the project may be published and will be available in the library but every attempt will be made to preserve anonymity. You are most welcome to request a copy of the results of the project should you wish. The data collected will be securely stored in such a way that only those mentioned below will be able to gain access

to it. At the end of the project any personal information will be destroyed immediately except that, as required by the University's research policy, any raw data on which the results of the project depend will be retained in secure storage for five years, after which it will be destroyed.

What if Participants have any Questions?

If you have any questions about our project, either now or in the future, please feel free to contact either:

Vicky Phillips	or	Dr Lynnette Jones
School of Physical Education		School of Physical Education
University Telephone Number: 479 8946		University Telephone Number: 479 8962

This project has been reviewed and approved by the University of Otago Human Ethics Committee



*Fat metabolism following endurance and resistance type aquatic exercise training
in overweight women*

CONSENT FORM FOR PARTICIPANTS

I have read the Information Sheet concerning this project and understand what it is about. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:

1. My participation in the project is entirely voluntary.
2. I am free to withdraw from the project at any time without any disadvantage.
3. The data will be destroyed at the conclusion of the project but any raw data on which the results of the project depend will be retained in secure storage for five years, after which it will be destroyed.
4. I am prepared to perform an aerobic fitness test and to complete a prolonged exercise programme as well as undergo blood sampling, and that these may cause some discomfort.
5. I understand that the investigation will be stopped if it should appear harmful to me.
6. I know whom to contact if I have any side effects to the study or have any questions.
7. The results of the project may be published and will be available in the library but every attempt will be made to preserve my anonymity.

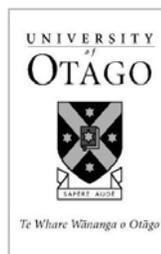
I agree to take part in this project.

.....
(Signature of participant)

.....
(Date)

This project has been reviewed and approved by the University of Otago Human Ethics Committee

Appendix C: Screening questionnaire for aquatic-based exercise study



*Fat metabolism following endurance and resistance type aquatic exercise training
in overweight women*

SCREENING QUESTIONNAIRE

Name: _____

Address:

Telephone:

Home - _____

Work - _____

Email - _____

Contact Person Phone - _____

Date of Birth: _____

Ethnic Group: _____

1. Has your doctor ever said that you have:

A heart condition

Yes/No

Diabetes

Yes/No

High blood pressure

Yes/No

Bone or joint problems

Yes/No

Any other health problem that may be affected by exercise

Yes/No

2. Have you ever felt pain in your chest during physical activity? Yes/No

3. In the past month have you had chest pain when you were not doing physical activity

Yes/No

4. Do you know of any other reason why you should not do physical activity? Yes/No
If Yes, please state
why_____
5. Are you currently taking any medication? Yes/No
If Yes, please state what medications you are taking

-
6. Are you currently taking either the oral contraceptive pill or on Depo Provera? Yes/No
7. Are you currently on hormone replacement therapy? Yes/No
8. Has your weight been stable over the past six months? Yes/No
9. If No, by how much has your weight changed (increased/decreased)?
_____ kg
10. How many periods have you had in the last six months? Please circle
More than 6 6 5 4 Less than 4
11. Date (approximately) of last period? _____
12. When did you start aqua-jogging (approximately)? _____
13. How many times per week (average) do you aqua jog? _____
14. How long is each typical aqua jog session? _____
15. Have you ever had any adverse reaction(s) during or following a blood test, eg fainting?
Yes/No, if Yes, what happened? _____

Signature.....

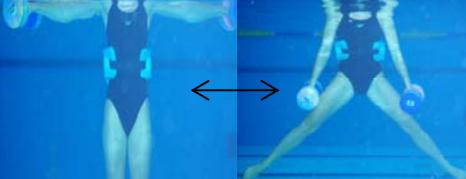
Date.....

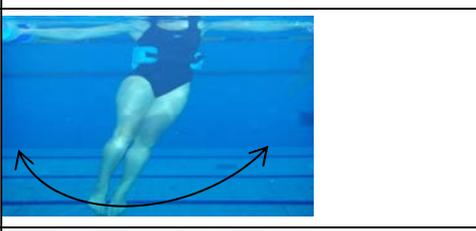
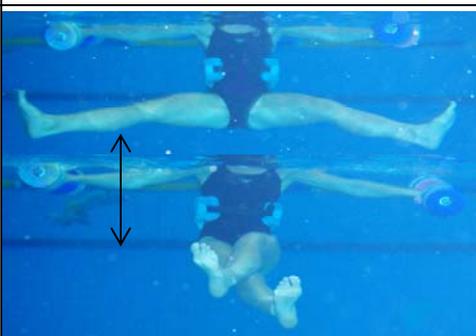
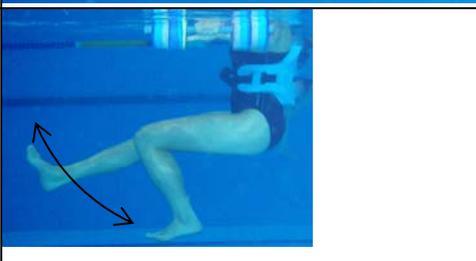
Appendix D: 24 h diet record

Appendix E: Endurance training for DWR

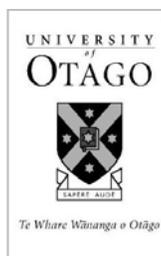
1. Traditional DWR = Feet flat, abdominals tight, head up, shoulders and torso in line
with hips, buttocks tight (running form)
2. Karate Chop = Traditional DWR, but hands flat not cupped
3. Punching (straight/upper cut) = Traditional DWR, but hands in fist
4. Cross Country Ski = Legs and arms straight swinging backwards and forwards
5. Breast stroke = Traditional DWR, but arms breast stroke motion
6. Alternate Breast Stroke = Traditional DWR, but one arm at a time
7. Tire Legs/ Alternate Breast Stroke = Same arms as above, legs striding wide like
running through tires
8. Elephant = Traditional DWR, but arms out in front clasped hands swinging side to side
9. Prayers = Traditional DWR, but hands clasped together tight to chest under water
10. Crunchies = Traditional DWR, but opposite elbow to opposite knee
11. Penguin = Tire legs and arms reach down to heel as heel comes up to hip
12. Tin Man = Straight arms and legs from vertical to horizontal
13. Mini Tin Man = Straight legs horizontal, doggy paddle arms, kicking from hip
14. Backward Arm Sweeps = Breast stroke arms, pushing water with the back of hand
15. Moonwalk = Like cross-country ski, but bending at knee and elbow when striding
16. Hurdles = High legs like jumping over something while running
17. Train = Wide cycles of arms and legs in running form
18. Chariot = Tow someone across pool while they keep legs pointed to bottom of pool
19. Seated Scissor Kick, Breast Stroke Arms = Legs horizontal, spread wide bring back
to centre and cross and repeat
20. Going Backwards by Breast Stroking = Moving in a backwards motion with opposite
breast stroke arms
21. Basketball dribbling = Regular running legs, upper arms tight to sides of body, bend
lower arms up to 90°, with straightening out using flat hands flat
22. Clapping = Regular running legs, or tire legs. Keep arms straight and bring out to
sides then bring together like clapping

Appendix F: Circuit training for DWR+RT

<p>Chest Press/ Row <i>Target muscles: Rhomboids/Pectoralis major and minor</i></p> <ul style="list-style-type: none"> Starting with arms at 90° press dumbbells downwards until arms are fully extended Raise arms up leading with elbows, squeezing shoulder blades together 	
<p>Flys/ Reverse Flys <i>Target muscles: Rhomboids/Pectoralis major and minor</i></p> <ul style="list-style-type: none"> Keeping arms extended with slight bend in elbow lower down and toward each other to meet Raise arms back up, squeezing shoulder blades together 	
<p>Lateral Raise/ Lateral Pull Down <i>Target muscles: Latissimus dorsi/deltoids</i></p> <ul style="list-style-type: none"> Keeping arms straight and extended lower down to meet in the middle of body Raise arms back up until horizontal with water 	
<p>Internal/External Rotation <i>Target muscles: Rotator cuff</i></p> <ul style="list-style-type: none"> Keeping upper arms close to body with forearms at a 90° angle bring forearms into the centre of body horizontally until hands touch Move forearms back to sides as far as possible 	
<p>Tricep Kickback <i>Target muscles: Triceps/Biceps</i></p> <ul style="list-style-type: none"> Holding on to side of pool keep upper arm close to body while only moving forearm until horizontal Move forearm down and under in line with the upper arm until nearly touching shoulder 	
<p>Press Down/Pull up <i>Target muscles: Biceps/Triceps</i></p> <ul style="list-style-type: none"> Starting with elbows up and hands close to the body press arms downwards until fully extended Move arms up, leading with elbows, until upper arm is at water level 	
<p>Bicep Curl <i>Target muscles: Biceps/Triceps</i></p> <ul style="list-style-type: none"> Beginning with arms fully extended downwards, curl forearm up in line with arm in front of the body Move forearm down toward the bottom of pool while keeping upper arm vertical and close to body 	
<p>Starjumps/T's and A's <i>Target muscles: Hip Adductors/Abductors</i></p> <ul style="list-style-type: none"> Begin in a 'T' position with arms horizontal and legs pointing straight down Move arms downwards, while bringing straight legs out laterally to meet arms in an 'A' position 	

<p>Scissor Kicks <i>Target muscles: Hip Flexors, Gluteus Maximus, Hamstrings</i></p> <ul style="list-style-type: none"> • Keep arms horizontal to the side and legs straight • Alternate legs so one moves forwards and one backwards as far as possible 	
<p>Can Can Dance <i>Target muscles: Quadriceps, Hamstrings, Gluteus Maximus</i></p> <ul style="list-style-type: none"> • Keeping arms out horizontally bring one leg up with bent knee and straighten to kick to the side • Bring leg back and down to the centre of the body and repeat with other leg 	
<p>Abdominal Curls - Normal and Oblique <i>Target muscles: Abdominals – rectus and oblique</i></p> <ul style="list-style-type: none"> • Lying on surface of water with hands out horizontally, bring knees through the centre into chest and straighten for normal curls • Repeat same movement but bring knees in and out to each side while twisting hips for oblique 	
<p>Pendulum <i>Target muscles: Oblique abdominals</i></p> <ul style="list-style-type: none"> • Begin with legs straight and pointing toward bottom of the pool • Tilt from the waist so legs move out and up, keeping straight, return to centre and out and up other side 	
<p>Pogo Stick/Squats <i>Target muscles: Quadriceps, Gluteus Maximus</i></p> <ul style="list-style-type: none"> • Place dumbbell between feet and begin with straight legs pointing towards the bottom of the pool • Bending at the knees bring feet upwards in the centre and push down until legs are straight 	
<p>Seated Scissors <i>Target muscles: Abductors/Adductors</i></p> <ul style="list-style-type: none"> • Keeping arms out horizontally, hold legs up and out straight to each side • Bring legs together in front of the body and cross over each other • Move legs back out straight to each side 	
<p>Seated leg kick <i>Target muscles: Hamstrings/Quadriceps</i></p> <ul style="list-style-type: none"> • Keeping arms out horizontally, bring thighs up to a 90° angle with lower legs hanging at a 90° angle • Alternate legs with one leg rising to be horizontal with the thighs, while the other stays at 90° 	

Appendix G: Participant information sheets for land-based exercise training study



Fat metabolism following endurance and resistance land based exercise training in overweight women

INFORMATION SHEET FOR PARTICIPANTS

Thank you for your interest in this project. Please read this information sheet carefully before deciding whether or not to participate. If you decide to participate we thank you. If you decide not to take part there will be no disadvantage to you of any kind and we thank you for considering our request.

What is the Aim of the Project?

The aim of the project is to compare the responses of a twelve week walking and circuit gym based exercise programme on fat metabolism in overweight women.

What Type of Participants are being sought?

- Healthy women volunteers who are identified as being overweight
- Aged between 25-70 years

You will be asked to complete a screening questionnaire to obtain information on your medical and exercise history prior to entry into the study. You may be excluded if you do not meet the age, exercise or body composition requirements, have a history of heart disease, diabetes, orthopaedic problems, are taking hormone replacement therapy or have any health problem which may interfere with exercise, regular use of any medications known to affect metabolism, or significant weight fluctuations within the six months prior to participation in this study. Women who meet one or more of the exclusion criteria set out above may not participate in this project, because in the opinion of the researchers and the University of Otago Human Ethics Committee, it may involve an unacceptable risk to them.

What will Participants be Asked to Do?

- Should you agree to take part in this project, you will be asked to complete the screening questionnaire. If you meet the entry criteria for admission into the study, you will be assigned to either the endurance walking or resistance circuit gym exercise group.
- You will be asked to participate in the exercise programme for 12 weeks, 3 days per week. Session duration will average 60 minutes. We will run group exercise

sessions at various times of the day to best suit your time schedule. The endurance walking session will involve walks around the Dunedin area, leaving from the School of Physical Education, University of Otago. The resistance circuit gym session will take place in the circuit gym at the School of Physical Education, 55 Union Street West, University of Otago. This session will involve exercises for all the major muscle groups interspersed with active recovery. Both exercise sessions will be performed at a moderate exercise intensity of 70% of your maximum heart rate.

- As a requirement we will ask you to undertake the following procedures before and after the exercise programme so we can get an indication of the effectiveness of the walking and circuit gym sessions on these measures.
 1. At one visit we will take anthropometric measures including waist and hip circumference, and weight and height. Following this you will be asked to perform a maximal graded exercise test on a treadmill to determine your walking fitness. You will begin walking at a comfortable pace while the gradient will be gradually increased until you decide that you have reached your maximum effort. The test will be supervised at all times. Continuous breath analysis and heart rate will be undertaken throughout the exercise test. You will also be asked to perform an upper body and lower body exercise test on land to determine your strength.
 2. You will also be asked to meet in the morning after an overnight fast of at least 10 hours. Following a 15 minute rest period where you will be sitting quietly, you will breathe through a mouthpiece attached to a metabolic cart for a further 15 minutes. This equipment measures the oxygen and carbon dioxide content of each breath. A measure of your resting metabolic rate will be calculated using this information which provides an estimate of how many calories you would burn if you were resting for 24 hours. The proportion of fat and carbohydrate you are using as a fuel at rest, and your rate of fat oxidation at rest will also be calculated. A person qualified in taking blood will obtain a small blood sample from your arm. This will be analysed to provide a lipid profile which will measure total cholesterol, high density lipoprotein, low density lipoprotein and triacylglycerol.
 3. At a final visit you will be asked to perform a 30 minute walk on a treadmill. You will wear a heart rate monitor throughout the exercise session and walk at a moderate intensity of 60% of your maximum heart rate. Every five minutes you will be asked to breathe into a mouthpiece for one minute while you are still walking. A small blood sample will be taken from your arm before and after the test. This will be used to analyse glycerol and free fatty acids which give an indication of fat use during exercise.
 4. A 30 minute appointment will be made with Dunedin Hospital to undertake a test to determine your body composition by assessing amounts of fat and lean body mass. This painless procedure is known as dual-energy x-ray absorptiometry (DXA) and will be undertaken and supervised by an experienced technician.

- Participants who are menstruating will need to monitor their menstrual cycle closely, as the pre- and post-intervention 30 minute walking tests will be conducted between days three and nine following the onset of your menstrual cycle.
- As the food that you eat may affect the results, we will ask you to record what you eat and drink over the 24 hour period prior to each 30 minute walking test. We would also ask that you do not eat anything in the four hour period before each exercise session and the exercise test. Water may be consumed as needed.
- Research staff with first aid certification will be present at all times throughout the exercise sessions and exercise test. All testing areas are body safe protected. Participants considered at medical risk will not be considered for entry into the study. Your heart rate will be continually monitored and any signs of exercise distress will be reason to terminate the exercise session or exercise test.
- There may be minimal discomfort during the blood sampling and the use of the mouthpiece used for the indirect calorimetry measures. However, we will use an individual qualified in collecting blood samples and the mouthpieces are available in different sizes, to ensure the study experience is not too uncomfortable.

Can Participants Change their Mind and Withdraw from the Project?

You may withdraw from participation in the project at any time and without any disadvantage to yourself of any kind.

What Data or Information will be Collected and What Use will be Made of it?

We will obtain information on body composition, fitness, strength, fat and carbohydrate metabolism, resting metabolic rate and lipid profiles. This information will only be obtained for research and no individual data which could be associated with an individual will be released. The results of the project may be published and will be available in the library but every attempt will be made to preserve anonymity. You are most welcome to request a copy of the results of the project should you wish. The data collected will be securely stored in such a way that only those mentioned below will be able to gain access to it. At the end of the project any personal information will be destroyed immediately except that, as required by the University's research policy, any raw data on which the results of the project depend will be retained in secure storage for five years, after which it will be destroyed.

What if Participants have any Questions?

If you have any questions about our project, either now or in the future, please feel free to contact either:

Vicky Phillips

School of Physical Education

University Telephone Number: 479 8946

or Dr Lynnette Jones

School of Physical Education

University Telephone Number: 479 8962

This project has been reviewed and approved by the University of Otago Human Ethics Committee



Fat metabolism following endurance and resistance land based exercise training in overweight women

CONSENT FORM FOR PARTICIPANTS

I have read the Information Sheet concerning this project and understand what it is about. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:

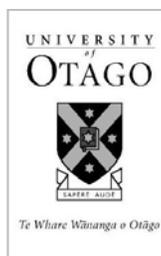
1. My participation in the project is entirely voluntary.
2. I am free to withdraw from the project at any time without any disadvantage.
3. The data will be destroyed at the conclusion of the project but any raw data on which the results of the project depend will be retained in secure storage for five years, after which it will be destroyed.
4. I am prepared to perform an aerobic fitness test and to complete a prolonged exercise programme as well as undergo blood sampling, and that these may cause some discomfort.
5. I understand that the investigation will be stopped if it should appear harmful to me.
6. I know whom to contact if I have any side effects to the study or have any questions.
7. The results of the project may be published and will be available in the library but every attempt will be made to preserve my anonymity.

I agree to take part in this project.

.....
(Signature of participant)

.....
(Date)

Appendix H: Screening questionnaire for land-based exercise training study



Fat metabolism following endurance and resistance land based exercise training in overweight women

SCREENING QUESTIONNAIRE

Name: _____

Address: _____

Telephone:

Home - _____ Work - _____

Email - _____ Contact Person Phone - _____

Date of Birth: _____

1. Which ethnic group(s) do you belong to? (Tick more than one if appropriate)

- New Zealand European
- Other European Please print your ethnic group _____
- New Zealand Maori
- Samoan
- Cook Island
- Tongan
- Chinese
- Indian
- Other Please print your ethnic group _____

2. Has your doctor ever said that you have:
- | | |
|---|--------------|
| A heart condition | Yes/No |
| Diabetes | Yes/No |
| High blood pressure | Yes/No |
| Bone or joint problems | Yes/No |
| Any other health problem that may be affected by exercise | Yes/No _____ |
3. Have you ever felt pain in your chest during physical activity? Yes/No
4. In the past month have you had chest pain when you were not doing physical activity
Yes/No
5. Do you know of any other reason why you should not do physical activity? Yes/No
If Yes, please state
why _____
6. Are you currently smoking cigarettes? Yes _____/day /No
7. Are you currently taking any medication? Yes/No
If Yes, please state what medications you are taking

8. Are you currently taking either the oral contraceptive pill or on Depo Provera? Yes/No
9. Are you currently on hormone replacement therapy? Yes/No
10. Has your weight been stable over the past six months? Yes/No
If No, by how much has your weight changed (increased/decreased)?
_____ kg

11. How many periods have you had in the last 6 months?

- More than 6 Please state how many _____
- 6
- 5
- 4
- Less than 4 Please state how many _____

12. Date (approximately) of last period? _____

13. How many times per week do you undertake some form of exercise? _____

14. What types of exercise do you partake in? _____

15. How long is each typical exercise session? _____

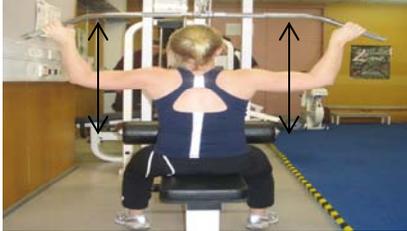
16. Have you ever had any adverse reaction(s) during or following a blood test, eg fainting?

Yes/No, if Yes, what happened? _____

Signature.....

Date.....

Appendix I: Circuit training for LBE+RT

<p>Chest Press Target muscles: pectoralis major/minor</p> <ul style="list-style-type: none"> • Lying on back with upper arms extended horizontally at shoulder level and forearms at 90°, press arms up until fully extended • Release arms to bring back down to 90° 	
<p>Flys Target muscles: pectoralis major/minor</p> <ul style="list-style-type: none"> • Lying on back with arms extended horizontally and a slight bend in the elbow bring arms up and together to meet above the head • Release arms to bring back to horizontal 	
<p>Lateral Raise Target muscles: deltoids</p> <ul style="list-style-type: none"> • Starting with arms extended and at the sides of the body, raise arms while straight until shoulder height • Lower arms back down to sides 	
<p>Internal/External Rotation Target muscles: rotator cuff</p> <ul style="list-style-type: none"> • Lying on one side, keeping upper arm at the side of the body with forearm at 90°, lower forearm down and across the body until touching the floor • Raise forearm keeping at 90° until vertical 	
<p>Tricep Kickback Target muscles: triceps</p> <ul style="list-style-type: none"> • Standing while bending at hips so back is horizontal, keep upper arm horizontal while straightening forearm and raising it so in line with upper arm • Lower forearm, keeping upper arm fixed horizontally, and bring up towards shoulder 	
<p>Lateral Pull Down Target muscles: latissimus dorsi</p> <ul style="list-style-type: none"> • While seated, pull bar down towards chest while squeezing shoulder blades together until elbows at 90° • Release bar to let arms straighten 	
<p>Bicep Curl Target muscles: biceps</p> <ul style="list-style-type: none"> • Holding barbell in front of the body begin with straight arms • Curl the bar up towards chest with forearms while keeping upper arms in place by sides of body 	

<p>Seated Row Target muscles: rhomboids</p> <ul style="list-style-type: none"> • While seated begin by gripping handles with arms extended • Move arms, leading with elbows, back while squeezing shoulder blades together 	
<p>Side Leg Raises Target muscles: abductors</p> <ul style="list-style-type: none"> • Begin lying on the side with legs out straight directly above each other and hips in line • Raise top leg while keeping straight to approximately shoulder height, keeping foot flat 	
<p>Leg Extension or Leg Press Target muscles: quadriceps</p> <ul style="list-style-type: none"> • Sitting with legs at 90° or hanging, either push feet or curl up until legs are nearly straight • Release so legs move back to starting position 	
<p>Leg Curl Target muscles: hamstrings</p> <ul style="list-style-type: none"> • Begin lying on stomach with legs straight out behind • Curl up lower leg until approximately 90° with thighs, then straighten 	
<p>Squats Target muscles: gluteus maximus/quadriceps</p> <ul style="list-style-type: none"> • Begin standing, leaning slightly on swiss ball resting in the small of back. • Bending at the knees, squat down so thighs are horizontal with the ground aiming for a 90° between lower leg and thigh 	
<p>Side Leg Pull Target muscles: adductors</p> <ul style="list-style-type: none"> • Standing with foot in elastic band move leg in towards the body keeping leg straight • Release leg back to standing position 	
<p>Abdominal Curls - Normal Target muscles: rectus abdominals</p> <ul style="list-style-type: none"> • Lying with swiss ball in the small of back, feet on floor, begin with trunk horizontal • Raise shoulders up while bringing upper body off swiss ball, release back to starting position 	

Appendix J: Phillips, V.K., Legge, M., & Jones, L. M. (2008). Maximal Physiological Responses between Aquatic and Land Exercise in Overweight Women. Medicine and Science in Sports and Exercise, 40, 959-964.